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Interactions between Two Gypsy Moth (Lepidoptera: Lymantriidae) Pathogens: Nucleopolyhedrovirus and *Entomophaga maimaiga* (Zygomycetes: Entomophthorales): Field Studies and a Simulation Model

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The sudden appearance of a fungal pathogen, *Entomophaga maimaiga*, in gypsy moth (*Lymantria dispar*) populations in the United States in 1989 raised questions concerning its effect on mortality from nucleopolyhedrovirus (LdMNPV), a pathogen that causes the collapse of defoliating gypsy moth populations. To determine the impacts of *E. maimaiga* on LdMNPV-induced larval mortality, we collected gypsy moth larvae from seven 0.04-ha plots in 1992 and four 0.04-ha plots in 1994. Two of the plots in 1994 were supplemented with artificial rain, and the gypsy moth larvae in those plots had a higher *E. maimaiga*-induced mortality (seasonal cumulative mortality of 81%) than those in unwatered plots (66%). The levels of LdMNPV mortality were similar in both watered and unwatered plots (seasonal cumulative mortalities of 35 and 39%, respectively). To elucidate the impact of *E. maimaiga* on LdMNPV-induced mortality, we modified a previously developed host–pathogen model for LdMNPV to include both pathogens and fit it to our observed data on *E. maimaiga* mortality. The model predicted that, at moderate densities of gypsy moths, as in our plots, the mortality induced by LdMNPV would not be very different with or without *E. maimaiga.* This occurred because gypsy moth mortality from *E. maimaiga* reaches the highest level only when the older instars are present.

***Key Words: Entomophaga maimaiga; Lymantria dispar;* baculovirus; epizootic; host–pathogen model; population dynamics.**

INTRODUCTION

Gypsy moth, *Lymantria dispar* (L.), is the most damaging defoliator of deciduous forests in the northeastern United States. Gypsy moth populations typically remain at low densities for many years, but occasionally experience outbreaks that cause extensive defoliation (Elkinton and Liebhold, 1990). Such outbreak populations usually collapse from naturally occurring epizootics of the gypsy moth nucleopolyhedrovirus (LdMNPV), and high mortality from LdMNPV sometimes continues in the year following such population collapses (Doane, 1969, 1970).

Entomophaga maimaiga Humber, Shimazu *et* Soper, a fungal pathogen of gypsy moth, appeared for the first time in North America in 1989 and decimated gypsy moth populations throughout New England (Andreadis and Weseloh, 1990; Hajek *et al.,* 1990). In subsequent years, *E. maimaiga* spread throughout the range of gypsy moth in eastern United States (Hajek *et al.,* 1996). Larvae killed by *E. maimaiga* produce two kinds of spores: early instars produce conidia that are short-lived and generally transported by the wind, whereas late instars produce resting spores (Hajek and Shimazu, 1996) that overwinter in the soil and germinate in subsequent years (Weseloh and Andreadis, 1997). Infection occurs when a larva comes in contact with the germinating spores (Hajek *et al.,* 1993). Infected larvae typically die within 4–7 days (Hajek *et al.,* 1993; Hajek and Shimazu, 1996), resulting in 6–7 cycles of death and transmission during one larval season. In contrast, gypsy moths acquire LdMNPV infection by ingesting contaminated egg chorion (Murray and Elkinton, 1989) or contaminated foliage (Doane, 1970). Larvae that die from LdMNPV release virions embedded in a protein matrix known as an occlusion body (OB) that serves as a protective coat. In the field environment, infected larvae take about 2 weeks to die (Woods and

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Elkinton, 1987). Typically, in a high-density gypsy moth population, the first cycle of LdMNPV-induced mortality is observed among the late 1st or 2nd instars that consumed LdMNPV-contaminated egg chorion, releasing a new generation of OBs into the environment. These OBs become inocula for the other healthy larvae when such larvae feed on OB-contaminated foliage to induce the second wave of mortality, peaking among the late instars (Woods and Elkinton, 1987).

Previous studies have shown that both *E. maimaiga* and LdMNPV coexist in gypsy moth populations in the field (Andreadis and Weseloh, 1990; Hajek and Roberts, 1992; Weseloh and Andreadis, 1992a). However, we have observed several high-density gypsy moth populations that experienced *E. maimaiga* epizootics and rebounded to high densities the following year (J.S.E., personal observations). Yerger and Rossiter (1996) reported that gypsy moth larvae hatched from eggs collected from several high-density populations in Massachusetts had very low levels of LdMNPV infections compared to larvae collected in other locations where *E. maimaiga* had not yet been established. They speculated that the presence of *E. maimaiga* in Massachusetts might have caused this difference. These observations suggest that *E. maimaiga* may, in some manner, suppress or interfere with LdMNPV mortality and thus allow the gypsy moth populations to rebound, but we know very little about the manner in which the two pathogens interact in the field. Laboratory studies (Malakar, 1997; Malakar *et al.,* 1999) showed that *E. maimaiga* can kill larvae that are already infected with LdMNPV due to the shorter incubation time of the fungal pathogen within its host, but beyond that, there was no evidence for antagonistic interactions between the two pathogens within the host. In field populations, mortality from *E. maimaiga* among early instars reduces the number of larvae that would otherwise have died from LdMNPV, thereby reducing the viral inoculum available to infect later instars. This might substantially affect the second wave (Woods and Elkinton, 1987) of mortality that predominates in LdMNPV epizootics in high-density populations.

To explore the possible interactions between LdMNPV and *E. maimaiga* in naturally occurring gypsy moth populations, we measured the levels of mortality caused by both pathogens in 1992 and 1994. We attempted to manipulate *E. maimaiga* levels by applying artificial rain in 1994. We developed a host–pathogen model to simulate the mortality caused by LdMNPV in the presence and absence of *E. maimaiga.*

MATERIALS AND METHODS

Experimental plots and estimation of initial densities of insects. We established seven 20 × 20 m plots in 1992 and four such plots in 1994 in the Holyoke Range State Forest in Amherst, Massachusetts. The plots were separated by at least 200 m. Red oak (*Quercus rubra* L.) and chestnut oak (*Q. prinus* L.) dominated the forest canopy, and witch hazel (*Hamamelis virginiana* L.) dominated the understory. Gypsy moth larvae defoliated most of the trees on these sites each year from 1990 to 1993 (J.S.E., personal observations). We estimated the density of egg masses in each plot by conducting a complete census of all egg masses on the ground, understory vegetation, and trees within the plots in mid-April 1992 and 1994, prior to egg hatch. In order to estimate the number of larvae per m^2 of ground area in each plot, we multiplied the total number of egg masses in a plot by the average number of larvae that hatched per egg masses, and divided by the area of the plot.

Estimate of initial viral and fungal inocula in the experimental sites. To estimate the percentage of egg hatch and virus infection among the neonates, we collected 10 egg masses from the vicinity of each plot. We removed egg masses from tree boles with a sterilized knife and transferred them individually into 60-ml empty diet cups, containing a piece of wet dental wick. The larvae that hatched from each egg mass were counted and reared in groups of 15 on artificial diet (Bell *et al.,* 1981) in 180-ml cups. They were held for 2 weeks at room temperature and monitored for mortality every other day. We examined tissues of each cadaver at 100× and 400× under a compound microscope to determine the cause of death (Hajek and Roberts, 1992). At that time we had little knowledge about how the fungal infection starts among the neonates; we simply brought in the feral egg masses and reared them as above with adequate moisture for the resting spores if any were present on the egg masses. None of the neonates from these egg masses died from *E. maimaiga.* Therefore, to estimate the initial number of *E. maimaiga* present in the environment, we used the proportion of larvae that died from *E. maimaiga* for the first time from the weekly larval collections. We did not make any distinction between *E. maimaiga* infections initiated by resting spores or conidial spores.

Measuring disease-caused larval mortality in the field. To estimate the gypsy moth larval mortality due to diseases, we collected about 50 larvae/plot each week from 25 May to 7 July in 1992. Similarly, in 1994, we collected ca. 100 larvae from each plot per week, starting 17 May, when most of the larvae were 1st instars. We continued the collections until 8 July, when most of the survivors had pupated. Early instars were collected from understory foliage and the later instars were collected from burlap bands wrapped around the tree trunks (McManus and Smith, 1984). Larvae were collected individually into 60-ml diet cups and reared for a week in an outdoor insectary. We checked mortal-

ity on alternate days and autopsied dead larvae to determine the cause of death. We tabulated the fraction of gypsy moth larvae that died within 1 week of collection and contained visible LdMNPV or *E. maimaiga* or both.

The cumulative percentage larval mortality from each agent was calculated as $100(1 - S_1 \times S_2 \ldots \times S_7)$, where S_i is the probability of surviving in week i and $(1 - S_i)$ is the weekly marginal probability of dying from each pathogen (Royama, 1981; Gould *et al.,* 1990; Elkinton *et al.,* 1992). The marginal probability of dying is equivalent to the proportion observed to die each week from each cause but is adjusted for the fact that death from one pathogen precludes dying from the other. We assumed that those larvae infected with both pathogens would die from *E. maimaiga,* and we used the calculation suggested by Elkinton *et al.* (1992), whereby the observed weekly proportion dying from fungus d_f equals the weekly marginal probability of dying m_f for *E. maimaiga*, and the weekly marginal probability of dying m_v for LdMNPV is given by $m_v = d_v/1 - m_f$, where d_v is the observed weekly proportion dying from LdMNPV.

Effect of rainfall on mortality of gypsy moths due to E. maimaiga. In 1994, we attempted to manipulate the levels of *E. maimaiga* infection by applying artificial rain to two of the four plots. Each plot was divided into four 10 × 10 m subplots. The artificial rain was applied for 30 min twice a week in each subplot with a hose affixed to a rotary garden sprinkler so that all the understory vegetation in the plot was completely soaked. Based upon the volume of water delivered and the area of ground covered, we calculated that this was equivalent to 4.2 mm of rainfall per week, in addition to the natural rainfall.

The model. We developed a host–pathogen model which is an extension of the single-pathogen model that Dwyer and Elkinton (1993) developed for LdMNPV epizootics. The Dwyer and Elkinton (1993) model is given by the Eqs. [1–3]

$$\frac{dS}{dt} = -\nu PS \qquad [1]$$

$$\frac{dI}{dt} = \nu PS - \nu P_{(t-\tau)}S_{(t-\tau)} \qquad [2]$$

$$\frac{dP}{dt} = \Lambda\nu P_{(t-\tau)}S_{(t-\tau)} - \mu P, \qquad [3]$$

where S is the density (number) of susceptible hosts, I is the density of infected hosts, P is the density of pathogens (LdMNPV), ν is the transmission constant, τ is the incubation time of the pathogen (i.e., time between infection and death of the host), Λ is the number of pathogen particles produced by a cadaver of an infected larva, μ is the decay rate of the pathogen, and t is time.

To extend the model to two pathogens, we defined two sets of constants analogous to the constants in the above model, one set for LdMNPV and another for *E. maimaiga.* The rate of change of susceptible (uninfected) host density due to the LdMNPV and *E. maimaiga* is given by

$$\frac{dS}{dt} = -(\nu_F F + \nu_V V)S, \qquad [4]$$

where S is the density of susceptible gypsy moth larvae which are neither infected by LdMNPV nor by *E. maimaiga, F* is the density of *E. maimaiga* inoculum, V is the density of LdMNPV inoculum, and ν_F and ν_V are the transmission constants of *E. maimaiga* and LdMNPV, respectively.

Previous studies have shown that within-host incubation time of *E. maimaiga* is about 7 days under field conditions (Hajek *et al.,* 1993), while that of LdMNPV is about 14 days (Woods and Elkinton, 1987). Laboratory studies (Malakar, 1997; Malakar *et al.,* 1999) have shown that larvae infected with LdMNPV could subsequently become infected with and die from *E. maimaiga,* provided that the fungal pathogen infected its host within about 1 week following infection with LdMNPV at 20°C. Therefore, in the model we allowed for dual infection, meaning that individuals could become infected by both pathogens and that the eventual cause of death depends on the relative timing of infection. We assumed that the larvae infected with LdMNPV remained susceptible to fungal infections up to 7 days following LdMNPV infection, and we defined these larvae as an intermediate class of infected individuals (I_i). We defined fungal infected (I_F) to be those individuals that would die from *E. maimaiga* and likewise viral infected (I_V) are those individuals that would die from LdMNPV. If a larva was infected with LdMNPV and then became infected with *E. maimaiga* within 7 days, it would die from *E. maimaiga* (Malakar *et al.,* 1999). In our model we included such larvae in the counts of fungal-infected individuals (I_F). Conversely, if an individual had been infected with LdMNPV for 7 or more days without being infected by *E. maimaiga,* then it would die due to LdMNPV, whether or not it was subsequently infected with *E. maimaiga.* In our model we included such larvae in our counts of LdMNPV-infected individuals (I_V). The rate of change of *E. maimaiga*-infected host density is

$$\frac{dI_F}{dt} = \nu_F F(S + I_i) - \nu_F F_{(t-\tau_F)}(S + I_i)_{(t-\tau_F)}, \qquad [5]$$

where I_F is the density of larvae infected by *E. maimaiga,* τ_F is the incubation period of *E. maimaiga* in the host, and I_i is the number of hosts in our previously defined intermediate class, that is, infected with LdMNPV but still susceptible to *E. maimaiga.* The equation for I_i is given in the Appendix. Larvae enter the class of individuals that die from *E. maimaiga* (I_F) at the rate of $\nu_F F(S + I_i)$, and leave by dying from *E. maimaiga* after the 7-day incubation period at the rate of $\nu_F F_{(t-\tau_F)} (S + I_i)_{(t-\tau_F)}$.

The change in LdMNPV-infected individuals I_V can be expressed as

$$\frac{dI_V}{dt} = \nu_V V_{(t-\Delta\tau)} S_{(t-\Delta\tau)} - \nu_V V_{(t-\tau_V)} S_{(t-\tau_V)} - \left[\int_{t-\Delta t}^{t} \nu_F F_{(s)} i_{(s,n)}\, ds - \int_{t-\tau_V}^{t-\tau_F} \nu_F F_{(s)} i_{(s,n)}\, ds \right], \quad [6]$$

where I_V is the density of larvae infected by LdMNPV, and τ_V is the incubation period of LdMNPV in the host. Derivation of Eq. [6], including the definition of $[i_{(s,n)}]$, is given in Appendix 1. The rate of change of *E. maimaiga* conidial density in the environment is given by:

$$\frac{dF}{dt} = \Lambda_F \nu_F F_{(t-\tau_F)} (S + I_i)_{(t-\tau_F)} - \mu_F F. \quad [7]$$

Here, Λ_F is the number of conidia produced by an infected larva that succumbed to *E. maimaiga* and μ_F is the decay rate of conidia in the environment. The rate of change of density of LdMNPV OBs in the environment is given by:

$$\frac{dV}{dt} = \Lambda_V i_{(t-\tau_F, t-\tau_V)} - \mu_V V. \quad [8]$$

Here, Λ_V is the number of OBs produced by an infected larva that succumbed to LdMNPV, $i_{(t-\tau_F,\ t-\tau_V)}$ is the number that enter I_i at time $(t - \tau_V)$ and remain in I_i stage until time $(t - \tau_F)$, and μ_V is the decay rate of OBs in the environment.

The values of the LdMNPV-related parameters, ν_V, Λ_V, μ_V, and τ_V, were taken from Dwyer and Elkinton (1993). The values of the *E. maimaiga*-related parameters, Λ_F and τ_F, were taken from Hajek *et al.* (1993) (Table 1). The transmission rate ν_F of *E. maimaiga* was adjusted by running the *E. maimaiga* model (Eq. [1–3], where F replaces P) with different ν_F until the predicted mortality from *E. maimaiga* matched the mortality that we recorded in the field. In the absence of data on conidial longevity, we assumed that $\mu_F = \mu_V$. We emphasize that our purpose was not to predict mortality from *E. maimaiga;* instead, our purpose was to predict the effects of *E. maimaiga* on the larval mortality from LdMNPV. We tuned the fungus model to fit the fungal mortality data.

TABLE 1

Values of Parameters from Eqs. [1–8], which Were Used in the Model

	Symbol/parameter	Value
ν_V	Transmission rate of the LdMNPV	1.45×10^{-12} m^2/day
ν_F	Transmission rate of *E. maimaiga* (unwatered plots, 1992)	3.99×10^{-8} m^2/day
ν_F	Transmission rate of *E. maimaiga* (unwatered plots, 1994)	1.67×10^{-7} m^2/day
ν_F	Transmission rate of *E. maimaiga* (watered plots, 1994)	2.50×10^{-7} m^2/day
τ_F	*E. maimaiga* incubation time	7 days
τ_V	LdMNPV incubation time	14 days
Λ_F	No. of conidia produced	2.12×10^5/cadaver
Λ_V	No. of occlusion bodies produced	2×10^9/cadaver
μ_F	Rate at which conidia break down in the environment	3×10^{-3}/day
μ_V	Rate at which occlusion bodies break down in the environment	3×10^{-3}/day

Note. The parameters related to *Lymantria dispar* nucleopolyhedrovirus (LdMNPV), ν_V, Λ_V, μ_V, and τ_V, were taken from Dwyer and Elkinton (1993) and the *Entomophaga maimaiga*-related parameters, Λ_F and τ_F, were taken from Hajek *et al.* (1993). The *E. maimaiga* transmission rate, ν_F, was taken from the *E. maimaiga* model. We ran the *E. maimaiga* model with different ν_F values until the model prediction was similar to the observed *E. maimaiga* mortality in each treatment group in the field.

The initial density of the host population, $S(0)$, was estimated from the number of larvae present per m^2 of ground area using the total number of egg masses present in the plot and the average number of larvae that hatched from the egg masses (see above). The initial fraction of larvae hatching with LdMNPV infections ($I_V(0)$) was estimated from the proportion of larvae that died from the field-collected egg masses. The density of *E. maimaiga*-infected larvae ($I_F(0)$) was estimated from the proportion of *E. maimaiga*-killed larvae from the first week of the larval collection. However, in 1992, we did not observe any *E. maimaiga*-induced mortality until the fourth collection week; so we used that value as $I_F(0)$ in the model. In each simulated year we adjusted the transmission parameter ν_F, using different values until the predicted values of *E. maimaiga* matched the observed weekly mortality. The model was implemented in the Pascal language with a 0.01-day time step. We used Euler's method (Haefner, 1996) to compute the number of new infections and the number dying in each time step.

To determine whether the LdMNPV mortality values predicted are different (a) with and without *E. maimaiga* (not including the dual infection, Eqs. [2–4]) and (b) with and without *E. maimaiga* dual infection (Eqs. [4–8]), we performed a paired *t* test.

TABLE 2

Estimates of Egg Mass Density, Larvae Hatched, and Percentage of the Neonates Initially Infected by *Lymantria dispar* Nucleopolyhedrovirus (LdMNPV) in the Research Plots of 1992 and 1994

Location/year	No. of plots	Mean no. of egg masses/ha (±SE)	Mean no. of larvae hatched/egg mass (±SE)	Initial no. of larvae/m^2 (±SE)	Mean % of neonates died from LdMNPV (±SE)
Holyoke 1992 (unwatered plots)	7	6260.0 (1368.2)	26.0 (1.6)	17.5 (4.7)	25.2 (4.0)
Holyoke 1994 (watered plots)	2	2100.0 (425.0)	128.7 (13.5)	27.0 (6.2)	10.2 (2.2)
Holyoke 1994 (unwatered plots)	2	2612.5 (187.5)	105.2 (10.6)	27.5 (2.3)	12.3 (3.5)

RESULTS

Initial density of gypsy moth larvae and viral and fungal inocula. Prehatched egg mass density is a measure of the population size of gypsy moth for the coming season. The egg mass counts in the plots (Table 2) correspond to high-density populations of gypsy moths (Campbell, 1981). However, the hatch rate was lower in 1992 than in 1994 and was lower than in a typical outbreak population (Campbell, 1981). In 1992, 25% of the hatched insects from the collected egg masses died from LdMNPV, and in 1994 10–12% died from LdMNPV. We did not observe any *E. maimaiga*-induced mortality in the larvae that hatched from the feral egg masses collected in either year.

Mortalities of gypsy moth larvae due to viral and fungal diseases in the field. In both years, we started the feral larval collections when >90% of the larvae were 1st instars. Among the larvae collected in the first week of 1992, 2–6% died from LdMNPV, but there was no mortality from *E. maimaiga.* In 1994, there was 3–5% mortality from LdMNPV and 1–7% mortality from *E. maimaiga* among the insects from the first week of collection. In 1992, the LdMNPV infection level became highest at the end of the larval season, but *E. maimaiga* infection remained at a low level. In 1994, *E. maimaiga* infections increased markedly among the late instars and LdMNPV-induced mortality remained at a very low level throughout the season. The overall cumulative mortality due to LdMNPV in 1992 was higher than in 1994 and *E. maimaiga* mortality was higher in 1994 (Table 3). When we censused egg mass in the spring of 1995, before the egg hatch, we had very few egg masses (38–113/ha) in the experimental plots.

Effect of rainfall. According to the National Oceanic and Atmospheric Administration (NOAA, 1964–1994) the total rainfall in Amherst in May 1994 was much higher than the 30-year average, whereas the rainfall in May of 1992 was close to the 30-year average (Fig. 1). The higher amount of rainfall in May 1994 compared to May 1992 probably explains the observation of higher mortality from *E. maimaiga* in 1994. In both 1992 and 1994, rainfall in June was very close to the 30-year average. In Fig. 1, we compare these values to those obtained in 1989, the first year that *E. maimaiga* was observed in the northeastern United States and when rainfall in May and June was the highest recorded in 30 years (Elkinton *et al.,* 1991).

In 1994, in our two artificial rainfall experimental plots, we applied a total of 8.4 mm of artificial rain in each plot in May and 16.8 mm in June. The cumulative weekly mortality due to *E. maimaiga* was significantly higher in watered plots than in the unwatered plots ($\chi^2 = 4.86$, $df = 1$, $P = 0.028$). However, we did not see any significant differences in LdMNPV mortality among the gypsy moth larvae in watered and unwatered plots ($\chi^2 = 0.99$, $df = 1$, $P = 0.32$) (Table 3).

TABLE 3

Cumulative Percentage Mortality of Gypsy Moth Larvae Due to *Lymantria dispar* Nucleopolyhedrovirus (LdMNPV) and *Entomophaga maimaiga*

Year	Cum. observed % larval mortality[a] due to		Model predicted total survivors/ha		Egg mass counts per ha in 1995 spring
	LdMNPV	*E. maimaiga*	Without *E. maimaiga*	With *E. maimaiga*	
Unwatered plots, 1992	63.7	27.0	157.8	52.8	No count
Unwatered plots, 1994	35.0	65.7	307.3	91.8	112.5
Watered plots, 1994	39.3	81.3	349.8	19.8	37.5

Note. The model predicted total survivors at the end of the 1992 and 1994 gypsy moth seasons.

[a] The cumulative percentage mortality was based upon the weekly marginal probability of dying from each pathogen (Elkinton *et al.,* 1992), but it is adjusted for the fact that death from one pathogen precludes dying from the other (see Materials and Methods).

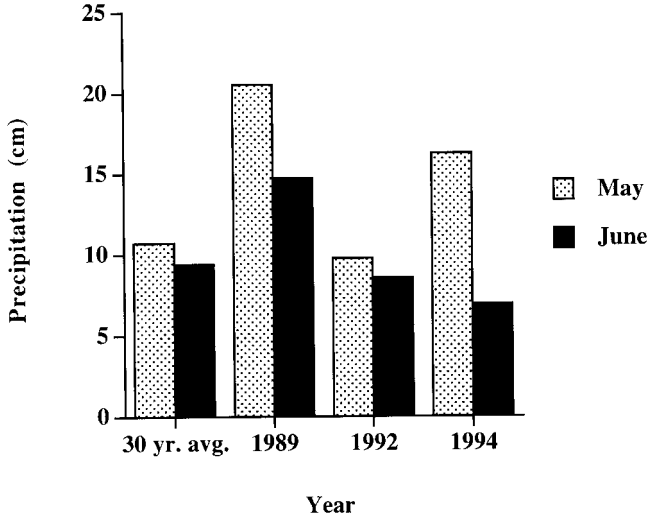

FIG. 1. Total recorded rainfall (cm) in Amherst, Massachusetts in the months of May and June.

The model. We predicted the mortality of gypsy moth larvae due to LdMNPV in the presence and absence of *E. maimaiga* using our simulation model. The overall impact of *E. maimaiga* on LdMNPV mortality was minor in our simulations at densities represented by our field data from both years 1992 and 1994 (Fig. 2). In 1992, when there was a low level of *E. maimaiga* infection (cumulative mortality at the end of the larval season was 15.3%), the LdMNPV models with dual infections showed a slightly higher LdMNPV mortality in the absence of *E. maimaiga* than when it was present (paired t test; $t = 2.79$, $df = 5$, $P = 0.04$), but in 1994 there was virtually no difference in LdMNPV mortality whether *E. maimaiga* was present or not (Figs. 2b and 2c).

In nature, it is probable that v_F varies depending upon natural rainfall (Hajek *et al.,* 1993). We ran the *E. maimaiga* model (Eqs. [1–3]) with different v_F values until the model prediction matched the observed

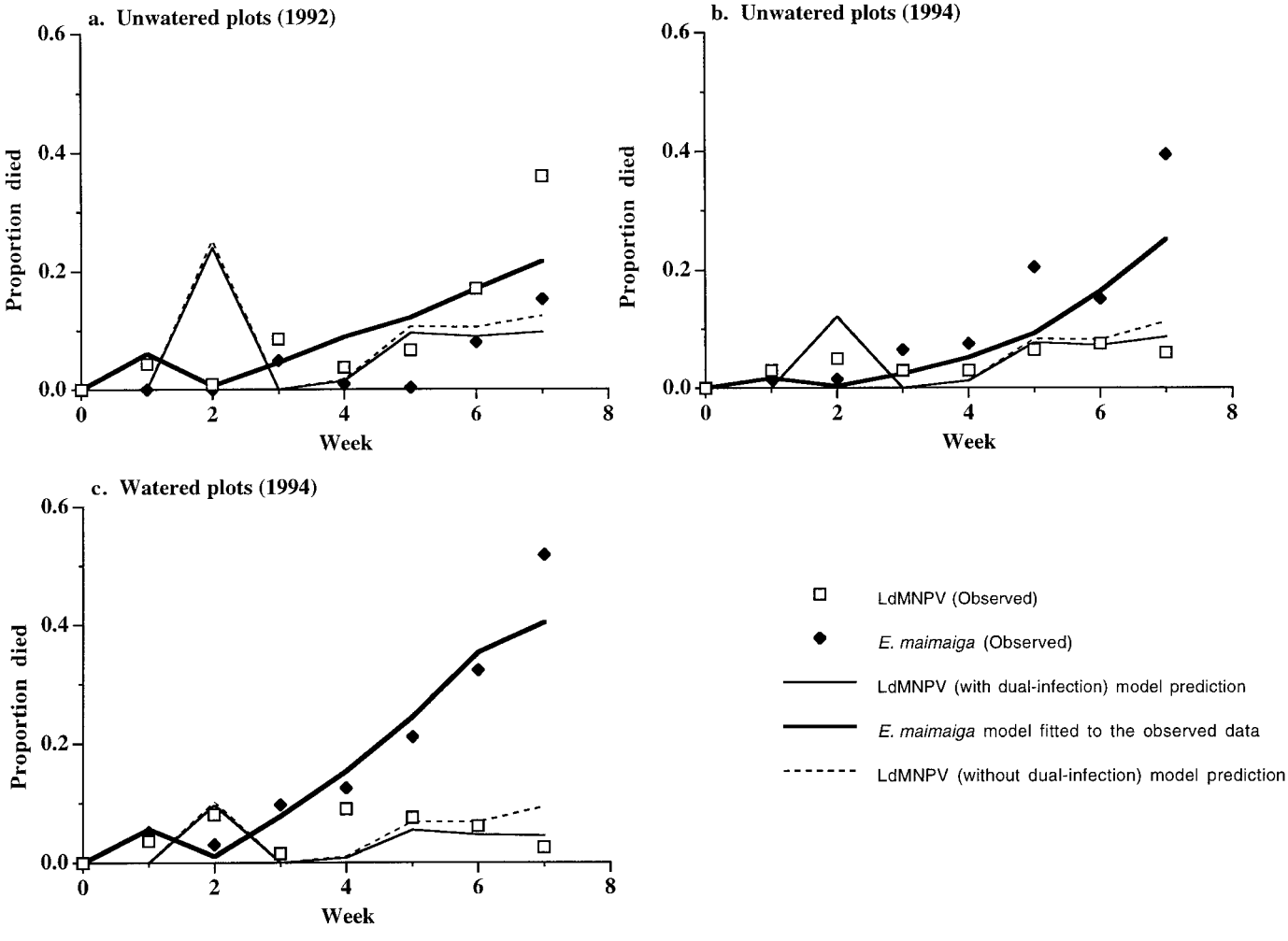

FIG. 2. Mean proportion of gypsy moth larvae dying each week from *Entomophaga maimaiga* and nucleopolyhedrovirus (LdMNPV) from field collections and as predicted by a simulation model in (a) unwatered plots (1992), (b) unwatered plots (1994), and (c) watered plots (1994). We ran the *E. maimaiga* model with different v_F values until the fitted line matched with the observed weekly *E. maimaiga* mortality.

E. maimaiga mortality in the field (Table 1). We used such estimated v_F values in the rest of the models (Eqs. [4–8]). However, our purpose was to model the impact of the observed levels of *E. maimaiga* on mortality of larvae due to LdMNPV, rather than predicting mortality from *E. maimaiga.* When we included the effects of dual infection in our model, in which we allowed larvae previously infected with LdMNPV to become infected and die from *E. maimaiga,* the effect of *E. maimaiga* on the mortality due to LdMNPV became noticeable only at the very end of the larval season, and our model predictions were similar to the observed LdMNPV mortality (Fig. 2). Without dual infection, there was no discernible effect of *E. maimaiga* on predicted levels of LdMNPV in the populations.

DISCUSSION

LdMNPV and *E. maimaiga* were the major causes of gypsy moth mortality in the population that we studied, because together they accounted for almost all the generational change in gypsy moth density. In 1992 LdMNPV became the dominant mortality factor among the late instars, whereas in 1994, *E. maimaiga* was the major source of mortality. In 1992, the gypsy moth egg mass density was higher, the initial infection level of LdMNPV was higher, and the rainfall was lower than in 1994. Despite the competitive advantage of a shorter incubation period of *E. maimaiga* over LdMNPV, our model predicted that *E. maimaiga* had only a limited impact on LdMNPV mortality. This occurred because *E. maimaiga* remained at a low level until the larvae entered the 5th or 6th instars. Larvae that acquire LdMNPV infections at the end of the larval stage pupate and survive (Murray *et al.,* 1991). The model predictions closely matched the levels of LdMNPV mortality actually observed in our plots.

Although the density of egg masses was high, the number of eggs per egg mass was quite low; so the larval populations in our research plots were only moderate in both years (Table 2). We observed a very small second peak of mortality due to LdMNPV in our plots. According to our simulation model, these larval densities were not sufficient to create a large second wave of LdMNPV mortality, even in the absence of *E. maimaiga* (Fig. 2). The model predictions from the 1994 data are consistent with the egg mass estimates that we made in the early spring before the egg hatch (Table 3). In spring 1995, we counted an average of 113 egg masses/ha in unwatered plots and 38 egg masses/ha in the watered plots, compared to 26,120 and 2100 egg masses/ha, respectively, the previous spring (Table 2). These data indicate a collapse of gypsy moth populations in the research plots during the 1994 season.

In a laboratory study, Malakar *et al.* (1999) have shown that gypsy moth larvae simultaneously inoculated with both LdMNPV and *E. maimaiga* usually succumbed to *E. maimaiga,* probably due to the shorter incubation time of *E. maimaiga* within the host. Thus, in nature, it is likely that some of the larvae infected with LdMNPV will become infected with and subsequently die from *E. maimaiga* instead. Even without this within-host interaction, larvae dying as early instars from *E. maimaiga* reduce the density of larvae available to die subsequently from LdMNPV, thereby reducing the LdMNPV inoculum required to cause the second wave of LdMNPV mortality among later instars (Woods and Elkinton, 1987). This is probably the mechanism by which applications of *Bacillus thuringiensis* Berliner (Bt) suppresses the LdMNPV-induced mortality in gypsy moths (Woods *et al.,* 1988). The effect of Bt in that study was much larger than the effect that we showed here for *E. maimaiga,* presumably because the Bt was applied at a very early larval stage (2nd instar), which suppressed the density of gypsy moths that would die from LdMNPV and thus the inoculum that triggers the second wave of LdMNPV mortality (Woods and Elkinton, 1987) among later instars.

During the study period, the weekly mortality from *E. maimaiga* increased steadily throughout the larval stage of gypsy moth, peaking at the end of the season. This result agrees with earlier studies of *E. maimaiga* in low- to moderate-density field populations, which showed that the highest *E. maimaiga*-induced mortality occurs among the late instars (Hajek, 1997; Weseloh and Andreadis, 1992a). Mortalities that peak at the end of the larval stage are also typical of LdMNPV (Campbell, 1967; Woods and Elkinton, 1987) because the number of infectious particles and the number of larvae becoming infected increase exponentially with each cycle of the pathogen in the population.

The greatest effect of *E. maimaiga* on LdMNPV may be on the level of LdMNPV in the environment near the time of pupation and on the inoculum present to be transmitted to the next generation. Environmental contamination is thought to be the principal route of transmission of LdMNPV in the next generation via egg masses deposited on LdMNPV-contaminated surfaces (Murray and Elkinton, 1990). Yerger and Rossiter (1996) found less than 1% LdMNPV-induced mortality in neonate gypsy moth larvae which were collected as egg masses from coastal and central Massachusetts in late summer 1991. However, when we collected eggs and reared neonates from exactly the same site in central Massachusetts in the same year (1992 larval season) that Yerger and Rossiter did, we observed 25.2% mortality from LdMNPV among the neonates reared from these egg masses (Table 2). In 1994 also we had 10–12% LdMNPV-induced mortality among the neonates collected as egg masses from the same site. The model indicates that the fraction dying from LdMNPV (3.5%) in the presence of *E. maimaiga* (and dual

infection) was about one-fourth that without *E. maimaiga* (12.9%) in the week just before pupation (Fig. 2C). Predicted amounts of LdMNPV inoculum left were 8.63×10^9 and 1.48×10^{10} OBs/m^2, with and without *E. maimaiga,* respectively. Webb *et al.* (1999) pursued a 3-year study to compare the virus and fungus mortalities among the newly established gypsy moth populations in Virginia. Their study shows that the *E. maimaiga*-induced larval mortality occurred among the late instars as in our observations of 1992 and 1994, and the second wave of viral mortality was highly suppressed. Further, they showed that the high *E. maimaiga*-induced larval mortality of 1995 greatly reduced the LdMNPV-induced larval mortality of the 1996 larval season. This negative impact of *E. maimaiga* on LdMNPV contamination might help to explain why gypsy moth populations sometimes appear to rebound following *E. maimaiga* epizootics.

A comprehensive model for *E. maimaiga* would have to include effects of humidity or rainfall, as well as distinguish between infection by conidia and resting spores. Such a model was beyond our objective here, which was to develop a simple model that provided a reasonably close fit to the observed mortality from *E. maimaiga* and to test the predictions of the model against the observed mortality from LdMNPV. We also wished to contribute an approach to modeling dual infections within the overall framework of Anderson/May type models developed by Dwyer and Elkinton (1993).

Previous studies have shown a positive correlation between rainfall and the mortality rates of gypsy moths from *E. maimaiga* (Elkinton *et al.,* 1991; Hajek, 1997; Hajek and Roberts, 1992; Smitley *et al.,* 1995; Weseloh and Andreadis, 1992a,b; Weseloh *et al.,* 1993). Secondary transmission via conidia is considered to be the major source of disease spread among the later instars, and it depends upon the pattern of rainfall (Weseloh and Andreadis, 1992b; Weseloh, 1998). Our study plots received a much higher than average natural rainfall in May 1994. This probably explains the higher cumulative mortality of gypsy moths from *E. maimaiga* in 1994 than in 1992 (Table 3). We found a higher cumulative mortality due to *E. maimaiga* in the watered plots than in the unwatered plots, which is similar to the findings of Hajek *et al.* (1996).

In conclusion, *E. maimaiga* at these gypsy moth larval densities has little effect on gypsy moth mortality induced by LdMNPV in the same gypsy moth generation. In all of our simulations, the combined mortality from LdMNPV and *E. maimaiga* was always higher than the mortality from LdMNPV alone. However, it may lower the probability of LdMNPV inoculum being available for cross-generational transmission, as suggested by the 50% reduction in the LdMNPV-induced mortality from the 1992 to the 1994 gypsy moth larval season.

APPENDIX

Here, we derive Eq. [6]. Note that τ_V, the incubation period of LdMNPV, is 2 weeks, while τ_F, the incubation period of *E. maimaiga,* is 1 week. The difference between these two incubation periods, $\Delta\tau = \tau_V - \tau_F = 1$ week, is the length of time that individuals in the I_i stage are susceptible to *E. maimaiga.* In order to define the rate of change of LdMNPV-infected host density, let us first define a two-dimensional function, $i_{(t,n)}$, which is the number of individuals initially infected with LdMNPV at time n that have escaped infection by *E. maimaiga* until time t. We assume that n is the time of entrance of hosts into the I_i stage; then $i_{(t,n)}$ can be defined on the subset of the $(t - n)$ plane, where $n \le t \le (n + \Delta\tau)$. In fact, $I_i(t) = \int_{t-\Delta\tau}^{t} i_{(t,n)}\, dn$. Using the rule of differentiation of integrals, $dI_i/dt = i_{(t,t)} - i_{(t,t-\Delta\tau)} + \int_{t-\Delta\tau}^{t} \partial i_{(t,n)}/\partial t\, dn$. By definition, $i_{(t,t)}$ = the number of individuals infected by LdMNPV at time t that have escaped infection by *E. maimaiga* until time t. As no time has elapsed, this is the number infected by *E. maimaiga* at time t, $(\nu_F F_{(t)} S_{(t)})$. Also, if we assume that *E. maimaiga* acts on subsets of susceptibles in the same manner as it does on the whole group, then $\partial i_{(t,n)}/\partial t = -\nu_F F_t i_{(t,n)}$. Thus,

$$\int_{t-\Delta\tau}^{t} \frac{\partial i_{(t,n)}}{\partial t}\, dn = \int_{t-\Delta\tau}^{t} -\nu_F F_t i_{(t,n)}\, dn$$

$$= -\nu_F F_t \int_{t-\Delta\tau}^{t} i_{(t,n)}\, dn = -\nu_F F I_i.$$

So, now we have:

$$\frac{dI_i}{dt} = \nu_V V S - \nu_F F I_i - i_{(t,t-\Delta\tau)}\,.$$

We developed this expression via a derivation but intuition confirms this expression. The change in I_i with respect to t is the number coming in minus the number going out. Individuals enter I_i from S (1st term) and can leave in one of two ways: (a) infection by *E. maimaiga* (2nd term) or (b) escaping infection by *E. maimaiga* for a time of $\Delta\tau$ (3rd term).

The number of individuals that were infected by LdMNPV 7 days earlier which have not been infected by *E. maimaiga,* $i_{(t,t-\Delta\tau)}$, is the number entering I_V at time t. This is the number that we used as the "new" viral infections at time t, although they were actually infected 7 days earlier, but we do not consider them viral infections until we know that they will die from LdMNPV. We can think of $I_V(t)$ as the summation of these new viral infections from $(t - \tau_F)$ to t because the

infections that were new at $(t - \tau_F)$ are dying at time t. Thus $I_V(t) = \int_{t-\tau_F}^{t} i_{(s,s-\Delta\tau)}\, ds$. The lower limit is $(t - \tau_F)$ because individuals entering I_V have been infected with LdMNPV for a time $\Delta\tau$. They need a total time of τ_V to die from LdMNPV; so, the remaining time they need to die is $\tau_V - \Delta\tau = \tau_V - (\tau_V - \tau_F) = \tau_F$.

Using the fundamental theorem of calculus,

$$\frac{dI_V}{dt}(t) = i_{(t,t-\Delta\tau)} - i_{(t-\tau_F,t-\tau_F-\Delta\tau)} = i_{(t,t-\Delta\tau)} - i_{(t-\tau_F,t-\tau_V)}.$$

The change in I_V with respect to time is the number coming in to I_V from I_i (1st term) minus the number dying from I_V (2nd term).

We can expand on this expression by deriving an alternative expression for $i_{(t,n)}$. By fundamental two-variable calculus, $i_{(t,n)} = i_{(n,n)} + \int_n^t \partial/\partial s\, [i_{(s,n)}]\, ds$. As before, $i_{(n,n)}$ is simply $\nu_V V_n S_n$. Also, from earlier, $\partial/\partial t\, [t_{(t,n)}] = -\nu_F F_t i_{(t,n)}$. This gives us:

$$i_{(t,n)} = i_{(n,n)} + \int_n^t \frac{\partial}{\partial s}\,[i_{(s,n)}]\, ds = \nu_V V_n S_n - \int_n^t \nu_F F_s i_{(s,n)}\, ds.$$

Substituting the above expression into our earlier expression, we get:

$$\frac{dI_V}{dt}(t) = i_{(t,t-\Delta\tau)} - i_{(t-\tau_F,t-\tau_V)} = \nu_V V_{(t-\Delta\tau)} S_{(t-\Delta\tau)}$$

$$- \int_{t-\Delta\tau}^{t} \nu_F F_s i_{(s,n)}\, ds - [\nu_V V_{(t-\tau_V)} S_{(t-\tau_V)} - \int_{t-\tau_V}^{t-\tau_F} \nu_F F_s i_{(s,n)}\, ds].$$

After rearranging terms we get:

$$\frac{dI_V}{dt} = \nu_V V_{(t-\Delta\tau)} S_{(t-\Delta\tau)} - \nu_V V_{(t-\tau_V)} S_{(t-\tau_V)}$$

$$- \left[\int_{t-\Delta t}^{t} \nu_F F_{(s)} i_{(s,n)}\, ds - \int_{t-\tau_V}^{t-\tau_F} \nu_F F_{(s)} i_{(s,n)}\, ds \right]. \quad [6]$$

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