JOURNAL OF INVERTEBRATE PATHOLOGY 74, 147–153 (1998)
ARTICLE NO. IN984773

Interactions between a *Nosema* sp. (Microspora: Nosematidae) and Nuclear Polyhedrosis Virus Infecting the Gypsy Moth, *Lymantria dispar* (Lepidoptera: Lymantriidae)[1]

Leah S. Bauer,* Deborah L. Miller,* Joseph V. Maddox,† and Michael L. McManus‡

*USDA Forest Service, North Central Forest Experiment Station, Pesticide Research Center and Department of Entomology, Michigan State University, East Lansing, Michigan 48823; †Office of Agricultural Entomology and Illinois Natural History Survey, Champaign, Illinois 61820; ‡USDA Forest Service, Northeastern Forest Experiment Station, Hamden, Connecticut 06514

Received August 15, 1997; accepted March 11, 1998

Simultaneous and sequential *per os* inoculations of gypsy moth larvae with the *Lymantria dispar* nuclear polyhedrosis virus (*Ld*NPV) and a *Nosema* sp. from Portugal demonstrated that the interaction of two pathogens during coinfection was variable, ranging from synergistic to antagonistic. Susceptibility of gypsy moth larvae to viral infection was unaffected by simultaneous and subsequent microsporidian infection. This resulted from the comparatively slow pathogenesis of the microsporidium when compared to the virus. Viral infectivity, however, increased 10-fold when larvae were preinfected with *Nosema* sp. *per os*, or through transovarial infection. Time to death decreased for larvae infected with both pathogens compared to larvae infected with the virus alone. Polyhedron production was significantly reduced by microsporidian infection preceding viral infection. In this infection sequence, larvae died at an earlier stage and were less than half the mass of cadavers infected with virus alone. The biological significance of these results on gypsy moth population dynamics and the implication for use of this *Nosema* sp. from Portugal in gypsy moth biological control are discussed in the context of viral epizootiology. © 1998 Academic Press

***Key Words: Lymantria dispar;* gypsy moth; *Nosema* sp. from Portugal; nuclear polyhedrosis virus; biological control; pathogen interactions; gypsy moth viral epizootic; population dynamics.**

INTRODUCTION

Following the accidental introduction of gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), into North America over a century ago, control efforts focused on the introduction and establishment of insect parasitoids and predators from its native range in Europe (Reardon, 1981). Only a single attempt to introduce a gypsy moth pathogen is documented. This early biological control effort by Speare and Colley (1912) involved the release of the "gypsy fungus," now known as *Entomophaga maimaiga* Humber, Shimazu, and Soper, from Japan into eastern Massachusetts.

Prior to the discovery of widespread *E. maimaiga* epizootics in North America (Andreadis and Weseloh, 1990; Hajek *et al.,* 1990), the collapse of local gypsy moth populations typically resulted from the *L. dispar* nuclear polyhedrosis virus (*Ld*NPV) (Lewis, 1981; Dwyer and Elkinton, 1993; Elkinton and Liebhold, 1990). First reported in North America in 1907, *Ld*NPV was inadvertantly introduced with gypsy moth, as a contaminant of gypsy moth parasitoids, or on imported host plants (Hajek *et al.,* 1995). Although both *Ld*NPV and *E. maimaiga* are now considered vital to the naturalization of gypsy moth in North America (Hajek *et al.,* 1993), these two pathogens represent a small proportion of the total number of microorganisms associated with gypsy moth in Eurasia (Weiser, 1987).

Microspora is a ubiquitous group of obligate, intracellular, protozoans commonly causing disease in insects and other invertebrates, and gypsy moth is no exception. Five species of microsporidia are described from European gypsy moths, and at least 10 different isolates were collected during the last decade in Europe (Solter *et al.,* 1997). These pathogens, however, are absent from gypsy moths in North America.

In their normal host, microsporidia typically cause chronic disease expressed as prolonged developmental time; reduced adult size, longevity, fecundity, mating, and egg fertility; and increased mortality in all developmental stages (Novotny and Weiser, 1993). Because of the debilitating, rather than acute nature of microsporidiosis, these pathogens are considered important cofactors in maintaining gypsy moth population densi-

[1] This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

0022-2011/98 $25.00

ties below tolerance thresholds for long periods of time (Novotny, 1988, 1989; Weiser, 1987; Weiser and Novotny, 1987; Zelinskaya, 1980).

A species of *Nosema,* collected from gypsy moths in Portugal (J.V.M., unpublished data), is a promising candidate for biological control of gypsy moth in North America (McManus *et al.,* 1989). This microsporidium is receiving considerable research attention; areas being investigated include taxonomy and genetics, epizootiology, tissue specificity, host range, impact on host bionomics, and interactions with other natural enemies (Jeffords *et al.,* 1987, 1988, 1989; Onstad *et al.,* 1993; Solter *et al.,* 1997). Favorable attributes of this microsporidian species include high infectivity and low virulence, permitting some larvae to reach the adult stage and transmission of the microsporidium within the host egg (transovarially) (L.S.B., unpublished data). Thus, gypsy moth larvae, hatching in the spring, sustain varying levels of infection. This strategy of vertical transmission (between generations) better limits the microsporidium to its gypsy moth host and ensures survival at low host population densities.

In contrast, the relatively inefficient vertical transmission of *Ld*NPV is achieved by the liberation of polyhedra into the host environment the previous season after larval cadavers lyse on host trees. Gypsy moth neonate larvae become infected by ingesting the viral polyhedra during egg hatch (Woods and Elkinton, 1987). Horizontal transmission (within a generation) is similar for both pathogens, involving ingestion of viral polyhedra or microsporidian spores that contaminate the environment. Because *Ld*NPV is highly virulent, both host and pathogen become scarce following a gypsy moth population collapse.

Field studies of gypsy moth population dynamics in Central Europe suggest a close association between *Ld*NPV and microsporidia. Following a viral epizootic, gypsy moth populations begin to recover and microsporidian infections increase, reaching 15 to 30% as viral infections begin to reappear. As host population density peaks, however, *Ld*NPV is the most prevalent pathogen, and microsporidian prevalence declines. Postmortem examination of cadavers reveals a high prevalence of mixed viral and microsporidian infections (Weiser, 1987; Novotny, 1989, 1991; David and Novotny, 1990; Pilarska and Vavra, 1991; Novotny and Weiser, 1993). Weiser (1987) suggests that microsporidiosis may act as a stressing agent, increasing the susceptibility of gypsy moth to virus and lowering the host density threshold required for viral epizootic induction. Understanding the dynamic interactions between viral and microsporidian infections is of particular interest in the present study.

Our objective was to evaluate the compatibility of two obligate, intracellular pathogens for biological control of the gypsy moth. Sequential and simultaneous laboratory bioassays were used to predict what might occur if this *Nosema* sp. were introduced and established in a gypsy moth population where *Ld*NPV is a primary controlling factor. We compared the effect of *Nosema* infection on (1) the infectivity of *Ld*NPV in microsporidia-free, *per os*-infected and transovarially infected gypsy moth larvae, (2) the viral mortality response, and (3) the impact of coinfection on viral and microsporidian pathogenesis in the gypsy moth.

MATERIALS AND METHODS

Insects. Gypsy moth egg masses, obtained from the USDA Forest Service, Northeastern Forest Experiment Station (USDA FS NEFES, Hamden, CN) laboratory colony, were surface sterilized (Bell *et al.,* 1981) and allowed to hatch. Neonate larvae were placed at a density of 10 per cup (60 ml clear polystyrene) containing high wheat germ diet (Bell *et al.,* 1981) and reared at 24°C, L16:D8.

Transovarially infected neonates were produced by inoculating parental stock as second-instar larvae with *Nosema* dosages ranging from 4×10^2 to 4×10^3 spores/larva. Only neonates hatched from egg masses laid by infected females were included in the viral bioassays of transovarially infected neonates.

Pathogens. Fresh *Nosema* spores were produced by inoculating second-instar gypsy moth larvae with ca. 1×10^3 spores/larva, as described below. This dosage assured 100% infection and a high yield of spores. Infected salivary glands and fat body were removed from late fifth-instar larvae, homogenized in a handheld glass tissue grinder with sterile tap water, and filtered through fine mesh fabric and thick cotton. The resulting spore suspension was centrifuged at 100*g* for 30 min. The pellet was resuspended in sterile tap water and centrifuged two more times; spores were further purified using a continuous density gradient of Ludox (0–100%) (Undeen and Alger 1971). The *Ld*NPV lyophilized powder (Hamden isolate, LDP 226), obtained from USDA FS NEFES, was rehydrated in sterile tap water using a 1-ml tissue grinder. Concentrations of spores and polyhedral inclusion bodies (polyhedra) were quantified using a Petroff–Hausser cell counting chamber, and dosages were prepared using serial dilutions.

Viral and microsporidian interactions in microsporidia-free larvae. Microsporidia-free gypsy moth larvae were inoculated *per os* with four dosages of two pathogens in all combinations, totalling 16 treatments per experiment with 24 larvae per treatment. The experiments included (1) simultaneous inoculation of *Ld*NPV and *Nosema* at third instar (dosages were 0, 3×10^1, 3×10^2, 3×10^3 spores/larva and 0, 3×10^3, 3×10^4, 3×10^5 polyhedra/larva); (2) sequential inoculation of *Nosema* at second instar (dosages were 0,

5×10^1, 5×10^2, 5×10^3 spores/larva) followed by *Ld*NPV inoculations at third instar (dosages were 0, 3×10^3, 3×10^4, 3×10^5 polyhedra/larva), resulting in *Nosema* inoculations preceding *Ld*NPV by 6 days; and (3) sequential inoculation of *Ld*NPV at third instar (dosages were 0, 3×10^3, 3×10^4, 3×10^5 followed by *Nosema* inoculation at fourth instar (dosages were 0, 4×10^2, 4×10^3, 4×10^4 spores/larva), resulting in *Ld*NPV inoculations preceding those of *Nosema* by 5 d.

Larvae were inoculated by applying 1-μl droplets, containing pathogen dosages, onto 7 × 1-mm diet discs placed singly in the wells of 24-well tissue culture plates. Homogeneous cohorts of microsporidia-free second-, third-, or fourth-instar larvae, harvested from diet cups less than 24 h after molt, were placed individually into each well. Only larvae that consumed the entire dose within 24 h were included in the assay. After inoculation, larvae were reared individually in 60-ml cups and monitored daily for development and mortality. Cadavers were removed from diet cups daily, placed individually in labeled vials, dried (75°C), weighed, and stored. Cadavers were rehydrated overnight in a known volume of water, homogenized in a 7-ml glass tissue grinder, and diluted sufficiently to facilitate counting of polyhedra and spores using a compound microscope (400×) with a Petroff–Hausser cell counter. Within each experiment, the number of polyhedra or spores was counted in cadavers sampled from the separate and dual treatments at the highest dosages only.

Viral bioassays of larvae transovarially infected with Nosema. A cohort of transovarially infected neonate larvae, harvested from egg masses 24–48 h after initial egg hatch, were inoculated with a viral suspension applied to the diet surface of 60-ml cups. Each bioassay included five 10-fold dilutions of virus (0.05–500 polyhedra/cm^2) and a water control and 24 larvae/concentration. Larvae were allowed to feed on the viral-treated diet in groups of 4 larvae/cup for 48 h and transferred individually to 60-ml diet cups. Each larva was monitored for development and mortality. Spores and polyhedra were quantified from frozen individuals at a later date. An uninfected cohort of neonates was also bioassayed simultaneously, in the same manner. Each bioassay was replicated four times. Since not all progeny were infected with the microsporidium, only those determined to be infected by postmortem examination were used in the analyses.

Statistics. Slope and maximum-likelihood estimate of viral median infective dosages (ID_{50}s) were calculated with the probit option of POLO-PC (LeOra Software, 1987). The criterion for significance was failure of the 95% confidence limits to overlap. The *Ld*NPV ID_{50} estimates were calculated at 20 days after inoculation with the virus. The Kruskal–Wallis test was used to compare differences in time to death between separate and dual treatments (Onstad *et al.*, 1986) with the Statistical Analysis System 6.03 (SAS Institute 1990). Student's *t* test was used to test for significant differences between the separate and dual pathogen treatments for larval dry mass, and production of infective units (spores and/or polyhedra) per larva with Systat 4.0 (Wilkinson, 1987).

RESULTS

Viral and microsporidian interactions. The ID_{50} of *Ld*NPV in gypsy moth larvae was significantly higher (10-fold) than in larvae infected 6 days before with 5×10^3 *Nosema* spores (Table 1). At the lower microsporidian dosages, viral infectivity tended to increase as the *Nosema* dosage increased. No significant differences in viral infectivity were measured when the two pathogens were inoculated simultaneously, or when *Ld*NPV infection preceded *Nosema* infection (Table 1). Slopes of the viral ID_{50}s were similar at each dosage and sequence of *Nosema*.

Time to death for gypsy moth larvae infected with only *Ld*NPV was similar within and between the three experiments, with means ranging from 12 to 14 days (Table 2). Viral lethal times were also similar to those measured for larvae with dual infections, except when *Nosema* infection preceded *Ld*NPV infection. In the latter inoculation sequence, larvae with dual infection

TABLE 1

Effect of *per os Nosema* Dosages on the Infectivity of *Ld*NPV in Gypsy Moth Larvae

Nosema dosages (spores/larva)	*Ld*NPV ID_{50}[a] (95% FL) (polyhedra/larva)	Slope ± SE
	Simultaneous *Nosema* and *Ld*NPV	
0	1.6×10^4 (9.1×10^3–3.0×10^4)	1.56 ± 0.28
3×10^1	7.8×10^3 (2.3×10^3–2.5×10^4)	1.18 ± 0.20
3×10^2	1.1×10^4 (1.9×10^3–3.8×10^4)[b]	1.50 ± 0.29
3×10^3	1.2×10^4 (5.6×10^3–2.2×10^4)	2.05 ± 0.50
	Sequential *Nosema* preceding *Ld*NPV	
0	1.9×10^4 (1.0×10^4–3.7×10^4)	1.29 ± 0.23
5×10^1	1.7×10^4 (3.4×10^3–5.8×10^4)	1.02 ± 0.28
5×10^2	1.2×10^4 (2.9×10^3–3.2×10^4)	0.92 ± 0.24
5×10^3	8.1×10^2 (7.2–2.7×10^3)[b]	0.98 ± 0.41
	Sequential *Ld*NPV preceding *Nosema*	
0	1.2×10^4 (6.8×10^3–2.1×10^4)	1.63 ± 0.29
4×10^2	1.3×10^4 (6.9×10^3–2.3×10^4)	1.48 ± 0.26
4×10^3	1.1×10^4 (3.2×10^3–4.0×10^4)[b]	1.35 ± 0.23
4×10^4	2.5×10^4 (1.3×10^4–5.0×10^4)	1.27 ± 0.23

Note. ID_{50}, median infective dose; FL, fiducial limits; SE, standard error.

[a] Each ID_{50} estimate includes mortality data from ca. 96 larvae per bioassay, and was calculated 20 days after inoculation with *Ld*NPV.

[b] 90% FL maximum resolution.

TABLE 2

Time to Death (Days) for Gypsy Moth Larvae Infected Separately, Simultaneously, and Sequentially with *Nosema* and *Ld*NPV

Bioassay	Time to death (days)[a] [mean (range)]	*n* (larvae)
	Simultaneous	
Nosema	25.3 (19–32)	24
*Ld*NPV	14.0 (13–19)	24
Nosema and *Ld*NPV	13.5 (11–19)	24
	Sequential	
Nosema	23.9 (5–34)	24
*Ld*NPV	12.0* (9–21)	22
Nosema preceding *Ld*NPV	10.0* (5–22)	23
	Sequential	
Nosema	29.6 (16–37)	23
*Ld*NPV	13.5 (10–16)	22
*Ld*NPV preceding *Nosema*	13.7 (12–23)	22

[a] Days to death (mean ± SE) was determined for larvae inoculated with the highest dosages within each experiment.

* Within an experiment, dual pathogen treatment differed significantly from single pathogen treatment ($\chi^2 = 7.62$, $P < 0.006$) using the Kruskal–Wallis test.

died within ca. 10 days, significantly less than the 12 days to death for larvae infected with virus alone. Time to death for larvae infected with only the microsporidium was significantly longer than for the viral or dual infections ($P < 0.0001$), with means ranging from ca. 24 to 30 days (Table 2).

Production of polyhedra was significantly affected when microsporidian infection preceded viral (Table 3).

TABLE 4

Infectivity of *Ld*NPV in Neonate Gypsy Moth Larvae Infected Transovarially with *Nosema* and Time to Death in Days

Larval health	*Ld*NPV ID_{50} (90% FL) (polyhedra/cm^2 diet)	Slope ± SE	Time to death [mean ± SE (days)]
Microsporidia-free	48.2 (8.7–1181.1)	0.70 ± 0.09	7.17 ± 0.09
Microsporidia-infected[a]	3.5 (0.4–24.7)	0.71 ± 0.14	7.62 ± 0.20

[a] Neonate larvae were infected transovarially with *Nosema* sp. from infected mothers.

Cadavers resulting from this inoculation sequence produced ca. 1000× fewer polyhedra than cadavers infected with virus alone. In addition, production of microsporidian spores was significantly lower in cadavers infected with both pathogens (Table 3). This reduction was greatest following simultaneous infection.

The larval stage at death and dry mass of coinfected cadavers were similar to those infected with virus alone, except when the microsporidian infection preceded viral. In the latter experiment, coinfected larvae died at an earlier stage of development and were less than half the mass of cadavers infected with virus alone (Table 3).

Viral infections in larvae transovarially infected with Nosema. Neonate larvae, transovarially infected with *Nosema,* were ca. 10-fold more susceptible to *Ld*NPV than were microsporidia-free neonates (Table 4). Variability in the initial spore load of transovarially in-

TABLE 3

Distribution of Gypsy Moth Larval Instars at Death (%), Larval Cadaver Dry Mass (Mg), Infective Units/Larva (Mean ± SE Spores and Polyhedra) from the Highest Dosages[a] within Separate and Dual Pathogen Bioassays

Bioassay	Larval stage (%) III	IV	V	Larval dry mass (mg) (*n*)	Infective units/larva: Spores (*n*)	Polyhedra (*n*)
				Simultaneous: *Nosema* and *Ld*NPV		
Nosema	0	89	11	80.43 ± 6.21 (18)*	$5.0 \times 10^9 \pm 5.1 \times 10^8$ * (5)	—
*Ld*NPV	0	100	0	22.81 ± 1.75 (19)	—	$4.8 \times 10^8 \pm 8.7 \times 10^7$ (8)
Simultaneous	0	100	0	20.08 ± 2.29 (24)*	$1.2 \times 10^8 \pm 7.5 \times 10^7$ * (14)	$6.2 \times 10^8 \pm 1.3 \times 10^8$ (14)
				Sequential: *Nosema* preceding *Ld*NPV		
Nosema	67	33	0	22.67 ± 2.90 (24)*	$2.3 \times 10^9 \pm 4.1 \times 10^8$ * (5)	—
*Ld*NPV	4	96	0	28.30 ± 2.75 (18)*	—	$7.7 \times 10^8 \pm 1.5 \times 10^8$ * (7)
Sequential	83	17	0	13.87 ± 2.17 (22)*	$1.0 \times 10^9 \pm 3.1 \times 10^8$ * (15)	$8.3 \times 10^5 \pm 8.3 \times 10^5$ * (15)
				Sequential: *Ld*NPV preceding *Nosema*		
Nosema	0	0	100	232.22 ± 14.96 (24)*	$5.7 \times 10^9 \pm 7.9 \times 10^8$ * (15)	—
*Ld*NPV	0	100	0	43.98 ± 3.51 (20)	—	$1.6 \times 10^9 \pm 2.1 \times 10^8$ (5)
Sequential	0	100	0	42.89 ± 3.67 (20)*	$2.2 \times 10^9 \pm 1.2 \times 10^9$ * (12)	$1.3 \times 10^9 \pm 3.0 \times 10^8$ (12)

[a] Results are from highest dosages (ID_{100}) within each treatment.

* Within an experiment, dual pathogen treatment differed significantly from single pathogen treatment at the $P \leq 0.05$ level of significance using Student's *t* test.

fected larvae is likely responsible for the variability in mortality response to viral inoculations, resulting in overlapping fiducial limits.

Viral time to death in gypsy moth neonates was ca. 7 days, irrespective of preexisting microsporidian infection. Larvae transovarially infected with microsporidium, however, died in ca. 35 days as late-stage larvae, almost five times later than virally infected larvae. Unlike *Ld*NPV which is a fatal infection, however, over half of the larvae infected with *Nosema* survived to the adult stage (58%).

DISCUSSION

Pest management objectives for areas of North America infested or threatened with infestation by gypsy moth attempt to (1) suppress gypsy moth population fluctuations to minimize adverse impacts on forest ecosystems, (2) reduce the nuisance of large, noxious caterpillars in urban forests and high-value recreation areas, (3) slow the spread of the gypsy moth to contiguous uninfested areas, and (4) eradicate infestations in noncontiguous uninfested areas. These objectives are accomplished primarily through the introduction and establishment of gypsy moth natural enemies, intensive monitoring of populations, and intermittent spraying with the relatively narrow spectrum microbial insecticide formulated with *B. thuringiensis* var. *kurstaki*. Because mortality caused by *B. thuringiensis* results from toxicity to several insecticidal proteins, it does not cycle in the field, and must be reapplied as needed. Natural enemies, on the other hand, once introduced from the gypsy moth's native lands, have the potential to sustain themselves permanently within the host and its environment.

The complex of introduced and native natural enemies of gypsy moth in North America, including pathogens, parasites, and predators collectively, have often failed to maintain gypsy moth populations below the human tolerance threshold, as evidenced by a history of episodic outbreaks. The gypsy moth pathogen complex in North America clearly lacks the diversity found throughout gypsy moth populations in Europe and Asia. The gypsy moth virus, *Ld*NPV, is long recognized as the primary agent responsible for collapse of high-density populations worldwide, including North America (Elkinton and Liebhold, 1990). Since 1989, the gypsy moth fungus, *E. maimaiga,* in North America, produced widespread epizootics, particularly during wet, spring weather (Hajek *et al.,* 1993). Conspicuously lacking in North American gypsy moth populations are microsporidia. Unlike *Ld*NPV and *E. maimaiga,* these entomopathogens typically cause chronic disease, resulting in increased mortality at each life stage, prolonged development, and reduced fecundity. Based on several European studies (Zelinskaya, 1980; Novotony, 1989), we feel that microsporidia possess characteristics that will facilitate the natural control of gypsy moths in North America.

One critical consideration prior to the release of microsporidia is whether their addition might have an antagonistic effect on gypsy moth's established natural enemies complex. Interactions between entomopathogens can be independent, antagonistic, or synergistic. The implications of these terminologies are quite different when discussing the attributes of a microbial insecticide as compared to a pathogen that was introduced for establishment as a biological control agent. Most studies are designed to find the pathogen mixture or formulation that achieves the highest mortality in the shortest time period (for reviews see Krieg 1971, Jaques and Morris 1981). However, such a strategy is not compatible with the inoculative release of insect pathogens introduced as biological control agents because periodic "extinctions" of the host may occur. Thus, sustainability of the introduced pathogen, as well as other natural enemies, would be periodically disrupted, leading to frequent and large-amplitude fluctuations of the host population.

Our laboratory bioassays indicate that the impact of this *Nosema* sp. from Portugal on the pathogenesis of *Ld*NPV in the gypsy moth was variable, ranging from synergistic to antagonistic, depending on the sequence of infection. Viral infectivity, time to death, and pathogenesis were unaffected by simultaneous and subsequent microsporidian infection. However, larvae with a preexisting microsporidian infection were more susceptible to *Ld*NPV, particularly at a high *per os* microsporidian dose or when infection occurred transovarially. Moreover, time to death was decreased, resulting in lower polyhedron production by the virus. Varying degrees of competition between microsporidia and NPV are documented in a variety of lepidopterans (Lipa, 1971; Nordin and Maddox, 1972; Fuxa, 1979; Cossentine and Lewis, 1984; Moawed *et al.,* 1987). Antagonism is not unexpected as both pathogens often invade the same tissues, perhaps resulting in competition for cellular resources or interference with cellular functions.

In Europe, where this species of *Nosema* is endemic, gypsy moth larvae hatch in the spring with varying levels of microsporidiosis from transovarial transmission (L.S.B., unpublished data). During eclosion, these neonates then ingest viral polyhedra which contaminate the egg chorion. Our results suggest that larvae which are first infected with microsporidia and then become infected with virus will contaminate the environment with a mixture of spores and polyhedra. Although we found that coinfection results in lower polyhedron production, we also learned that larvae infected with microsporidia tend to be more susceptible to viral infection. Indeed, mixed infections common in larval populations of gypsy moths in Europe may

depress the density threshold required for induction of a viral epizootic.

The release of polyhedra and spore mixtures from gypsy moth cadavers suggests that simultaneous inoculation may occur through horizontal transmission in the field. In our laboratory study, simultaneous inoculations confirmed that the rate of pathogenesis for *Nosema* was slower than for *Ld*NPV, minimizing the impact of microsporidiosis on both viral infectivity and lethal time. On the other hand, the rapid rate of viral pathogenesis and short lethal time antagonized the microsporidium life cycle by reducing the number of spores produced. Antagonism of the microsporidium infection was dose dependent and more evident when viral infection preceded that of the microsporidium. Similar antagonism by the virus on microsporiosis is also reported in another study (Lipa, 1971). Although the actual mechanisms of these observed pathogen interactions were beyond the scope of this study, it would be interesting to investigate how these very different pathogens utilize the resources, cellular machinery, and space within coinfected host cells. Studies using cell culture would greatly facilitate our understanding of these interactions and their underlying mechanisms.

Sustainability of effective and viable populations of these and other host-specific natural enemies of gypsy moth depends upon the availability of an adequate population of susceptible hosts. Virulent pathogens such as *Ld*NPV theoretically may kill too quickly and become too prevalent, to the detriment of other natural enemies. In fact, gypsy moth viral epizootics are so devastating that few, if any, residual egg masses can be found the following year. Antagonism of virulent pathogens such as the gypsy moth virus by microsporidia may dampen volatile population oscillations and provide a continuous supply of host necessary to support a diversity of natural enemies, thus generating consistent, long-term control. Indeed, microsporidia, because of their chronic nature, high infectivity, and efficient vertical transmission, are one of the few host-specific natural enemies detectable when host population densities are low (Onstad and Maddox, 1989; Onstad *et al.*, 1990). A greater diversity of natural enemies that includes microsporidia may reduce the frequency, amplitude, and duration of outbreaks, thereby maintaining gypsy moth populations at endemic levels. Such permanent and self-sustaining control would greatly minimize the combined damage caused by both gypsy moth and human interventions on the biological diversity and health of North American forests.

ACKNOWLEDGMENTS

We thank G. Heaton, J. Rae, and R. Oyer for laboratory assistance during this project, and Drs. R. A. Haack, C. N. Koller, and D. W. Onstad for their critical reviews of the manuscript.

REFERENCES

Andreadis, T. G., and Weseloh, R. M. 1990. Discovery of *Entomophaga maimaiga* in North American gypsy moth, *Lymantria dispar*. *Proc. Natl. Acad. Sci. USA* **87,** 2461–2465.

Bell, R. A., Owens, C. D., Shapiro, M., and Tardiff, J. R. 1981. Mass rearing and virus production. *In* "The Gypsy Moth: Research Toward Integrated Pest Management" (C. C. Doane and M. L. McManus, Eds.), pp. 599–600. USDA, Washington. [U.S. Dept. Agric. For. Serv. Tech. Bull. 1584].

Cossentine, J. E., and Lewis, L. C. 1984. Interactions between *Vairimorpha necatrix, Vairimorpha* sp., and a nuclear polyhedrosis virus from *Rachiplusia ou* in *Agrotis ipsilon* larvae. *J. Invertebr. Pathol.* **44,** 28–35.

David, L., and Novotny, J. 1990. Laboratory activity and field effect of *Nosema lymantriae* Weiser (Microsporida) on larvae of the gypsy moth, *Lymantria dispar* L. *Biologia (Bratislava)* **45,** 73–79.

Dwyer, G., and Elkinton, J. S. 1993. Using simple models to predict virus epizootics in gypsy moth populations. *J. Anim. Ecol.* **62,** 1–11.

Elkinton, J. S., and Liebhold, A. M. 1990. Population dynamics of gypsy moth in North America. *Annu. Rev. Entomol.* **35,** 571–576.

Fuxa, J. R. 1979. Interactions of the microsporidium *Vairimorpha necatrix* with a bacterium, virus, and fungus in *Heliothis zea*. *J. Invertebr. Pathol.* **33,** 316–323.

Hajek, A. E., Humber, R. A., and Elkinton, J. S. 1995. Mysterious origins of *Entomophaga maimaiga* in North America. *Am. Entomol.* **41,** 31–42.

Hajek, A. E., Humber, R. A., Elkinton, J. S., May, B., Walsh, S. R. A., and Silver, J. C. 1990. Allozyme and restriction fragment length polymorphism analyses confirm *Entomophaga maimaiga* responsible for 1989 epizootics in North American gypsy moth populations. *Proc. Natl. Acad. Sci. USA* **87,** 6979–6982.

Hajek, A. E., Larkin, T. S., Carruthers, R. I., and Soper, R. S. 1993. Modeling the dynamics of *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) epizootics in gypsy moth (Lepidoptera: Lymantriidae) populations. *Environ. Entomol.* **22,** 1172–1187.

Jaques, R. P., and Morris, O. N. 1981. Compatibility of pathogens with other methods of pest control and with different crops. *In* "Microbial Control of Insects and Mites" (H. D. Burges and N. W. Hussey, Eds.), pp. 695–715. Academic Press, New York.

Jeffords, M. R., Maddox, J. V., and O'Hayer, K. W. 1987. Microsporidian spores in gypsy moth larval silk: A possible route of horizontal transmission. *J. Invertebr. Pathol.* **49,** 332–333.

Jeffords, M. R., Maddox, J. V., McManus, M. L., Webb, R. E., and Wieber, A. 1988. Egg contamination as a method for the inoculative release of exotic microsporidia of the gypsy moth. *J. Invertebr. Pathol.* **51,** 190–196.

Jeffords, M. R., Maddox, J. V., McManus, M. L., Webb, R. E., and Wieber, A. 1989. Evaluation of the overwintering success of two European microsporidia inoculatively released into gypsy moth populations in Maryland. *J. Invertebr. Pathol.* **53,** 235–240.

Krieg, A. 1971. Interactions between pathogens. *In* "Microbial Control of Insects and Mites" (H. D. Burges and N. W. Hussey, Eds.), pp. 459–468. Academic Press, New York.

LeOra Software. 1987. "POLO-PC. A User's Guide to Probit or Logit Analysis." LeOra, Berkeley, CA.

Lewis, F. B. 1981. Gypsy moth nucleopolyhedrosis virus. *In* "The Gypsy Moth: Research Toward Integrated Pest Management" (C. C. Doane and M. L. McManus, Eds.), pp.454–455. USDA, Washington. [U.S. Dept. Agric. For. Serv. Tech. Bull. 1584]

Lipa, J. J. 1971. Studies on the interactions of various pathogens in one insect host (cutworms). Institute of Plant Protection, Poznan, Poland. [Final Rep. E21-Ent-17, FG-PO-194]

McManus, M. L., Maddox, J. V., Jeffords, M. R., and Webb, R. E. 1989. Evaluation and selection of candidate European microsporidia for

introduction into U.S. gypsy moth populations. *In* "The Lymantriidae: A Comparison of Features of New and Old World Tussock Moths" (W. E. Wallner, Ed.), pp. 455–468. USDA, Washington. [U.S. Dept. Agri. For. Serv. Gen. Tech. Rep. NE-123].

Moawed, S. M., Saleh, M. R., and Salem, S. A. 1987. Interaction of the microsporidium *Vairimorpha ephestiae* with nuclear polyhedrosis virus and their effect on Egyptian cotton leafworm, *Spodoptera littoralis*. *Acta Entomol. Bohemoslov.* **84,** 422–430.

Nordin, G. L., and Maddox, J. V. 1972. Effect of simultaneous virus and microsporidian infection on larvae of *Hyphantria cunea*. *J. Invertebr. Pathol.* **20,** 66–69.

Novotony, J. 1988. The use of nucleopolyhedrosis virus (NPV) and microsporidia in the control of the gypsy moth. *Folia Parasit.* **35,** 199–208.

Novotony, J. 1989. Natural disease of gypsy moth in various gradation phases. *In* "The Lymantriidae: A Comparison of Features of New and Old World Tussock Moths" (W. E. Wallner, Ed.), pp. 101–111. Washington, DC. [U.S. Dept. Agri. For. Serv. Gen. Tech. Rep. NE-123]

Novotony, J. 1991. Influence of gypsy moth larvae age on the development of microsporidian diseases. *Biologia (Bratislava)* **46,** 105–112.

Novotony, J., and Weiser, J. 1993. Transovarial transmission of *Nosema lymantriae* (Protozoa: Microsporidia) in the gypsy moth *Lymantria dispar* L. *Biologia (Bratislava)* **48,** 125–129.

Onstad, D. W., and Maddox, J. V. 1989. Modelling the effects of the microsporidium, *Nosema pyrausta,* on the population dynamics of the insect, *Ostrinia nubilalis*. *J. Invertebr. Pathol.* **53,** 410–421.

Onstad, D. W., Maddox, J. V., Cox, D. J., and Kornkven, E. A. 1990. Spatial and temporal dynamics of animals and the host-density threshold in epizootiology. *J. Invertebr. Pathol.* **55,** 76–84.

Onstad, D. W., Maddox, J. V., Jeffords, M. R., McManus, M. L., Bauer, L. S., Webb, R. E. 1993. Modeling horizontal transmission of microsporidia in gypsy moth populations. *In* "Proceedings: Interagency Gypsy Moth Research Forum," Jan. 19–22, 1993, Annapolis, MD, p. 87. USDA, Washington. [U.S. Dept. Agri. For. Serv. Gen. Tech. Rep. NE-179]

Onstad, D. W., Reissig, W. H., and Shoemaker, C. A. 1986. Influence of apple cultivar, tree phenology, and leaf quality on the development and mortality of *Choristoneura rosaceana* (Lepidoptera: Tortricidae). *Can. Entomol.* **118,** 123–132.

Pilarski, D., and Vavra, J. 1991. Morphology and development of *Nosema serbica* Weiser, 1963 (Microsporida, Nosematidae), parasite of the gypsy moth *Lymantria dispar* (Lepidoptera, Lymantriidae). *Folia Parasitol.* **88,** 115–121.

Reardon, R. C. 1981. Alternative controls. Parasites. *In* "The Gypsy Moth: Research Toward Integrated Pest Management" (C. C. Doane and M. L. McManus, Eds.), pp. 299–421. USDA, Washington. [U.S. Dept. Agric. For. Serv. Tech. Bull. 1584]

SAS. 1990. "Procedures Guide Version 6." SAS Institute, Cary, NC.

Solter, L. F., Maddox, J. V., and McManus, M. L. 1997. Host specificity of microsporidia (Protista: Microspora) from European populations of *Lymantria dispar* (Lepidoptera: Lymantriidae) to indigenous North American Lepidoptera. *J. Invertebr. Pathol.* **69,** 135–150.

Speare, A. T., and Colley, R. H. 1912. "The Artificial Use of the Brown-Tail Fungus in Massachusetts, with Practical Suggestions for Private Experiment, and a Brief Note on a Fungous Disease of the Gypsy Caterpillar." Wright & Potter, Boston, MA.

Undeen, A. H., and Alger, N. E. 1971. A density gradient method for fractionating microsporidian spores. *J. Invertebr. Pathol.* **18,** 419–420.

Weiser, J. 1987. Patterns over place and time. *In* "Epizootiology of Insect Diseases" (J. R. Fuxa and Y. Tanada, Eds.), p. 215–242. Wiley, New York.

Weiser, J., and Novotny, J. 1987. Field application of *Nosema lymantriae* against the gypsy moth, *Lymantria dispar* L. *J. Appl. Entomol.* **104,** 58–62.

Wilkinson, L. 1987. "SYSTAT: The System for Statistics." SYSTAT, Evanston, Illinois.

Woods, S. A., and Elkinton, J. S. 1987. Bimodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth (*Lymantria dispar*) populations. *J. Invertebr. Pathol.* **50,** 151–157.

Zelinskaya, L. M. 1980. Role of microsporidia in population dynamics of the gypsy moth in the forests of the lower Dniepr Valley. *Vest. Zool.* **1,** 57–62.