Quantitative Analysis of a Pathogen-Induced Premature Collapse of a "Leading Edge" Gypsy Moth (Lepidoptera: Lymantriidae) Population in Virginia[1]

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Abstract The population dynamics of a "leading edge" (= at the edge of the expanding gypsy moth invasion) gypsy moth, *Lymantria dispar* (L.), population was monitored for 3 years (1995-97), with emphasis on the interactions of the gypsy moth nuclear polyhedrosis virus (LdNPV) and the fungus *Entomophaga maimaiga* Humber, Shimazu, & Soper. Gypsy moth populations in the woodlots varied from very sparse to high (potentially defoliating) levels. LdNPV was strongly density dependent, being confirmed only from the higher populated woodlots. In contrast, the fungus was similarly active in both sparse and highly-populated woodlots. In 1995, the fungal epizootic developed late in the season, with most larvae succumbing during stadia 5-6 and producing mainly resting spores (azygospores). Estimated mortality due to fungus averaged 68% in high-density plots and 85% in low-density plots. LdNPV mortality occurred in a two-wave epizootic, although second-wave LdNPV mortality was undoubtedly reduced because of the reduction of late-season larvae due to fungus activity. Estimated mortality due to LdNPV averaged 14% in highly-populated plots and 1% in low-population plots. In 1996, high levels of fungal-induced mortality occurred earlier in the gypsy moth season than in the previous year. Most gypsy moth larvae in 1996 died in a mid-season wave of fungal-induced mortality, with necropsied cadavers containing only conidia. This resulted in relatively few larvae surviving to late instars. At this time, a second wave of fungus-induced mortality occurred, with over half of the necropsied cadavers containing resting spores. The depletion of the gypsy moth populations by the fungus in 1995 resulted in a greatly reduced first wave of LdNPV in all plots in 1996, and perhaps due to the early appearance of the fungus in 1996, LdNPV was nearly absent from late-season larvae collected from all plots. In 1997, gypsy moth populations were uniformly low, and no dead larvae were found in any of the plots.

Key Words *Lymantria dispar*, *Entomophaga maimaiga*, gypsy moth NPV, nuclear polyhedrosis virus, forest entomology, insect pathogen, insect population dynamics.

Southwestern Virginia is currently experiencing its first cycle of defoliation from the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera:Lymantriidae), which recently invaded the area (Sharov et al. 1995). As gypsy moth populations become established and increase, natural enemies are moving into the area, including the gypsy moth nuclear polyhedrosis virus (LdNPV). LdNPV typically occurs at low levels until gypsy moth populations increase; then, LdNPV becomes the dominant factor leading to gypsy moth population collapse (Elkinton and Liebhold 1990). LdNPV tends to remain

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prominent in post-collapse populations, leading Woods et al. (1991) to conclude that transovum transmission is density-independent after an epizootic, whereas larva-to-larva transmission remains density-dependent. An intriguing new (to North America) pathogen of the gypsy moth is the fungus *Entomophaga maimaiga* Humber, Shimazu, and Soper, which was first recorded in North America in 1989 (Andreadis and Weseloh 1990, Hajek et al. 1990b) and was introduced into northwestern and western central Virginia in 1991, where it is now widely established (Hajek et al. 1996). Under appropriate conditions in the spring, overwintered resting spores (azygospores) germinate to produce a second type of spore (germ conidia) that can cause infections at any time from egg hatch until about 2 wks before pupation (Hajek et al. 1993, Hajek and Humber 1998). The fungus penetrates the larval cuticle and produces protoplasts and hyphal bodies that grow vegetatively within the larva, killing it. Under appropriately moist weather conditions, hyphae grow through the outer wall of the insect, producing enormous numbers of conidia that are forcibly discharged and presumably disseminated by the wind, spreading the disease to surrounding gypsy moth populations. Infections arising from azygospores only produce conidia, never azygospores (Hajek et al. 1997a). Those hosts subsequently infected by these conidia can produce all conidia, all resting spores, or a mixture of conidia and resting spores, depending in part on host age (Hajek and Shimazu 1996, Hajek 1997b). Resting spores must overwinter to be infective (Hajek and Humber 1998).

In 1993 and 1994, we monitored gypsy moth populations for pathogen occurrence in selected woodlots near Lexington, VA, on the "leading edge" of the expanding North American gypsy moth infestation, and found LdNPV at significant levels only in woodlots with high gypsy moth populations, but recovered *E. maimaiga* from both high- or low-population woodlots. In 1995 and 1996, we conducted studies in 10 of the woodlots to quantify the impact the two diseases were having on gypsy moth populations as a function of population density.

From the literature we understood that *E. maimaiga* should operate at lower gypsy moth population densities than LdNPV (Hajek 1997b); the fungus should be considerably less density dependent than the virus (Hajek 1997b), and, due to different modes of action, any impact of the fungus on the virus should be indirect and due solely to fungal effects on gypsy moth population density (Malakar et al. 1994). The specific objectives of our 1995 study were (1) to document the comparative impacts of *E. maimaiga* and LdNPV; (2) to monitor the higher density plots to determine timing of disease onset; and (3) to quantify the impact of *E. maimaiga* in a "leading edge" gypsy moth population. Our objective in 1996 was to quantify the course of the disease epizootics for an additional year. Based on the observed buildup of resting spores at the end of 1995, we hypothesized that fungal levels should remain high in 1996, while LdNPV should be somewhat suppressed because of reductions in gypsy moth populations, but should still be detectable throughout the season because heavy fungal attack was not expected until stadia 5 and 6 (as per Weseloh and Andreadis 1992b, Weseloh et al. 1993). Finally, burlap larval-monitoring bands were monitored once in 1997, just after the onset of pupation, to assess additional pathogen impact.

Methods and Materials

Site selection and characterization. Ten gypsy moth-infested woodlots near Lexington, VA, were chosen to reflect a range of gypsy moth population densities. A

one-ha evaluation plot was established at an appropriate location (accessible and composed predominately of oak, *Quercus* spp). Egg mass numbers were estimated prior to eclosion in 1995, 1996, and 1997 from five 0.01-ha fixed-radius egg mass surveys per plot as described in Liebhold et al. (1994). Burlap larval-monitoring bands (Liebhold et al. 1986) (burlap bands, 0.3 m in width, wrapped entirely around the tree, secured with staples, folded once and slit 2 to 4 times for ease of observation) (50 in 1995, 150 in 1996 and 1997) were placed at a height of 1.5 m on dominant host trees (oak, primarily white oak, *Q. alba* L. and chestnut oak, *Q. prinus* L.) within the 1-ha evaluation area of each woodlot. Bands showing wear were replaced in 1996 and 1997. In 5 "high-population" plots (mean of 2280 egg masses per ha, SE = 1146), 25 bands were used for population monitoring, while the remaining bands were used for weekly sampling of later instar larvae as per Hajek (1997b). In 5 "low-population" plots (mean of 267 egg masses per ha, SE = 149), all bands were used for population monitoring. In the high-density plots in 1995 and 1996, we collected two separate data sets: (1) weekly collections of live larvae, and (2) weekly counts of all larvae, living and dead, under burlap bands, with cadavers discarded after the count. In the low-density plots in 1995 and 1996, we made weekly counts of larvae, living and dead, under burlap bands, with cadavers collected for necropsy.

Weekly live larval collections. These collections were similar to ones made by Woods and Elkinton (1987) and Webb et al. (1994), with total mortality and mortality caused by LdNPV and *E. maimaiga,* assessed by the death-rate analysis method (Van Driesche et al. 1991). Live larvae (100 per plot in 1995, 50 per plot in 1996) were sampled weekly from the 5 high-density plots. Because most early instars remain in the lower canopy or in the understory during the day (Ticehurst and Yendol 1989), and later instars become quite mobile (Liebhold et al. 1986), collections were made from lower foliage early in the season, and from foliage, tree trunks, and the burlap bands established for this purpose for later-instar larvae. All collected live larvae were reared (one per cup) on an artificial diet (Bell et al. 1981) in 30-ml creamer cups with paper lids. These cups were held under natural conditions in a field insectary at Beltsville, MD. After each collection, the larvae from the previous collection were assessed for mortality, and mortality was recorded. Larvae collected on 15 June 1995 or on 20 June 1996 (at which times pupation exceeded 50% in the field) were periodically assessed for mortality until all individuals had died or pupated. We considered pupation to represent survivorship for the purpose of computing % cumulative mortality. Dead larvae were subjected to necropsy by light microscopy, with the presence of occlusion bodies considered positive for LdNPV and characteristic conidia and/or azygospores (Hajek and Shimazu 1996) considered positive for *E. maimaiga.* Care was taken to record the type of spore present in fungal-killed cadavers and the instar of all cadavers (by measuring head capsules). Based on the number of larvae that died during the week after each collection, season-long cumulative larval mortality (all sources) was calculated as per Wieber et al. (1995) by formula 1:

$$T_{Mort} = 100 - [100 - (100 \cdot M_1) - (S_2 \cdot M_2) - \ldots - (S_n \cdot M_n)] \quad (1)$$

Where T_{mort} = % total season-long mortality, M_x = Proportion of mortality recorded for time period x, S_x = % survivorship at start of time period x, and n = number of time periods. The relative prevalence of LdNPV and *E. maimaiga* in the plots was calculated as the percent of the total number of cadavers that was positive for the respective pathogens. Cadavers with mixed infections were scored positive for both pathogens.

Weekly burlap larval-monitoring band counts. For the 5 higher-population plots, the weekly counts of dead larvae found under the burlap larval-monitoring bands were divided into mortality source categories based on the necropsy results for the preceding week's larval collection. For the 5 lower-population plots in 1995, mortality source was estimated from the necropsy results from a sample of cadavers collected directly from the burlap bands; however, numbers in 1996 were so low that an assessment of mortality sources was not attempted for these plots. Burlap larval-monitoring bands were monitored once a week beginning in late May for the low-density plots. Relative population density was expressed as per Webb et al. (1989) as the peak number of immatures (live or dead larvae or pupae) found under the bands of a given plot at any one count, divided by the number of bands used for the survey. Total season-long larval mortality was estimated from the burlap counts as follows. For each count, all live life stages were recorded and left under the bands, while dead individuals were counted and removed. Total mortality was estimated by formula 2:

$$T_{mort} = (DL/L_{Max}) \cdot 100 \quad (2)$$

That is, season-long % larval mortality (T_{mort}) equals the total number of dead larvae (DL) divided by the peak number of life stages (L_{Max}) (maximum number of live or dead larvae or pupae found under the bands of a given plot at any one count) times 100. The relative prevalence of LdNPV and *E. maimaiga* in the plots was calculated as the percent of the total number of cadavers that was positive for the respective pathogens. Cadavers with mixed infections were scored positive for both pathogens.

Statistical analysis. Data were analyzed as appropriate by paired *t*-tests, unpaired *t*-tests, or correlation analysis using SAS Version 6.10 for Windows (SAS Institute 1994). Data were left untransformed when, using the SAS PROC UNIVARIATE test for normality, the probability of normality was > 0.10; otherwise, an appropriate transformation was chosen to stabilize the variance and to normalize residuals (Carrol and Ruppert 1988). Accordingly, data comparing egg mass counts in 1995 for high-density versus low-density plots, were converted to $\log_{10}(x)$. Data for the following comparisons were converted to $\log_{10}(x + 1)$: preseason egg mass counts in 1996 for high versus low plots, preseason egg mass counts for 1997 for high versus low plots, preseason egg mass counts for high-density plots for 1995 compared to 1996, and preseason egg mass counts for high-density plots in 1996 compared with 1997.

Results and Discussion

Higher population plots, 1995. Pertinent parameters determined for the 5 higher population plots are given in Table 1. Preseason gypsy moth populations averaged 2280 egg masses per ha (SE = 1146, range = 320-6540). The peak number of gypsy moth larvae found beneath burlap bands averaged 70.0 larvae per band (SE = 15.6, range = 30.5-119.9), and was not significantly correlated with preseason egg mass density (n = 5; r = 0.18 $P = 0.77$). This was an expected result because it has been shown that egg mass counts do not correlate with burlap counts at high densities (Wallner et al. 1989), probably due to differential early-larval survival. Estimated season-long larval mortality (all sources) was high across all plots as computed both from weekly larval collections using formula 1, averaging 98.8% (SE = 0.5, range = 97.3-99.9%), and when using burlap band data and formula 2, averaging 95.0% (SE = 1.9, range = 87.9-98.3%); it should be noted that formula 1 covers the entire season while formula 2 covers only the period when larvae seek refugia such as burlap

Table 1. Gypsy moth population density and mortality (Avg ± SE) from LdNPV and *E. maimaiga* based on necropsies of cadavers from serial collections or from under burlap bands in five higher-population and five low-population woodlots near Lexington, VA, 1995, 1996

	1995				1996			
	High-plots		Low-plots		High-plots		Low-plots	
Parameter	Avg	(SE)	Avg	(SE)	Avg	(SE)	Avg	(SE)
Preseason egg mass/ha:	2280	(1146)	267	(149)	364	(191)	16	(7.5)
Peak no. larvae/burlap:	70.0	(15.6)	8.3	(3.5)	0.69	(0.42)	0.08	(7.5)
% cumulative larval mortality, collections:	98.8	(0.5)			99.3	(0.3)		
% total mortality of larvae under burlap larval traps:	95.0	(1.9)	90.3	(2.0)	17.7	(8.0)	8.2	(3.8)
Est. larval mortality due to LdNPV, collections:	14.1	(5.5)			5.0	(4.0)		
Est. larval mortality due to LdNPV, burlaps:			1.2	(1.0)			*	
Est. larval mortality from *E. maimaiga,* collections:	68.4	(2.2)			58.4	(12.2)		
Est. larval mortality due to *E. maimaiga,* burlaps:			84.6	(4.9)			*	
Post-season egg mass/ha:	364	(191)	16	(7.5)	40	(22.3)	26	(20.1)

* The few larvae that died where not necropsied.

larval-monitoring bands (instars 4-6). The percentage of larvae found positive for LdNPV averaged 14.1% (SE = 5.5, range = 6.4-35.7%); NPV-induced mortality was significantly correlated with higher preseason egg mass populations (n = 5; r = 0.88; P = 0.046). The positive association of LdNPV-induced mortality and population density agrees with published findings (Woods and Elkinton 1987) and with a "simple" model for virus epizootics in gypsy moth populations (Dwyer and Elkinton 1993). The percentage of larvae found positive for *E. maimaiga* averaged 68.4% (SE = 2.2, range = 58.1-72.8%), and was significantly negatively correlated with gypsy moth population density (n = 5; r = −0.98; P = 0.004). These percentages are close to levels of *E. maimaiga* reported from similar studies conducted in New York State in 1991 and 1992 (Hajek 1997b). We found mixed infections in 4.5% of the cadavers scoring positive for the two pathogens, which agrees closely with the 4.4% rate of mixed infection reported by Hajek and Roberts (1992).

The negative correlation between population density and *E. maimaiga*-induced mortality may simply reflect the greater number of larvae succumbing to virus as gypsy populations increase, leaving a smaller percentage of the population available for the fungus. Another possible explanation is that a lesser percentage of the larval population encounter germinating resting spores at the base of trees as population densities increase. A higher percentage of late-instar gypsy moth larvae migrate from the tree during the day at lower population densities than at higher population densities (Lance et al. 1987) and, thus, would be more likely to encounter germinating azygospores at the base of trees. Azygospores germinate during most of the period of time the gypsy moth larvae are in the field (Hajek and Roberts 1991). The model of Hajek et al. (1993) has the resting spores exerting a constant infection rate season-long; however, more recent work indicates that new infections from azygospores may cease about 2 wks before the onset of pupation (Hajek and Humber 1998).

As seen in Fig. 1, total mortality occurring during week "x" in larval collections were significantly correlated (n = 5; r = 0.89; P = 0.041) with total mortality found under burlaps during burlap counts made at the beginning of week "x + 1", suggesting that the two sampling methods were equally appropriate under the conditions pertaining during 1995 in the high-population plots. Post-season 1995 (preseason 1996) egg mass densities were significantly lower (t = 10.22; df = 8; P = 0.0005) than preseason 1995 egg mass densities, averaging 364 (SE = 191, range = 20-840) egg masses per ha. The data shown in Fig. 2 indicate that the bulk of the population decline can be attributed to *E. maimaiga,* although LdNPV was clearly important in the collapse of population in the plot with the highest population. A few parasitoids were recorded from collections, but in numbers indicating little impact on overall population reductions.

Low-population plots, 1995. Pertinent parameters determined for the 5 low-population plots are given in Table 1. Preseason gypsy moth populations averaged 267 egg masses per ha (SE = 149, range = 83-480), which was significantly less than that in the high-population plots (t = 2.88, df = 8; P = 0.02). The peak number of gypsy moth larvae found beneath burlap larval-monitoring bands averaged 8.3 larvae per band (SE = 3.5, range = 1.5-20.9), and was positively but not significantly correlated with preseason (n = 5; r = 0.82; P = 0.089) or post-season (n = 5; r = 0.79; P = 0.11) egg mass numbers. Estimated season-long larval mortality (all sources) as computed from weekly counts, made from the burlap larval-monitoring bands, using formula 2 averaged 90.3% (SE = 2.0, range = 82.9-94.3%). The percentage of larvae scoring positive for LdNPV averaged 1.2% (SE = 1.0, range = 0.0-5.3%); while not compared

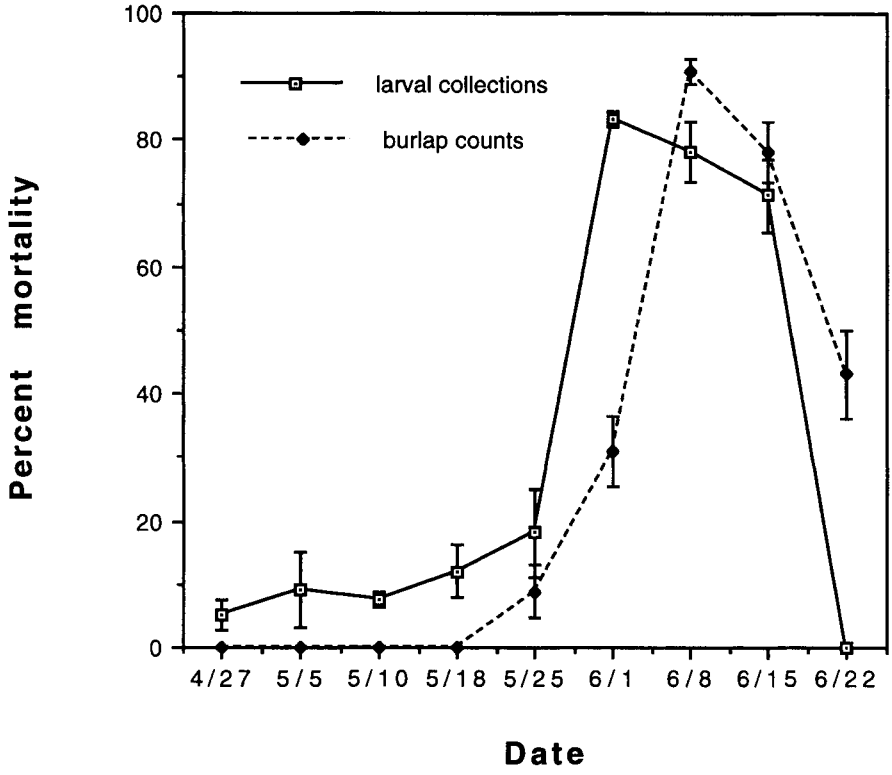

Fig. 1. A comparison of total percent gypsy moth larval mortality on the indicated dates evaluated by two different techniques in gypsy moth populations in 5 "higher-population" woodlots near Lexington, VA, in 1995. One technique measured the mortality occurring to live larvae collected weekly and held for 7 days on artificial diet. The second technique computed the percentage dead for counts of gypsy moth larvae resting under burlap bands during weekly counts. Data for each of 8 weekly samples or counts.

statistically with per cent virus computed for the high-population plots because of the different method of computation (burlap mortality vs collection mortality), LdNPV levels were substantially less in the low-population plots than in the higher-population plots, as expected (Woods and Elkinton 1987). The percentage of larvae scoring positive for *E. maimaiga* averaged 84.6% (SE = 4.9, range = 69.5-95.2%), which was greater than that seen in the higher density plots. LdNPV-induced mortality was positively correlated with preseason egg mass density, but not significantly so ($n = 5$; $r = 0.80$; $P = 0.10$). This lack of significance may be due to low sample size, reflecting both the low overall NPV levels in the plots and the smaller range of egg mass densities in the low-population compared with the higher-population plots. *Entomophaga maimaiga*-induced mortality was again negatively correlated with preseason egg mass density, but not significantly so ($n = 5$; $r = -0.67$; $P = 0.21$). This lack

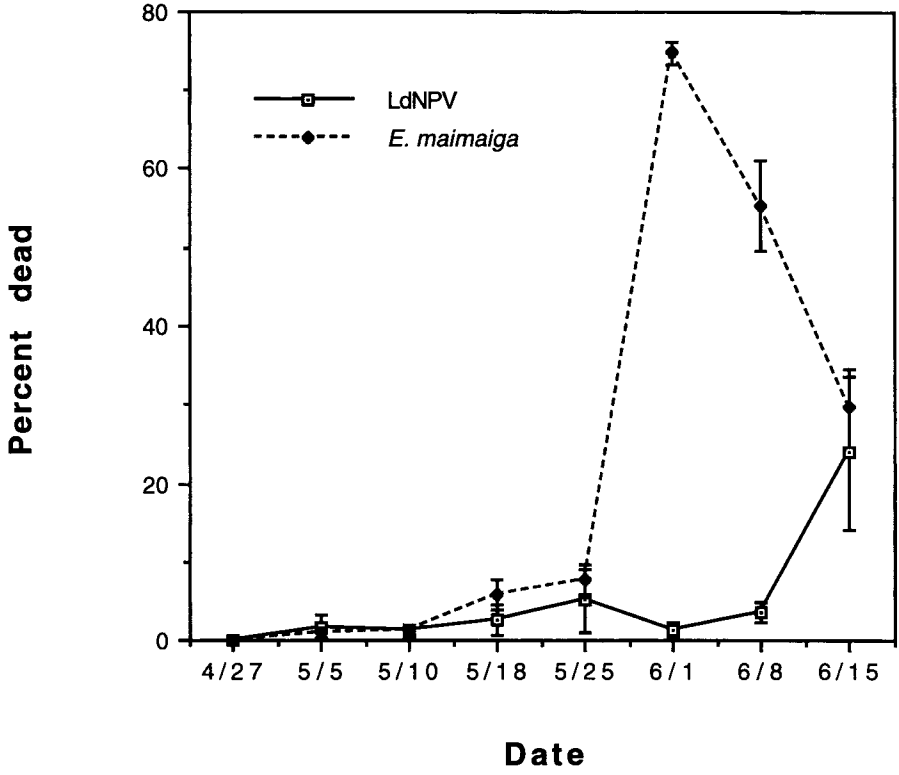

Fig. 2. Occurrence of the fungus *E. maimaiga* and LdNPV in gypsy moth populations in 5 "higher-population" woodlots near Lexington, Virginia, in 1995. Data for each of 8 weekly samples expressed as percent of each sample dying within one week of collection and subsequently found positive by necropsy for the indicated pathogen.

of significance may result from the smaller range of egg mass densities in the low-density compared with the high-density plots. Post-season 1995 (preseason 1996) egg mass densities were significantly lower (t = 3.55, df = 8; P = 0.024) than pre-season 1995 egg mass densities, averaging 16 (SE = 7.5, range = 0-40) egg masses per ha. The data indicate that virtually all the population decline can be attributed to *E. maimaiga,* with little contribution by LdNPV or parasitoids.

Fungus levels and weather, 1995. *Entomophaga maimaiga* expression is known to be affected by weather events, and is favored by high humidity and high levels of rainfall (Elkinton et al. 1991, Hajek et al. 1990a, 1993, 1996, Weseloh and Andreadis 1992a, 1992b, Weseloh et al. 1993). Given adequate moisture, conidial germination and sporulation occurs between 2 to 25°C, with maximum rates seen between 20 to 25°C (Hajek et al. 1990a). In 1995, April temperatures were normal (13°C, departure from normal = 0°C), but rainfall totalled only 3.6 cm for the month, a departure of −3.7 cm from normal. May temperatures were near normal (16.9°C, departure from normal

= 0.8°C), and rainfall was somewhat above normal for the month (10.4 cm). June was characterized by near normal temperatures (20.7°C, departure from normal = 1.1°C) and heavy rainfall totalling 43 cm, 35 cm above normal. *Entomophaga maimaiga* levels were low until the June 1 collection (Fig. 2), when, probably in response to a period of warm, wet weather that occurred from 26 May to 29 May (25.4 cm total rainfall), fungus-induced mortality soared. The resulting mortality curve for the high-density plots (Fig. 2) is similar to that reported by Weseloh and Andreadis (1992b).

High-population plots, 1996. Pertinent parameters determined for the 5 high-population plots are given in Table 1. The peak numbers of gypsy moth larvae found beneath burlap bands in 1996 averaged 0.69 larvae per band (SE = 0.42, range = 0.04-2.28). This average was significantly less than the average peak numbers found in 1995 (t = 4.50; df = 8; P = 0.005), and was not significantly correlated with preseason 1996 egg mass density (n = 5; r = 0.82; P = 0.09), perhaps due to the low numbers for these parameters compared with those in 1995. Estimated season-long larval mortality (all sources) was high across all plots as computed by weekly larval collections using formula 1, averaging 99.3% (SE = 0.5, range = 98.4-100%). The percentage of larvae found positive for LdNPV averaged 5% (SE = 4.0, range = 0.0-21.1%); LdNPV-induced mortality in 1996 was significantly correlated with LdNPV-induced mortality recorded in 1995 (n = 5; r = 0.98; P = 0.0024), due mostly to residual virus (21% in 1996) in the plot with the highest level of LdNPV (35%) in 1995, despite the fact that post-season egg mass counts revealed the population had collapsed during 1995 leaving few new egg masses for the 1996 season. However, all LdNPV mortality occurred early in the season (Fig. 3), and the usual second peak did not occur. The percentage of larvae found positive for *E. maimaiga* averaged 58.4% (SE = 12.2, range = 21.1-84.7%). Contrary to results obtained in 1995, fungus-induced mortality was positively but not significantly correlated with gypsy moth population density (n = 5; r = 0.64; P = 0.25); fungus levels were relatively low in two higher-population plots (c3, t3), while remaining high in the other 3 plots (Fig. 4). *Entomophaga maimaiga*-induced mortality in 1996 was not significantly correlated with 1995 *E. maimaiga* mortality or with preseason or post-season egg mass density, indicating a density-independence that agrees with previous reports (Elkinton et al. 1991, Hajek et al. 1996).

Low-population plots, 1996. Egg mass populations began the season low, averaging 16 (SE = 7.5, range = 0-40) per ha, and ended the season low, averaging 26 (SE = 20.1, range = 0-104) per ha. Again, preseason 1996 egg mass levels were significantly lower in the low-population plots than in the higher-population plots (t = 2.77, df = 8; P = 0.03). The peak number of gypsy moth larvae found beneath burlap bands averaged 0.08 larvae per band (SE = 0.008, range = 0.06-0.10). Only three cadavers (cause of death unknown) were found season-long. Most of the larvae under the bands survived to the end of the season and pupated. Post-season egg mass counts were not significantly different (t = 0.61; df = 8; P = 0.56) between higher-population (average = 40, SE = 22.3, range = 0-122) and low-population plots (average = 25.6, SE = 20.1, range = 0-104), indicating that populations had declined to low levels in all plots.

Fungus levels and weather, 1996. In 1996, April precipitation was near normal at Lexington (6.5 cm, a departure of 0.6 cm from normal). This amount of rainfall was almost twice that seen in 1995. The higher rainfall in April 1996 may partly explain the earlier appearance of the fungus in 1996 compared with 1995, since findings by Hajek et al. (1993) suggested that early rainfall initiates epizootics of *E. maimaiga* by al-

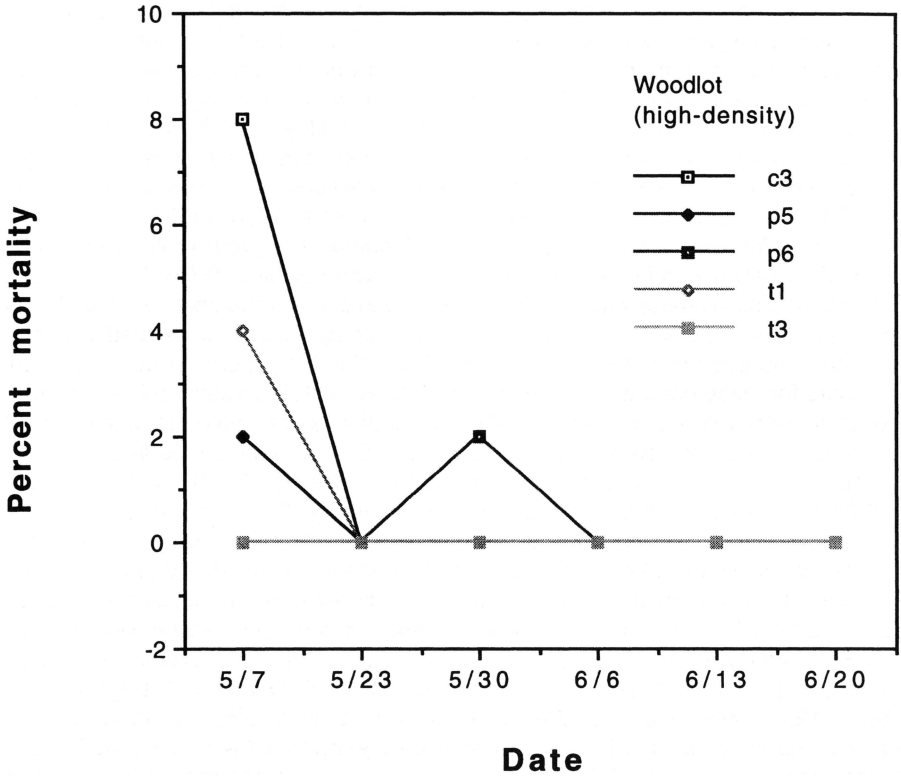

Fig. 3. LdNPV-induced mortality in gypsy moth populations in 5 "higher-population" woodlots near Lexington, Virginia, in 1996. Data for each of 6 weekly samples expressed as percent of each sample dying within one week of collection and subsequently found positive by necropsy for LdNPV.

lowing the azygospores to germinate and infect. Moreover, *E. maimaiga* infection levels are known to increase when soil containing azygospores are given supplementary water (Hajek et al. 1996). The large input of azygospores documented in 1995 would also have aided the 1996 epizootic. A total of 12.5 cm of rainfall was recorded for Lexington in May 1996, 3.3 cm above normal, which would encourage onset of fungal epizootics. However, the first large incidence of fungal-induced mortality was recorded from the 30 May collection (Fig. 4), apparently in response to a period of warm, wet weather from 24 to 28 May. Little rain fell during the period from 29 May until 6 June, perhaps explaining the decrease in fungal infection observed in the 6 June collection (Fig. 4). Then, a warm, wet, period from 8 to 11 June is associated with a second peak of *E. maimaiga* infection recorded from a 13 June collection. Smitley et al. (1995) has reported a correlation between *E. maimaiga* infection and rainfall and humidity in Michigan, but Hajek et al. (1996) failed to find such an association. The massive kill of larvae prior to the point in their life cycle that they regularly visit burlap resulted in very low burlap counts for these plots in 1996. Mor-

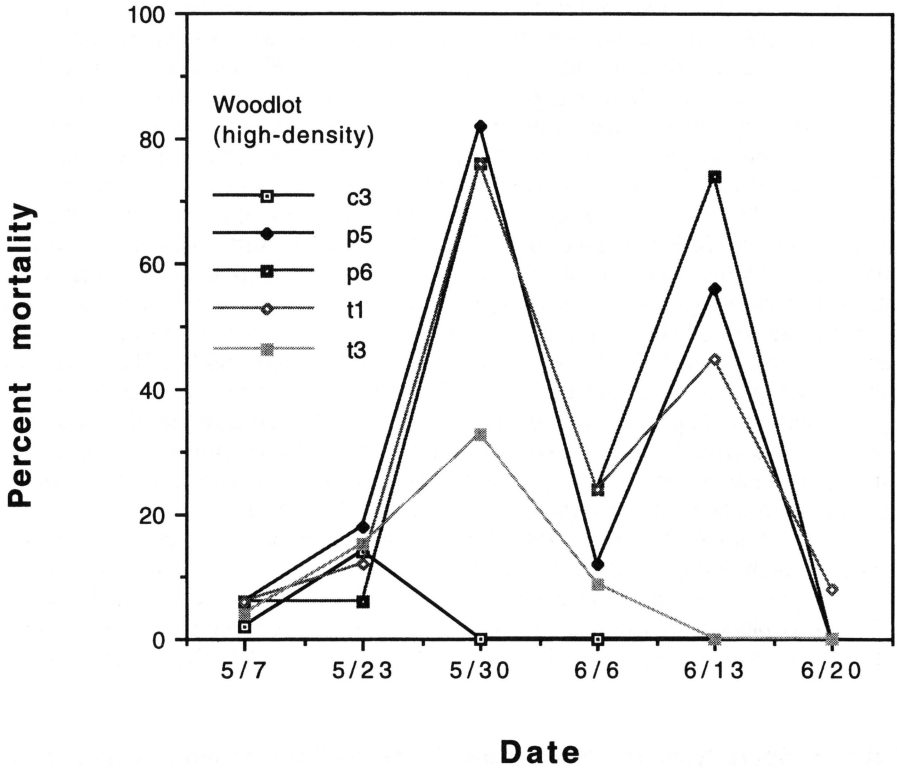

Fig. 4. Mortality induced by the fungus *E. maimaiga* in gypsy moth populations in 5 "higher-population" woodlots near Lexington, Virginia, in 1996. Data for each of 6 weekly samples expressed as percent of each sample dying within one week of collection and subsequently found positive by necropsy for *E. maimaiga*.

tality curves (Fig. 4) for 1996 were bimodal and considerably different from that seen in 1995 (Fig. 2) for the same plots.

Fungals spore types, 1995 vs 1996. In 1995, fungal-infected cadavers from the weekly larval collections in the higher-population plots contained only conidia (Table 2) until the 1 June collection. After that date, a large percentage of the cadavers contained azygospores as well as conidia, indicating that the infections primarily resulted from wind-dispersed conidia (Hajek and Humber 1998). Because most of the fungus-induced mortality occurred after 1 June, it is likely that a large inoculum of azygospores was produced for the next field season. A total of 867 cadavers were found infected with *E. maimaiga* in collections from the higher-population plots in 1995; 38.4% contained only conidia, 21.3% contained only azygospores, while 40.3% contained both spore types. In contrast, in 1996, only conidia were found in cadavers from the weekly larval collections in the higher-population plots prior to the 13 June collection, indicating that infections were primarily due to germinating azygospores

(Hajek and Humber 1998); by this time, most larvae had died in a mid-season general population collapse, leaving few larvae to produce azygospores for the following season. A total of 272 cadavers was found infected with *E. maimaiga* in the collections made from the high-density plots in 1996; 83.5% contained conidia only, 8.4% contained azygospores only, while 8.1% contained both spore types. A second mechanism that might partially explain the disproportionate number of cadaver containing conidia only concerns the earlier timing of the gypsy moth population in 1996 compared with 1995. The gypsy moth population was about a week behind in its development in 1996 compared to 1995, and the fungus struck earlier, probably in response to higher April rainfall. Infections arising from conidia will yield early-instar cadavers containing conidia only; as larval instar number increases, infections arising from conidia will yield increasing proportions of cadavers containing azygospores (Shimazu and Soper 1986, Hajek and Shimazu 1996, Hajek 1997a). Therefore, the reduced azygospore production recorded in 1996 may be due in part to the earlier appearance of the epizootic in 1996. However, the higher percentage of larvae containing only conidia in 1996 versus 1995 was not due to the presence of younger larvae, because the percentage of larvae in older instars was similar for the 2 years in both the mid-season and late-season collections (Table 2).

High- and low-population plots, 1997. The peak number of gypsy moth larvae found beneath burlap bands averaged 0.02 per band (SE = 0.011, range = 0-0.06), for the high-density plots, and 0.018 per band (SE = 0.007, range = 0-0.04) for the low-density plots. Residual populations now appeared to be marginally more abundant in the "low density" plots than in the "high density" plots. No cadavers were

Table 2. Spore types (percentage and Standard Error) found in gypsy moth larval cadavers infected by the fungus *Entomophaga maimaiga* early, mid, and late in the season, Lexington, VA, 1995 and 1996

Time Period	Year	% Conidia	% Azygospore*	SE	% Cadavers by larval stadia: Early I-II	Mid III-IV	Late V-VI
Early-season							
(May 6-May 26)	1995	100	0	—	25	75	—
	1996	100	0	—	33	42	25
Mid-season							
(May 27-June 9)	1995	60.7	39.3	5.8	—	18	82
	1996	100	0	—	—	23	77
Late-season							
(June 10-June 28)	1995	11.2	88.8	2.6	—	2	98
	1996	45.9	54.1	10.5	—	—	100

* Includes cadavers with mixed spore types (conidia + azygospores) as well as cadavers with azygospores only.

found (all larvae and pupae appeared healthy) during the one burlap count (at 40% pupation). Although a low residual population remained in 1997, there was no sign of a population rebound. On the other hand, there was no sign of pathogen activity, indicating that populations might have been too low to sustain an epizootic. While few *E. maimaiga* azygospores were produced in 1996, and few if any in 1997 in these plots, azygospores are known to persist for several years (Hajek et al. 1993, Weseloh and Andreadis 1997), and the heavy input of azygospores in these plots in 1995 may provide inoculum to initiate an epizootic should gypsy moth populations rebound in these plots.

Previous studies have recorded levels of *E. maimaiga* similar to those we recorded in 1995 (Elkinton et al. 1991, Hajek et al. 1990b, Weseloh and Andreadis 1992b); however, this is only the second study to follow the fate of the fungus in the same study blocks in subsequent years. Hajek (1997b) documented the activity of *E. maimaiga* in plots in central New York State from 1991-96, with the plots having outbreak gypsy moth populations in 1991 and 1992, and low populations thereafter. Although similarities were found between the two studies (for instance, both studies documented a pathogen-induced gypsy moth population collapse over a 2-year period, with *E. maimaiga* playing a key role in both studies), there were also substantial differences. In the New York study, a combination of LdNPV and *E. maimaiga* caused the collapse of the outbreak populations by the end of 1992. LdNPV was the more important pathogen in 1991, with dry conditions occurring during the period of gypsy moth activity. However, *E. maimaiga* was the more important pathogen in 1992, with normal rainfall recorded for that period. In contrast, our Virginia study documented a collapse over 2 years with *E. maimaiga* the more important pathogen in both years (1995, with normal rainfall, and 1996, with greater than normal rainfall), and LdNPV playing only a minor role. The New York study was conducted in an established gypsy moth population, while the Virginia study was conducted in a newly-established leading edge population. In both cases, *E. maimaiga* was new to the area. A third difference was that LdNPV remained important during the second year of the New York study, while virtually disappearing during the second year of the Virginia study. However, in New York, almost no LdNPV was found in the plots in the four following years; therefore, the two studies are probably in basic agreement in that levels of virus receded to below detectable levels (in year 3 of the Virginia study and in years 4-6 in the New York study) following a year or so of high *E. maimaiga* impact. A fourth difference was that *E. maimaiga* remained detectable in the New York populations in all four years following the gypsy moth population collapse, while *E. maimaiga* was not detected in any plots during 1997, the third year of the Virginia study.

Hajek et al. (1990b) reported that, in 1989, *E. maimaiga* caused 60 to 88% mortality in 4 research plots in Massachusetts. Despite these high levels of mortality, there was relatively little change in egg mass density in the Massachusetts study. In the present study, egg mass densities remained high in some plots at the end of 1995; however, in the subsequent year, we document the further impacts of the continuing epizootic of *E. maimaiga* that left low egg mass levels in all plots at the end of 1996. In 1995, the LdNPV epizootic developed in a typically bimodal fashion despite the large concurrent *E. maimaiga* epizootic. LdNPV has an early advantage by infecting larvae as they emerge from contaminated egg masses (Murray and Elkinton 1989, 1990). In contrast, *E. maimaiga* infections are not known to originate from contaminated egg masses (Yerger and Rossiter 1996), although such contamination undoubtedly occurs (as per Aoki et al. 1976). In 1996, LdNPV only occurred early in the

season, and the usual second peak did not materialize, probably because late-season gypsy moth population was too low to sustain the epizootic. LdNPV has been shown to persist in high concentrations in soil, litter, and on bark for at least one year after an epizootic (Podgwaite et al. 1979), and this probably explains the early-season residual effects seen. Since LdNPV-infected early-instar larvae produce relatively few occlusion bodies (Shapiro et al. 1986), it is likely that little new inoculum was introduced into the plots in 1996. The greatly reduced presence of LdNPV in the higher-population plots in 1996 as the season progressed may partially explain findings by Yerger and Rossiter (1996) that NPV was curiously absent from neonates emerging from gypsy moth egg masses taken from populations with histories of *E. maimaiga* infections.

In 1995, levels of the fungus were high in all plots regardless of gypsy moth density, while virus levels were clearly higher in the higher-population plots than the low-population plots. In 1996, the virus was not a factor in the continued decline of the gypsy moth population in all plots, while fungal impact remained strong. At the beginning of 1995, the highly susceptible, never-before-defoliated woodlots used in this study extended from the defoliating front (high density plots) into the "leading edge", where the (low density) plots were still several years away from serious defoliation. The populations in all woodlots fell dramatically without causing the high levels of defoliation that had been expected in the higher-population plots on the defoliating front, nor did the low-population plots just within the leading edge undergo the normal progression from "release" to "progradation" to "culmination" as expected, but instead, gypsy moth populations went from low to non-detectable. Put another way, the moving front of defoliation that should have passed through all plots simply disappeared. The gypsy moth has been advancing southward and westward at about 20 km a year since 1966 (Liebhold et al. 1992). The finding that *E. maimaiga* can indeed cause premature population collapse of gypsy moth populations in never-before-defoliated woodlots on the defoliating front and just within the leading edge may have profound implications for the future rate-of-spread in North America of this serious forest defoliator by drastically reducing population pressure at and beyond the defoliating front.

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