Expression of *Entomophaga maimaiga* at Several Gypsy Moth (Lepidoptera: Lymantriidae) Population Densities and the Effect of Supplemental Watering[1]

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Abstract Assessment of gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), populations in western Virginia during the year 2000, showed that larval mortality factors differed as a function of population density. Larval mortality, primarily due to the gypsy moth-specific entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu and Soper, was 28.7% in high-density plots (averaging 476.8 larvae per 5 burlap bands) but only 16.5% in low-density plots (averaging 60.8 larvae per 5 burlap bands). On the contrary, "missing" was the dominant mortality factor in low-density plots (6.5% in high-density plots vs 65.6% in low-density plots). This study was designed to assess the potential for inducing an earlier epizootic by facilitating the early germination of resident resting spores by spraying water around the base of trees. Eight of the high-density plots with measured natural *E. maimaiga* resting-spore loads were selected for a supplemental watering study conducted in 2001. Four plots received weekly watering to supplement natural rainfall, and four received only natural rainfall (control plots). More *E. maimaiga* occurred in watered plots than in control plots; however, treatment effects for watering were not significant. For a 01 June collection, fungal levels were 17% (ground) and 12% (canopy) for the watered plots vs 12% (ground) and 10% (canopy) for control plots. For a 15 June collection, fungal levels were 74% (ground) and 29% (canopy) for the watered plots vs 60% (ground) and 14% (canopy) from control plots. Height effects were significant for the second date. Egg mass populations in watered plots declined significantly (78%) compared with control plots (4%) ($P = 0.0433$), possibly reflecting further mortality occurring after the second collection.

Key Words *Lymantria dispar, Entomophaga maimaiga,* gypsy moth, entomopathogenic fungus, biological control

Gypsy moths, *Lymantria dispar* L., are serious defoliators of forest, park and residential shade trees. While effective registered pesticides exist, many landowners would prefer to rely, if possible, on an effective natural control agent such as the fungus *Entomophaga maimaiga* Humber, Shimazu and Soper. This gypsy moth-specific entomopathogenic fungus was responsible for the region-wide collapse of the gypsy moth in Virginia during the late 1990's (Webb et al. 1999). Epizootics of this

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fungus result in the accumulation of resting spores (= azygospores) in the soil at the base of trees (Hajek et al. 1998a,b). In this paper we use the term "resting spore" as per Hajek et al. (1998a) to emphasize the ecological function of these spores. Resting spores remain in the environment for a number of years (Weseloh and Andreadis 1997) and eventually germinate in the spring just prior to gypsy moth egg hatch. Germination continues until mid-June when late larval instars are present (Hajek and Humber 1998) so that infection can occur throughout the period when larvae are present. However, the fungal epizootic generally peaks late in the season when infections arise primarily from airborne conidia (Weseloh and Andreadis 1992a, Webb et al. 1999). Resting spores become infective upon the emergence of hyphae bearing the germ conidia, which is dependent on the presence of free water. Natural rainfall will eventually provide such water, but is unpredictable. Weseloh and Andreadis (1992a) suggested that sprinkler irrigation might hasten the activation of resting spores, leading to increased fungal activity on small sites, possibly in conjunction with augmentation of resting spores if these are not abundant. Hajek and Webb (1999) introduced resting spores into four treatment plots on the eastern shore of Maryland in 1995 and 1996 to augment the naturally-occurring fungal populations. Plots were watered weekly to facilitate activation of the spores. Fungal activity was always higher in the treatment plots compared to such activity in matched control plots, but due to high variability among plots, these results were not significant. The present study tests whether fungal activity at sites containing naturally-occurring resting spores, if measured and found abundant, could also be enhanced by supplemental watering.

In 2000, the gypsy moth populations along Skyline Drive in the Shenandoah National Park in western VA were increasing, and fungal activity was expected. However, a preliminary early-spring evaluation of the status of the gypsy moth populations along Skyline Drive indicated that the populations in 2000 were not at the level where a proposed study of supplemental watering could be effectively evaluated without more precise characterization. We, therefore, conducted such a characterization in 2000 in preparation for a supplemental-water study in 2001. Goals for 2000 were (1) to characterize the gypsy moth populations at candidate watering sites, and (2) to quantify levels of *E. maimaiga* present in the park as a function of gypsy moth population. Goals for 2001 were to determine the effect of supplemental watering on the level of infection caused by *E. maimaiga* on the increasing gypsy moth populations in the Shenandoah National Park.

Materials and Methods

Year 2000 plot characterizations. Twenty-four roadside strips (approximately 25 × 3 m = 75 m^2) each containing 5 dominant host trees were established along Skyline Drive in Shenandoah National Park, beginning at milepost 6 (near Front Royal) and ending near milepost 29 (near Luray). Trees were characterized by species, and the diameter at breast height (dbh) was measured. A burlap band larval trap was wrapped around each tree on 24 May at 1.5-m height. Beginning 2 June, all gypsy moth life stages occurring under burlap bands were counted and recorded as per Webb et al. (1999). Dead larvae and pupae were counted and removed, but living life stages were not removed. Weekly counts were taken through 24 July. Relative population density was expressed as per Webb et al. (1989, 1999) as the peak number of immature insects (L_{max}) (live or dead larvae or pupae) found under the 5 burlap bands for each plot. Per cent season-long larval mortality (L_{mort}) is expressed by formula 1:

$$L_{mort} = (DL/L_{max}) \cdot 100, \quad (1)$$

where DL equals the total number of dead larvae during the season. Similarly, per cent season-long missing larvae ($L_{missing}$), or the number of larvae present in the peak counts that were subsequently unaccounted for either as cadavers or as pupae, is expressed by formula 2:

$$L_{missing} = (ML/L_{max}) \cdot 100, \quad (2)$$

where ML equals the difference between L_{max} and the total number of life stages accounted for at the end of the season. Per cent dead as pupae (P_{mort}) is expressed by formula 3:

$$P_{mort} = (DP/L_{max}) \cdot 100, \quad (3)$$

where DP equals the total number dead pupae (DP) found during the season. This is different from per cent dead pupae, which is the number of dead pupae divided by the total number of pupae. Per cent missing as pupae ($P_{missing}$) is expressed by formula 4:

$$P_{missing} = (MP/L_{max}) \cdot 100, \quad (4)$$

where MP equals the total number of pupae present in the peak pupal counts that were subsequently unaccounted for as cadavers or as adults. Finally, per cent of population surviving to adulthood ($A_{survival}$) is expressed by formula 5:

$$A_{survival} = (A_{total}/L_{max}) \cdot 100, \quad (5)$$

where A_{total} equals the total number of adult males and females (A_{total}) (represented by empty pupal cases).

Dead larvae were sampled and returned to the lab for necropsy as per Webb et al. (1999). Cadavers having one or both types of characteristic (Hajek and Shimazu 1996) *E. maimaiga* spores (resting spores or conidia) were counted positive for fungus. The type of spore found in the cadaver was considered diagnostic for the spore type initiating the infection. Cadavers containing conidia, but no resting spores are usually the result of encounters with germ conidia from resting spores (Hajek 1997, Hajek and Humber 1998). Cadavers containing resting spores (with or without the presence of conidia) are the product of infection by (usually air-borne) conidia, although a small percentage of such infections will contain just conidia, with no resting spores (Hajek 1997, Hajek and Humber 1998).

In March 2001, 0.01-ha egg mass surveys were conducted just outside of the plots, one survey per plot, by the method of Liebhold et al. (1994). We felt that an egg mass survey within the plot would have been compromised by the presence of the burlap bands during the season. During this time, soil was sampled from the base of 3 trees that were within this 0.01-ha survey area. Soil was taken from four points around the base of each tree, within 5 cm of the junction of the bark and the soil. This is where resting spore numbers should be highest (Hajek et al. 1998a,b). Reported spore counts are, thus, the average of twelve 30-ml samples per plot. Spore extraction from the soil and spore counts followed a modification of the procedures of Hajek and Wheeler (1994). Briefly, all soil samples were stored in sealed plastic bags at approximately 4°C until the spores were extracted and enumerated. The soil was gently broken up and homogenized by manually shaking while still in the original sample bag. A soil subsample of 7.5 ml volume was placed in a 50-ml centrifuge tube,

and a washing solution containing the surfactant Triton x-100 (0.1%) was added to bring the volume up to 25 ml. This was mixed with a vortexer, then sonicated for 2 min and vortexed again. The soil was then washed through a series of sieves using tap water. The last fraction of this (particle size 20-63 μ) was collected and 0.15 M NaCl added to make 25 ml in volume. This was tinted with a trace of red food coloring (red FD&C 40). A 5-ml layer of Renographin-76 (Squibb Diagnostics) adjusted to 1.25 g/ml was placed in a centrifuge tube. On top of this was carefully layered a 5-ml aliquot of the washed sample. This was centrifuged at 4,000 rpm for 10 min. The supernatant plus interface were carefully removed by pipette and transferred to a counting chamber. The counting chamber was a 90-mm diam polystyrene Petri dish with an etched grid. Three bands of the grid (20.9% of the total surface area) were counted. We determined the dry weight of each soil sample so that we could report our results as g/ml of soil.

Supplemental watering study, spring 2001. Gypsy moth populations collapsed to undetectable levels in 15 of our 24 study plots, leaving us with 9 potential plots for a supplemental watering study. We chose four northern plots (sites 1 to 4) and four southern ones (sites 19 to 22) as study plots for 2001. Plots 1, 3, 19, and 21 were designated as supplemental-water plots, and plots 2, 4, 20, and 22 were designated as controls. Beginning on 15 April, water was applied at the rate of 1 liter per 2.5 m^2 (= 30 liters per plot with a goal of 3.78 liters around the base of each tree as per Hajek and Webb [1999]). However, the amount per tree was adjusted for tree diameter, with larger trees receiving somewhat more water, and smaller trees receiving somewhat less. The water was applied as a course mist around the base of the trees. Applications were made each week until 15 June.

On 01 June, and again on 15 June, 50 live larvae were sampled from each site, of which 25 were collected from foliage or the lower tree bole at "ground level" (0 to 2 m), and 25 were collected from the mid-canopy or higher using a bucket truck. All larvae were placed on artificial diet (Bell et al. 1981) in 30-ml plastic cups with paper lids, one larva per cup. The rearing cups were held on shelves in a wooden outdoor insectary (368 cm long, 215 cm wide, 92 cm deep, with hardware cloth covering the front to allow natural conditions of light, temperature, and humidity but not rain) at the Beltsville Agricultural Research Center, Beltsville, MD. All larvae in the insectary were observed every 2 to 3 d for mortality for 35 d. In November 2001, 1/100-ha egg mass surveys were again conducted just outside of the plots, one survey per plot, by the method of Liebhold et al. (1994).

Statistical analysis. Differences in site parameters, gypsy moth population parameters, and *E. maimaiga* spore counts among gypsy moth population density categories, and the effects of supplemental watering and collection height on *E. maimaiga* infection rate in collected gypsy moth larvae, were tested using PROC MIXED (SAS Institute 1996). Means were separated at a comparison-wise error rate of 0.05 using the least significant difference (LSD) procedure. Results of the egg mass surveys were analyzed by a Kruskal-Wallis Test using PROC NPAR1WAY (SAS Institute 1999).

Results and Discussion

Site parameters, 2000. Weekly burlap band counts indicated that two very different dynamic processes were occurring to gypsy moth populations among the 24 plots along Skyline Drive in 2000. Examination of the data suggests that gypsy moth

populations in higher density plots fared very differently from populations in lower density plots. In this paper we arbitrarily divide the plots into 3 categories based on their peak burlap count numbers to illustrate the dynamic differences occurring in the plots (Table 1). Nine plots with peak burlap larval populations (determined for 5 burlap bands per plot) ranging from 835 to 189 (average 474.8) are termed "high". Nine plots with peak populations ranging from 121 to 18 (mean 60.8) larvae per 5 burlap bands are termed "low". Finally, 6 plots with populations ranging from 10 to 1 (average 5.0) larvae per 5 burlap bands are termed "sparse". Population effects (= peak larval counts) were significant among the population categories ($F = 33.0$; df = 2, 21; $P < 0.0001$). Elevation effects were also significant ($F = 22.5$; df = 2, 21; $P < 0.0001$). High-population plots occurred at significantly lower elevations (average elevation =

Table 1. Plot parameters examined for Skyline Drive study in 2000 and 2001

Parameter*	High(se)	Low(se)	Sparse(se)	*F*	*P* > *F*
No. plots	9	9	6		
Avg. peak N/plot	476.8 (69.1)a	60.8 (11.8)b	5.0 (1.4)b	33.0	<0.0001
Avg. elevation (m)	684.4 (21.3)a	814.7 (23.3)b	915.2 (26.9)c	22.5	<0.0001
Avg. dbh (cm)	34.0 (1.6)	41.1 (5.3)	38.7 (4.5)	0.9	0.4
Avg. no. chestnut oak	3.8 (0.6)a	0.9 (0.6)b	0.0b	12.4	0.0003
Avg. no. white oak	1.2 (0.6)	1.9 (0.8)	0.0	1.9	0.2
Avg. no. red oak	0.0a	2.2 (0.9)b	5.0 (0.0)c	17.1	<0.0001
Avg. no dead larvae	129.0 (14.5)	9.8 (2.7)	0.3 (0.2)		
Avg. % dead as larvae	28.7 (8.7)a	16.5 (12.3)b	4.0 (6.4)b	56.9	<0.0001
Avg. no missing larvae	28.8 (15.0)	42.7 (11.1)	2.7 (1.1)		
Avg. % missing as larvae	6.5 (8.6)	65.5 (21.7)	63.7 (33.5)	2.4	0.1
Avg. no pupating	319.1 (58.5)	8.3 (2.3)	2.0 (0.8)		
Avg. % pupation	64.8 (3.6)a	18.0 (0.6)b	32.3 (13.1)b	13.0	0.0002
Avg. no. dead pupae	194.1 (30.5)	3.4 (1.0)	0.3 (0.2)		
Avg. % dead as pupae	40.2 (0.2)a	6.9 (0.2)b	4.4 (0.3)b	67.3	<0.0001
Avg. no. missing pupae	12.4 (2.7)	2.3 (0.8)	1.2 (0.7)		
Avg. % missing as pupae	3.1 (0.1)	5.0 (0.1)	19.0 (10.0)	3.3	0.06
Avg. no. adult males	61.1 (14.9)	1.7 (0.9)	0.0		
Avg. no. adult females	51.4 (18.0)	0.9 (0.3)	0.5 (0.3)		
Avg. % surviving to adult	21.6 (0.4)a	6.0 (0.3)b	10.0 (0.6)b	5.0	0.2
Avg. no. egg masses/ha (March, 2001)	2173.3 (807.5)a	4.4 (4.4)b	0.0b	5.9	0.009
Avg. no. egg masses/ha (November, 2001)	653.3 (220.2)a	0.0b	0.0b	7.2	0.004

* Values are averages based on counts from 5 burlap bands per plot.
Means within a row followed by the same letter are not significantly different at the 0.05 level.

684.4 m) than low-population plots (average elevation = 814.7 m), which occurred at significantly lower elevations than sparse-population plots (average elevation = 915.2 m) (Table 1). Tree size effects, expressed in terms of diameter at breast height, were not significant (Table 1). Host tree composition differed significantly among the population classes, with high-population plots containing mainly chestnut oaks (*Quercus prunus* L.) (76%) with some white oaks (*Q. alba* L.) (24%), while low-population plots contained 18% chestnut oak, 38% white oak, and 44% red oak group (several species but primarily *Q. rubra* L.). "Sparse" population plots contained 100% red oak group trees.

Gypsy moth population dynamics in 2000. *Entomophaga maimaiga,* while present, apparently played little role in the extensive erosion of gypsy moth larvae from low-density plots during the critical period JD 161-175 (Table 2). These larvae, representing 65.5% of peak N (Table 1), disappeared from subsequent band counts, leaving no cadavers or pupae. An additional 16.5% of peak N larvae died in association with the bands, leaving cadavers, and 18% of the peak N were recorded as pupae. Of the pupae, 41% died; this represented 6.9% of the total peak N. Also, 28% of the pupae disappeared (recorded as missing); this represented 5.0% of the total peak N. The dead and missing accounted for 94% of peak N, leaving 6.0% surviving to adulthood. Figures for the sparse plots were similar; of 30 larvae recorded as visiting the burlap bands for the 6 sparse plots, 16 disappeared as larvae, 2 died leaving cadavers, and 12 pupated under the bands. Two of the pupae died and 7 disappeared, so that a total of 3 gypsy moths (all females) survived to adulthood under the burlap bands in the 6 sparse plots. In contrast, of the 4292 larvae recorded as visiting burlaps in the 9 higher-density plots, 4033 (94%) were accounted for as

Table 2. Larval fate versus per cent pupation as a function of population density at three critical dates

Parameter	High(se)	Low(se)	Sparse(se)	*F*	*P* > *F*
Julian Date 161					
% dead larvae	0.4 (0.1)a	0.0b	0.0b	7.1	0.004
% missing larvae	0.0	1.7 (1.2)	0.0	1.6	0.21
% pupation	0.3 (0.1)a	0.0b	0.0b	4.7	0.02
Julian Date 168					
% dead larvae	10.3 (2.8)a	0.5 (0.4)b	0.0b	10.1	0.0008
% missing larvae	1.9 (0.9)a	33.2 (8.2)b	0.0a	12.4	0.0003
% pupation	44.8 (4.2)a	0.0b	0.0b	89.5	<0.0001
Julian Date 175					
% dead larvae	27.9 (2.8)a	14.0 (2.8)b	4.0 (3.4)b	15.5	0.0001
% missing larvae	5.9 (7.9)a	57.2 (7.9)b	10.0 (0.9)a	12.5	0.0003
% pupation	55.2 (3.1)a	3.5 (1.7)b	0.0b	179.9	<0.0001

Means within a row followed by the same letter are not significantly different at the 0.05 level.

either cadavers or pupae. The fate of burlap-visiting gypsy moths in the high-density plots was calculated as 28.7% dead as larvae, 6.5% missing as larvae, 40.2% dead as pupae, 3.1% missing as pupae, leaving 21.6% surviving to adulthood (Table 1).

Timing and severity of mortality factors, 2000. A total of 263 of the 305 cadavers sampled and necropsied during 2000 were positive for *E. maimaiga*. Cadavers containing conidia, but no resting spores (21.7% of fungus-positive cadavers) were probably the result of infection by resting spores, while the 78.3% of cadavers containing resting spores were the result of infection by conidia (Table 3). Infection by resting spores occurred throughout the season but was most prominent early in the season, while infection from conidia dominated later in the season. Only two cadavers, from the highest-density plot, were positive for the gypsy moth nuclear polyhedrosis virus (LdMNPV) (Table 3). The relative success of the gypsy moth populations in the higher density plots was largely due to the paucity of natural enemies other than the fungus, and to the late attack of the fungus (Table 2). The onset of infection corresponded with the onset of pupation, and many larvae successfully pupated. The source of the substantial mortality noted during the pupal stage was not determined, although the predator *Calosoma sycophanta* L. was active in the plots. Pupation was delayed in the low-density plots (Table 2) which tended to be at higher elevations (Table 1). *Entomophaga maimaiga* was active in these plots (Table 3), and given the slower development of the gypsy moth population at these sites, might have infected considerably more insects had the larvae not disappeared. The disappearance of larvae in the lower-density plot was likely due to the action of predators, which are known to dominate gypsy moth populations at lower densities but whose effects are much less prominent at higher gypsy moth densities (Campbell 1981, Smith and Lautenschlager 1981).

Weather conditions and fungal activity in spring, 2000. *Entomophaga maimaiga* epizootics are known to be favored by high humidity and rainfall (Elkinton et al. 1991, Hajek et al. 1990, 1993, 1996, Weseloh and Andreadis 1992a,b, Weseloh et al. 1993). With appropriate moisture, conidial germination and sporulation occurs between 2 to 25°C, with maximum rates seen between 20 to 25°C (Hajek et al. 1990). Temperature varied considerably among the plots according to their elevation on the

Table 3. Necropsy results and analysis of soil samples for *E. maimaiga* resting spores, Spring 2000

Parameter	High	Low	Sparse
No. larvae necropsied:	235	68	2
No. positive for *E. maimaiga:*	201 (86%)	60 (88%)	2 (100%)
Conidia only:	46	11	0
Resting spores:	155	49	2
No. positive for NPV:	2	0	0
No. plots evaluated for resting spores in soil:	9	7	1
Avg. no. spores per g soil	7,936.2	2,360.9	697.7

mountain (Table 4). Temperatures at Luray, VA (elevation 430 m) and at Front Royal (elevation 284 m) were similar in April, May and June 2000) (Table 4). Higher on the mountain, Big Meadows (elevation 1070 m) recorded cooler averages for those 3 months (Table 4). Thus, both the gypsy moth populations and the fungal pathogen would be expected to develop faster at the lower elevations. Monthly totals for rainfall were similar for the three areas for the 3 months. Precipitation events were frequent in April, with 6 events recorded for the months, but totals were generally 1 cm or less except for one 2.5 cm event on Julian Date (JD) 100, when about 2.5 cm of rain fell over the area. Rainfall was spotty in early May, however, with perhaps 2.5 cm of precipitation occurring over the area from JD 120 until the period of JD 141 to 147, when 4 to 5 cm of rain fell over the area. Then, except for 1 to 2 cm that fell on JD 159, little rain fell in the area until JD 166. The frequent but modest rain events recorded in April and mid-May should have provided enough moisture to encourage *E. maimaiga* resting spore activity by the time gypsy moth eggs hatched in early May. However, our data (no mortality noted on JD154, and modest fungal activity noted on JD 161 [Table 2]), indicated that the gypsy moth population might have remained in the trees, and did not interact with the germinating resting spores at this time. Beginning on JD 165 and continuing through JD 175, major rain events accounted for considerable precipitation, with recorded totals of 9.2 (Big Meadows), 9.5 (Front Royal) and 17.1 cm (Luray). This rainfall, plus generally warmer temperatures, would have encouraged both the germination of resting spores in the soil and the formation and distribution of conidia from early-season fungus-killed gypsy moth cadavers. This is reflected in the mortality figures given in Table 2 for the critical dates JD 168 and JD 175.

March 2001 egg mass surveys. Egg mass surveys conducted just outside the plots in March 2001 reflected the successful reproduction of the gypsy moth populations in the high-density plots in 2000, averaging 2173.3 per ha (Table 1). This was considered adequate for our supplemental watering study planned for 2001. On the other hand, just one egg mass was detected in the surveys conducted just outside the

Table 4. Monthly weather totals for three locations near the Skyline Drive study area

		April		May		June	
Location	Altitude (m)	Temp. (°C)	Rain (cm)	Temp.	Rain	Temp.	Rain
2000							
Luray	430	12.4	8.5	18.4	8.9	22.7	23.6
Front Royal	284	11.1	7.4	18.2	6.8	22.5	11.4
Big Meadow	1070	7.2	11.8	14.3	7.0	18.3	15.8
2001							
Luray	430	13.8	5.1	16.7	7.2	21.8	17.0
Front Royal	284	12.6	4.8	16.2	12.8	21.9	12.4
Big Meadow	1070	9.3	5.2	12.3	13.6	17.1	17.5

low-density plots. Thus, the missing larvae recorded from these plots did not simply leave the plots and settle in neighboring trees. No egg masses were found in surveys conducted just outside sparse-density plots. Gypsy moth populations in the 15 low-population and sparse-population plots were judged to be too low for our supplemental-watering study planned for 2001.

March 2001 soil spore counts. The number of resting spores in the soil is an important factor of consideration in the use of supplemental water as a control strategy. Post year-2000 spore counts from soil samples revealed at least some resting spores in all sampled plots with high numbers of resting spores in all of the high-density population plots and lesser numbers in the low-density plots (Table 3). Spore loads in the high-density plots (averaging 7,936.2 spores per g soil) were considered adequate for our supplemental-watering study planned for 2001 without the provision of additional spores from outside of the plots. Spore loads have generally been reported as a function of soil weight (Hajek et al. 1998a, Gillock and Hain 2002a,b), although Weseloh and Andreadis (2002) developed a physical spore-count method based on soil volume.

2001 supplemental watering study. Most of the cadavers were positive for *E. maimaiga.* However, one cadaver from the first collection, and 7 cadavers from the second collection, were positive for NPV, indicating that virus levels remained low along Skyline Drive in 2001. Somewhat more larvae died due to *E. maimaiga* in watered plots than in control plots for both sample dates (Table 5), and this was true at both sample heights. However, the treatment effect for watering was not significant for either date. Only one larval cadaver from larvae collected on the 01 June collection contained resting spores, the rest contained only conidia. This indicated that the infections arose mainly from activated resting spores within the plots. F values for

Table 5. Necropsy results from larvae sampled from 8 plots along Skyline Drive on two dates in 2001. Samples were taken at ground level and from canopy vegetation. Four of the plots received supplementary water each week, and 4 received only natural rainfall*

Site status	Collection N	Collection Date	Height	Mean larvae sampled	% larvae with *E. maimaiga* (se)
Supplement water	4	01 June	Ground	25	17.0 (7.7)
	4		Canopy	25	12.0 (5.7)
No suppl. water	4		Ground	25	12.0 (3.7)
	4		Canopy	25	10.0 (2.6)
Supplement water	4	15 June	Ground	25	74.0 (14.4)
	4		Canopy	25	29.0 (13.3)
No suppl. water	4		Ground	25	60.0 (13.4)
	4		Canopy	25	14.0 (2.0)

* For 01 June collection, F value for water = 0.44 ($P > 0.05$); F value for height = 0.44 ($P > 0.05$); F value for water*height = 0.08 ($P > 0.05$). For 15 June collection, F value for water = 1.48 ($P > 0.05$); F value for height = 14.62 ($P = 0.0024$); F value for water*height = 0.01 ($P > 0.05$).

watering were 0.44 ($P > 0.05$) for the 01 June collection, and 1.48 ($P > 0.05$) for the 15 June collection. In the 15 June collection, 89% of the cadavers positive for *E. maimaiga* contained resting spores, indicating that airborne conidia were the main source of infection at this time. Interestingly, 30% of the cadavers resulting from larvae collected in the canopy in the second collection contained just conidia, while only 7% of such cadavers from ground collections contained just conidia. This suggests that larvae receiving their infections via air-borne conidia, and hence containing resting spores, may tend to descend the tree to die. This would help the fungus by getting the resting spores closer to the soil. On the other hand, fungal infections arising from resting spores result in cadavers filled with conidia. Having the larvae die in the trees would aid in the airborne dispersal of these conidia.

On 01 July, larvae sampled from the ground exhibited slightly higher levels of fungus than larvae sampled from the bucket truck. The effects of watering on collection height were not significant for this date, nor were water*height interactions. In contrast, for the 15 June collection, larvae sampled from the mid/upper canopy had significantly lower levels of fungus than did larvae sampled from the ground. F value for height was 14.62 ($P = 0.0024$), and the F value for water*height was 0.01 ($P > 0.05$). Similar indications that levels of *E. maimaiga* infection vary with collection height were reported by Hajek and Webb (1999). Future research should address the obvious sampling problems suggested by these results.

Egg mass counts declined in most of the plots during 2001 (Table 1). However, egg mass populations in watered plots declined an average of 78%, while egg mass population in control plots declined only 4%, giving a significant difference for population change using the Kruskal-Wallis Test (Chi-square = 4.0833; df = 1; $P = 0.0433$). This difference in egg mass counts between treated and control plots may reflect differential mortality occurring after the second collection, since *E. maimaiga* strikes hardest late in the season.

Weather conditions and fungal activity, spring, 2001. Temperature again varied considerably among the plots according to their elevation on the mountain (Table 4). Temperatures at Luray and at Front Royal were again similar in April, May and June 2000) (Table 4). Big Meadows again recorded cooler averages for those 3 months (Table 4). Monthly totals for rainfall were similar for the three areas for the 3 months. April was relatively dry, with one period of significant rainfall occurring on Julian Dates (JD) 101 to 103, when about 2.5 cm of rain fell over the area. No significant rainfall occurred in early May, and at this point, conditions seemed ideal for our supplemental watering study. However, a considerable amount of moisture fell over an 8 day period from JD 137 to JD 144 (6.4 cm at Luray, 11.2 cm at Front Royal, and 11.6 cm at Big Meadows), which provided enough moisture to encourage early *E. maimaiga* resting spore activity. In June, 3 evenly-spaced rainfall events occurred on JD 156 through JD 160, JD 166 through 169, and JD 172 through 176. This rainfall would have encouraged both the germination of resting spores in the soil as well as the formation and distribution of conidia from early-season fungus-killed gypsy moth cadavers. This may have obviated the need for supplemental watering in this particular location for this particular year.

Summary. The study tested a simple, inexpensive biological control strategy against gypsy moths that takes advantage of a natural control agent already in many environments. The study assessed the potential for inducing an earlier epizootic of *E. maimaiga* by facilitating the early sporulation of resting spores by supplemental watering. Assuming that the fungus is already in the soil at the base of trees, it will kill

gypsy moths whether we add supplemental water or not. However, assuming a below normal level of precipitation during the gypsy moth field season, fungal kill may occur at low rates until late in the season, providing little impact on gypsy moth populations. An earlier epizootic may promote earlier episodes of airborne conidia that can move beyond the water-application site to affect gypsy moth populations throughout a wood lot. Timing of rainfall events during the study was sufficient to insure a considerable fungal epizootic, which may have been aided by supplemental watering. Supplemental watering is an inexpensive, common sense strategy that may especially reward the land manager in dry years.

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