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HOST DISPERSAL AND THE SPATIAL SPREAD OF INSECT PATHOGENS[1]

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Abstract. Empirical studies of the spatial spread of insect pathogens have emphasized the importance of high dispersal rates, but typically rely strictly on observational data. In contrast, existing mathematical models on the spatial spread of infectious diseases have suggested that a highly infectious disease can spread rapidly even if the dispersal rate of its host is low; such models, however, are largely untested. To understand how host dispersal and disease infectiousness affect the spatial spread of infectious diseases, we performed a field experiment in which we released a nuclear polyhedrosis virus (NPV) into two experimentally established, disease-free gypsy moth (*Lymantria dispar*) populations. We simultaneously and independently measured the long-distance dispersal of ballooning, first-instar, gypsy moth larvae, the stage in which dispersal is by far the greatest. Surprisingly, dispersal by ballooning was a good predictor of NPV spread only in the first few weeks of spread. Subsequently, the virus spread much farther and less directionally than did ballooning larvae. No infections appeared in control plots that started with only uninfected larvae, confirming that the virus that we released caused the epizootic in our experimental populations. On the theory that the additional spread was due to a combination of small-scale larval dispersal and a high rate of disease transmission, we compared our data to the predictions of a mathematical model for the spatial spread of insect pathogens that combines disease transmission and small-scale host dispersal. By using independent estimates of each of the model parameters, we used the model to make post hoc predictions of the rate of spread of the virus from an initial distribution dictated by larval ballooning. Although the model included both initial long-distance dispersal by larval ballooning and subsequent short-distance dispersal by larval crawling, it poorly matched the observed distribution of the virus. An alternative and as yet untested hypothesis is that the observed spread of the virus may be due to mechanical vectoring by a parasitoid fly, a mechanism for which there are as yet no available mathematical theory or relevant field data.

Key words: biological control; disease modelling; dispersal; field experiment; gypsy moth; host–pathogen; nuclear polyhedrosis virus; spatial spread; transmission.

INTRODUCTION

The importance of spatial structure in animal host–pathogen systems is not widely recognized, in spite of its clear importance in many other kinds of interspecific interactions (Paine and Levin 1981, Kareiva and Odell 1987, Kareiva 1990, Morris 1993), including plant–pathogen systems (van den Bosch et al. 1988*a, b, c*). This inattention is probably due at least in part to the predominance of theory over empiricism in animal pathogen ecology (Dobson and Hudson 1986); in particular, there have been few quantitative field experiments involving animal host–pathogen systems (but see Myers 1990, Dwyer 1991, Grosholz 1992). We performed two field experiments designed to understand the spatial spread of an insect pathogen, the nuclear polyhedrosis virus (NPV) of gypsy moth, *Lymantria dispar*. Our expectation was that host dispersal will be a key to understanding the spatial dynamics of this and other animal pathogens, because pathogens are rarely motile.

Existing published experiments on the spatial spread of insect pathogens usually come from the release of pathogens as biological control agents (Young 1974, Entwistle et al. 1983, Otvos et al. 1987*a, b*). Because of the emphasis on biological control, efforts to explain the rate of spatial spread of such pathogens are usually limited to lists of possible dispersal agents of the pathogen, including the host, abiotic factors, and vectors. To approach the problem more quantitatively, we simultaneously measured the spatial spread of the gypsy moth virus and the dispersal of gypsy moth larvae. This design allowed us to directly compare the pattern of virus spread to the pattern of larval dispersal. Because ballooning in first instars is the major method of dispersal in gypsy moths (females in North America are flightless), our initial hypothesis was that the pattern of spread of the virus would match the pattern of first-instar ballooning.

Our alternative hypothesis was that initial ballooning would be a poor predictor of the ultimate pattern of spread of the virus. Instead, we suspected that ballooning might only determine the initial distribution of the virus, and that the virus would subsequently spread far beyond this initial distribution, due to successive

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rounds of transmission accompanied by larval crawling. This alternative is an application of mathematical theories of disease spread, which have suggested that horizontal disease transmission can play an important role in determining the rate of spatial spread of a pathogen (Murray et al. 1986, Mollison 1991, van den Bosch et al. 1988*b*, Dwyer 1992, Dwyer 1994). Specifically, in most models the rate of spatial spread of a pathogen increases rapidly as the pathogen increases in infectiousness. Because NPVs are very infectious diseases of hosts that are often not very mobile (Evans and Entwistle 1987), our alternative hypothesis was thus that measurements of host dispersal alone would not be sufficient to understand the spread of the NPV; that is, we would have to make use of measurements of the transmissibility of the disease as well. To do this, we used a mathematical model that incorporates both host dispersal and disease transmission, and for which we had previously estimated the transmission rate of gypsy moth NPV (Dwyer and Elkinton 1993).

Natural history of gypsy moth and its NPV

Gypsy moth was introduced into the northeastern United States in 1868, and has become one of the most significant pests of hardwood forests in North America. Like many forest-defoliating insects, gypsy moths undergo sporadic outbreaks, in which densities can increase by as much as 4 or 5 orders of magnitude (Campbell 1981). Although the forces leading to outbreaks remain obscure, declining populations are usually associated with NPV epizootics, suggesting that the NPV causes populations to collapse (Elkinton and Liebhold 1990).

NPVs are distinguished by a polyhedral inclusion body that contains the double-stranded viral DNA (Evans and Entwistle 1987). The inclusion body enables the virus to survive outside of its host for periods as long as four decades (Thompson et al. 1981). The virus is transmitted when host larvae consume virus particles, or inclusion bodies, on contaminated foliage. Within 1 to 3 wk, infected larvae die, releasing virus particles into the environment shortly after death, completing the cycle of transmission (Woods and Elkinton 1987). Because adults and pupae do not feed, they cannot become infected by contacting the virus. Similarly, if a larva that is exposed to the virus survives, the virus does not appear to replicate in, or kill, the resulting pupa or adult (Murray et al. 1991).

For gypsy moth as well as for other Lepidoptera, NPV epizootics are initiated when larvae hatch from eggs laid on virus-contaminated surfaces (Murray and Elkinton 1989, 1990). Like other Lymantriidae, first-instar gypsy moth larvae can disperse long distances by ballooning (Mason and McManus 1981). During ballooning, larvae hang down from the egg mass or from foliage on silken strands and are carried long distances by the wind. Because adult females in North America are flightless, first-instar ballooning is the most significant contributor to gypsy moth dispersal. Because epizootics are initiated by hatching larvae, and because hatching larvae balloon long distances, one would expect that dispersal would be very important in the spatial dynamics of the virus. Our initial hypothesis was thus that the spread of disease through an uninfected gypsy moth population from a local source of NPV would be very similar to the pattern of first-instar dispersal; that is, both would be determined by the prevailing winds. We set out to test this hypothesis first by quantifying the dispersal of first-instar larvae, and second by quantifying virus spread through a population of uninfected larvae.

Methods

Experimental protocols

Part I. Larval dispersal experiment.—We began a dispersal experiment on 9 May 1991, the same day that we began the virus-spread experiment. For the dispersal experiment, we stapled 41 packets of surface-disinfected egg masses to a central overstory red oak, *Quercus rubra,* yielding about 2.25×10^5 larvae (see *Part II. Virus release experiment*). We used a plot near the virus-spread plots to ensure that background densities were close to zero (see *Part II,* below), and so that wind direction would be similar to the spread plots, but far enough away (500 m or more) to reduce the chances that there would be any cross-contamination from the other plots. Tree species composition and density of trees in this area were essentially the same as in the release plots (see *Part II,* below). We sampled larvae for this experiment by suspending sticky traps covered with Tanglefoot (Forestry Suppliers, Inc., Jackson, Mississippi, USA) from branches of overstory red oak trees at roughly the height of the mid-canopy (≈15 m). Trap locations relative to the central tree are given in Fig. 1. The traps were cylinders (0.9 m in height × 0.3 m in diameter) so that the wind would hit them in the same way from all directions, and were made of hardware cloth so that the wind could pass through easily. The central trap was raised and lowered usually every other day to monitor ballooning. When the number of newly collected larvae on the central trap had dropped to nearly zero (total of six larvae, Fig. 2), we lowered all the traps and counted all of the larvae on them.

During this experiment, we measured wind speed and direction at a weather station ≈200 m from the center of the dispersal experiment, and a few hundred m from the virus-spread plots. The weather station was located at canopy height (≈10 m above the ground). Wind direction was measured every 60 s by a Met-One O24A wind vane (Campbell Scientific), and recorded on a Campbell Scientific (Logan, Utah, USA) CR21 weather station data-logger.

Part II. Virus release experiment.—To quantify virus spread in an area like Massachusetts, where the virus

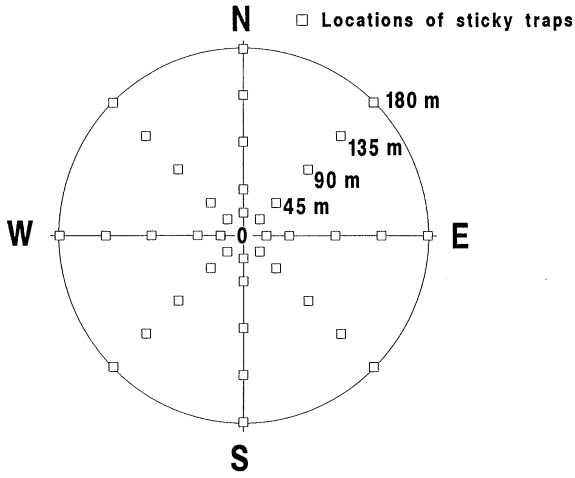

FIG. 1. Setup of the larval dispersal experiment (in Cadwell Memorial Forest, Massachusetts, USA). 2.25×10^5 uninfected gypsy moth larvae were released in the form of disinfected eggs on an overstory red oak at the point marked "0." Sticky traps were hung in the canopy from limbs of overstory red oak trees at each square plus one at the central release tree. The distance of each ring of traps from the center is indicated, except that the inner ring was located at ≈22.5 m from the center.

is already established in the host population, the first requirement was to establish a disease-free host population. In previous experiments, our laboratory had demonstrated that it is possible to establish a disease-free population within a summer by releasing disinfected eggs into low-density areas (Gould et al. 1990). Although the virus can survive in the soil for long periods, apparently acquisition of such virus by larvae feeding in the canopy is a rare event. We therefore felt safe in using an area that had a very low density of gypsy moths, as determined by egg mass counts, and that had not had an outbreak for several years, so that the density of indigenous larvae would be negligible. We located our plots in Cadwell Memorial Forest near the University of Massachusetts campus in Amherst, Massachusetts, USA. Naturally occurring gypsy moths had not attained outbreak densities in Cadwell since 1981, and densities in 1991 were extremely low. Standardized egg mass counts in March of 1991 (Woods and Elkinton 1987), well before hatch, turned up no egg masses after an exhaustive search of five 10-m^2 areas distributed uniformly over the center hectare of each plot. An additional 40 such searches conducted for another project that took place in the same area turned up only one egg mass. Densities were thus extremely low throughout the entire forest.

Given that we can establish disease-free populations, the second requirement was to establish these populations at a high-enough density to permit disease spread; in other words, a density roughly equivalent to outbreak gypsy moth densities. Between February and mid-April of 1991 we collected ≈5 kg of egg masses from two sites in Massachusetts that had high gypsy moth densities, 10–30 km from Cadwell forest. We calculated the number of larvae per gram of eggs by hatching 25 samples of ≈0.5 g each (466.7 ± 22.43 larvae per 0.5 g [mean ±1 SE]). The eggs were then placed into rectangular packets made of doubled-over 2.25-mm mesh fiberglass screening, with each packet containing ≈5.34 g/packet, corresponding to ≈5000 larvae/packet. Lab tests have shown that the size of this mesh is large enough to easily allow hatching larvae to get out, but small enough to retain the eggs (Gould et al. 1990).

To eliminate any preexisting virus on the eggs, we surface-sterilized the egg masses in 10% formalin for ≈1 h, and then washed them in distilled water for ≈3 h to wash off the formalin (Bell et al. 1981). This formalin wash may not prevent trans-ovarial transmission, in which the virus is somehow transmitted within the egg (Fuxa and Richter 1991, 1992). Attempts to demonstrate this kind of disease transmission in gypsy moth, however, have been unsuccessful (Murray et al. 1991); moreover, numerous tests in our laboratory have shown that a formalin wash is sufficient to prevent infection in large samples of hatching larvae. To introduce the virus into the population, we infected one third of the egg packets by immersing them in an extremely concentrated virus solution. We tested this dosing procedure by rearing larvae from samples of 10 eggs from each of 10 virus-dosed egg packets. These larvae all apparently died from the virus before reaching the second instar, although subsequent tests at this dosage level have suggested that some of these larvae may have died of non-disease factors.

We established two experimental plots containing both infected and disinfected eggs, and two control plots containing only disinfected eggs. Each plot was at least 450 m from the edges of the other plots, to minimize any chances that larvae would be able to balloon between plots. The results of our ballooning

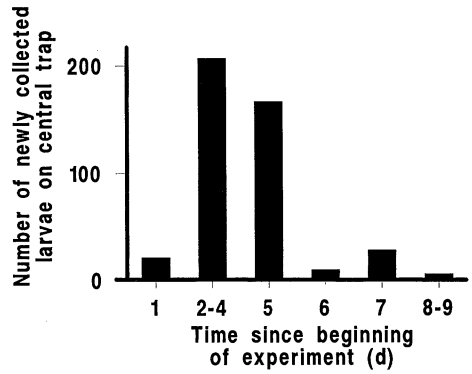

FIG. 2. The timing of larval dispersal in the dispersal experiment. The central trap in the larval dispersal experiment was raised and lowered every few days, at which time any larvae on the trap were counted and removed. Time zero is 9 May 1991.

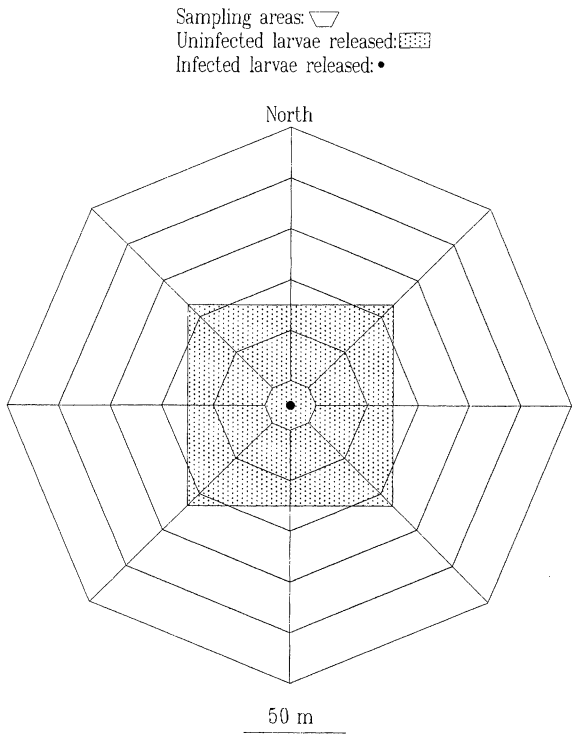

FIG. 3. Setup of the two experimental virus-spread plots. The infected gypsy moth larvae were released on a single tree at the center of each plot; the uninfected larvae were released uniformly over the stippled 1-ha square. During each week of the virus-spread experiment, two people sampled in each trapezoidal area for 20 min or until they found 25 larvae. The total sampling area was a little less than 6 ha. Each control plot was equivalent in area to the stippled square alone.

experiment demonstrate that very few larvae balloon even this distance (see *Results: Larval dispersal*). The predominant overstory trees in the plots are the oaks *Quercus rubra* and *Q. alba*. The plots also contained substantial quantities of red maple, *Acer rubrum*, with lesser amounts of black birch (*Betula lenta*), white birch (*Betula papyrifera*), and white pine (*Pinus alba*).

We deployed the egg packets on 10 May 1991, ≈1 wk after hatch began in natural populations in the Amherst area. To create the uninfected populations in the experimental plots, we used 180 disinfected packets per plot, corresponding to a density of $\approx 9 \times 10^5$ larvae/hectare. These uninfected packets were distributed as uniformly as possible over the central hectare of each plot (Fig. 3); specifically, packets were stapled to the overstory tree nearest to each point on a 10 × 10 grid, with 10 m between grid points. Because there were 100 release trees and 180 packets in each plot, there were 20 trees that received only one packet; these trees were scattered more or less uniformly throughout the plot. 100 virus-dosed packets for each plot were stapled to the overstory red oak that was closest to the center of each plot to introduce the virus at the center of each uninfected population. This procedure resulted in an initial infection rate of about 30% of the population of hatching larvae. We chose this high level of infection because our intuition before the experiment was that, even with this high an initial input of virus, the virus would not spread far from the release point (an intuition that later turned out to be incorrect).

Hatch began immediately, and lasted ≈1 wk. We began sampling larvae on 24 May, *after* the last day that we collected ballooning larvae in the dispersal experiment. The 1st wk of collections thus represents the 3rd wk of the experiment. We collected larvae over an area of ≈6 ha around the central tree of each plot, in sampling areas that were arranged as in Fig. 3. In each trapezoidal sampling area, two people collected for either 20 min or until they had collected 25 larvae, whichever came first. Only live larvae were collected. Due to the enormous area sampled, elaborate sampling schemes were not logistically feasible; larvae were therefore collected by sight, by workers on the ground. As much as possible, we attempted to distribute sampling efforts uniformly within each sampling area. To avoid extraneous infections in the field, we collected all of the larvae by gently knocking them into a cup, using the cup's inner lid surface to touch the larvae, thus avoiding any contact of larvae by the hands of collectors. To avoid contamination in the laboratory, we reared all of the larvae individually in 60-mL cups containing artificial diet (Bell et al. 1981). We reared larvae for 1 wk in an insectary at ambient temperatures in the field, and for an additional 1 wk at ≈26°C in the laboratory. Dead larvae were autopsied for the presence of the virus using light microscopes at 400× (Woods and Elkinton 1987).

To assess the possibility that indigenous, or "wild," virus would appear in our experimental populations, we established two control plots that had only uninfected larvae. Each control plot was also 1 ha in size (100 × 100 m). The control plots were intentionally located between plots with released virus (some of which were from another study), in order to make sure that infected larvae could not balloon between plots. In addition, one of the two control plots was located on one of the highest ridge lines in the surrounding forest, thus maximizing the likelihood that it would be exposed to larvae ballooning in from other plots, or from long distances away. Because of the logistics of collecting large quantities of eggs, we used only 100 packets in each of the two control plots. This corresponds to a density of $\approx 5 \times 10^5$ larvae/ha, or about 55% of the density in the experimental plots. Control larvae were treated identically to larvae from the experimental plots; they were collected in the same fashion, and reared using the same type of diet cups, in the same rearing facilities, by the same personnel, for the same length of time.

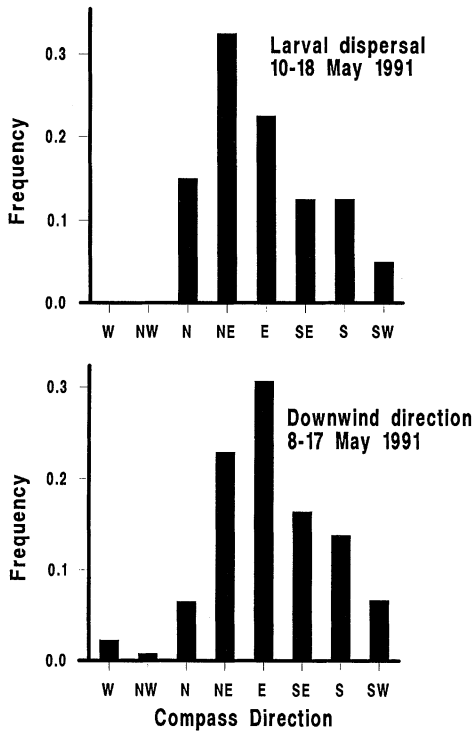

FIG. 4. Results of the larval dispersal experiment. (a) The fraction of larvae that were collected on traps at different directions from the central release tree. Larvae collected at different distances within each direction were pooled. (b) The fraction of samples made by an electronic anemometer of downwind direction that fell into each category of compass direction. These data strongly suggest that larval dispersal direction is dictated by wind direction.

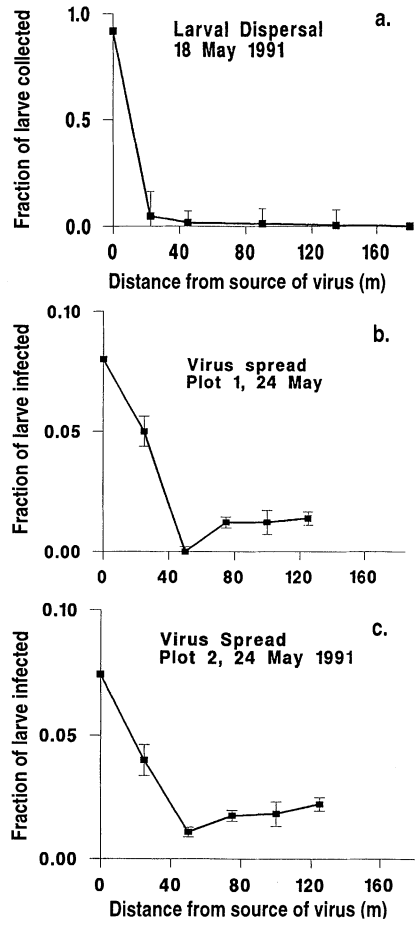

FIG. 5. Comparisons of first-instar gypsy moth larval ballooning distance with the distance that the virus spread in the virus-spread experiment. (a) Larval ballooning distances in the dispersal experiment; other than for the zero distance point, larvae from different directions within each distance were pooled. The error bars represent ± 1 SE around the mean of the number of larvae captured in different directions at each distance. (b), (c) The fraction of captured larvae that were infected, vs. distance from the source of the virus, in the 3rd wk of the virus-spread experiment (the 1st wk of collections), in plots 1 and 2, respectively. For (b) and (c) we first pooled larvae over directions in each plot. The error bars represent ± 1 SE around the mean of samples in different directions. In both cases, the respective distributions drop off rapidly with distance, suggesting that initial virus spread is dictated by larval ballooning.

RESULTS

Larval dispersal

The results of the larval dispersal experiment are summarized in Figs. 4 and 5. Fig. 4a shows larval dispersal summarized by pooling distances, while Fig. 4b shows downwind direction. We used Batschelet's circular correlation coefficient to assess the extent to which larval dispersal and wind direction were associated with a particular direction (Batschelet 1981). Both larval dispersal ($r^2 = 0.786$, $\chi^2_2 = 31.44$, $P < 0.001$) and the wind ($r^2 = 0.862$, $\chi^2_2 = 5223.8$, $P < 0.001$) were strongly directional. Moreover, a linear regression of the number of larvae dispersing in each direction, on the number of wind samples in each direction, was highly significant ($F = 15.14$, df = 1, 6, $P = 0.0081$). In other words, during the 11 d after the release there was a consistent west wind, and larval dispersal was directional as well, apparently in response to the wind.

Fig. 5a shows larval dispersal summarized by pooling directions. As in previous dispersal experiments (Mason and McManus 1981), the distribution of collected larvae dropped off strongly with distance.

Virus spread

No infections appeared in either of the control plots, suggesting that the infections that occurred in the spread plots were due to the virus that we released.

The results of the virus-spread experiment are shown in Figs. 6 and 7, and are summarized in Figs. 5b and c, 8, and 9. In Figs. 6 and 7 the left column of plots represents the fraction of larvae collected in each sector that proved to be infected, and the right column of plots gives the total number of larvae collected in each sec-

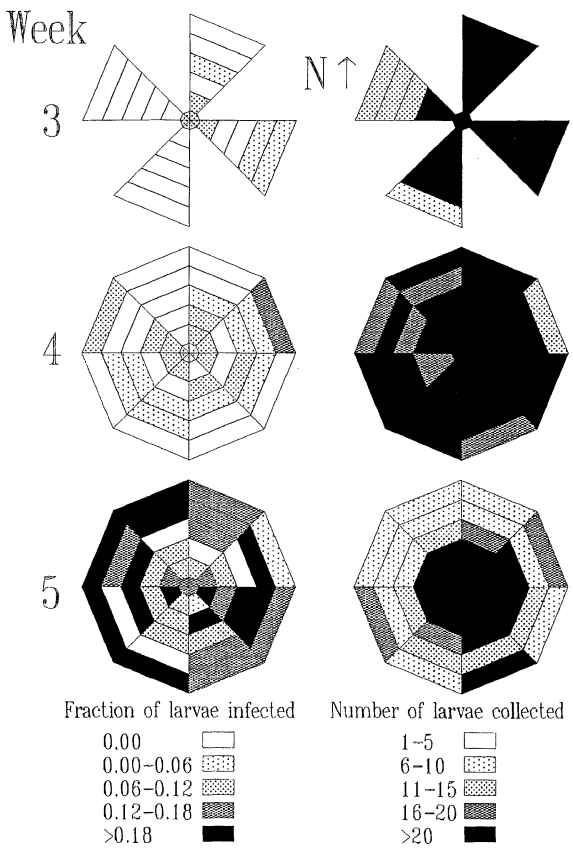

FIG. 6. Results of the virus-spread experiment in experimental plot 1. The three octagonal figures in the left column represent the spatial distribution of infected larvae in the plot at each week of collection. The figures in the right column represent the number of larvae collected in each sector in each week. In the 1st wk of collections, which was the 3rd wk of the experiment, we only had enough personnel available to sample half of the plot; otherwise, the figures are intended to represent the octagonal area shown in Fig. 3. Notice that although there is a suggestion of a directional effect in plot 1 in the 3rd wk, the effect rapidly dissipates.

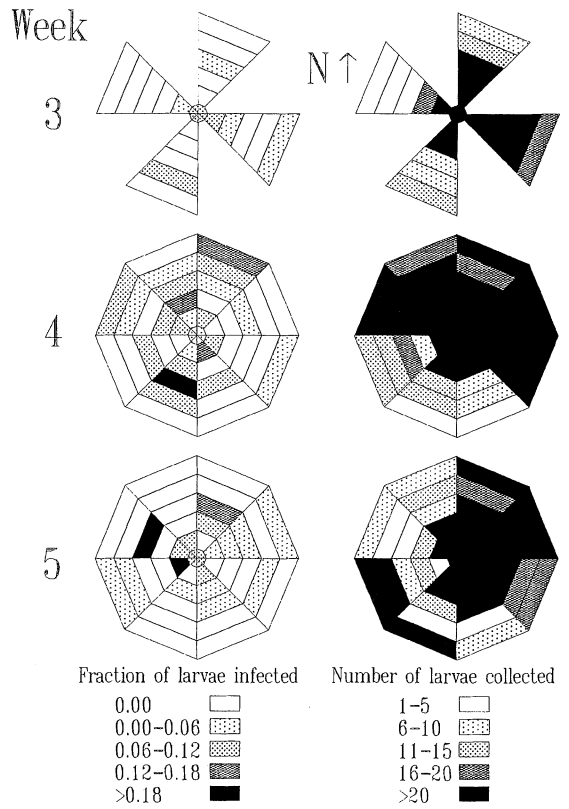

FIG. 7. Results of the virus-spread experiment in experimental plot 2. Details as in Fig. 6. Notice that any effect of direction is even less evident than in Fig. 6.

tor. Visual inspection of Fig. 6 suggests that there was an effect of direction in the 3rd wk of the experiment (the 1st wk of our collections) in plot 1, in that all of the infected larvae were found in the eastern half of the plot, but this effect disappeared by the 4th wk. Fig. 7 shows that there also may have been some effect of direction in plot 2 in the 3rd wk, but any such effect similarly disappeared by the 4th wk. Variation in sample sizes presented in the plot summaries on the right does not materially affect this conclusion; that is, there is no obvious underlying pattern of high or low sample sizes that might be driving the lack of pattern in weeks 4 and 5 of the infection data.

To make it easier to see the relationship between fraction infected and distance, the data from week 3 in Figs. 6 and 7 are summarized in Figs. 5b and c, 8, and 9 by pooling larvae from different directions at each distance in each plot. In any statistical treatment of these data, including calculation of standard error bars and linear regression, an individual data point consists of a sample of insects at a particular distance and direction; that is, the fraction infected that were collected from one of the trapezoidal sectors. Figs. 5b and c show that, in the 3rd wk, virus infection dropped off strongly with distance in both virus-spread plots, as did larval dispersal. The total number of infected larvae, however, was too small to permit meaningful statistical analysis. Figs. 8 and 9 show the 4th and 5th wk. Fig. 8 suggests that, in plot 1 in week 4, the percentage infection declined less strongly with distance, although a linear regression of arc-sine square-root transformed fraction infected against distance was significant ($F = 4.15$, df $= 1, 39$, $P = 0.048$). By week 5, however, percentage infection was as high or higher at the edges of the plot than it was closer to the source of the virus, and there was no effect of distance upon infection rate ($F = 1.25$, df $= 1, 39$, $P = 0.27$). In plot 2, the percentage infection similarly did not decline significantly with distance in week 4 ($F = 1.69$, df $= 1, 39$, $P = 0.20$), although by week 5 there was again a significant distance gradient ($F = 5.74$, df $= 1, 39$, $P = 0.022$).

Finally, in Fig. 10 we have plotted the distribution of larvae in the two spread plots with respect to instar. This figure demonstrates that by the time we began

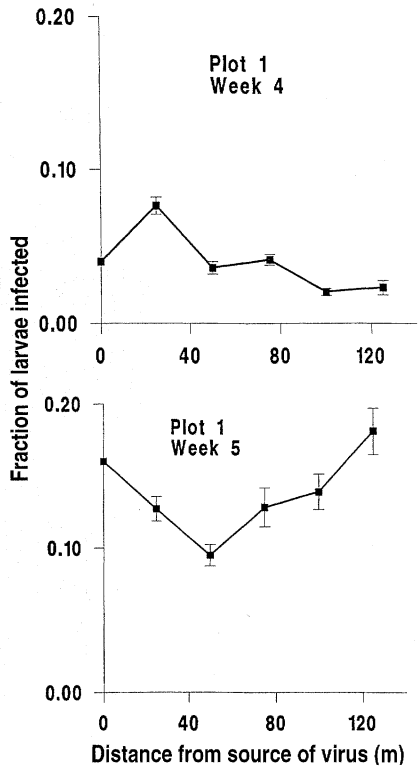

FIG. 8. Results of the last 2 wk of the virus-spread experiment in plot 1, summarized with respect to direction. The upper graph shows week 4, and the lower graph shows week 5. As in Fig. 5b, we first pooled larvae over directions. Note that, in comparison with Fig. 5b, the distribution of the fraction of infected larvae flattens out over time, suggesting that initial larval ballooning had little effect on long-term virus spread in plot 1. The error bars are as in Fig. 5b.

collecting larvae in the virus-spread experiment, essentially the entire population (≈95%) had passed beyond the first instar. Since larvae only balloon in the early days of the first instar, these data demonstrate that ballooning had ceased by the time we began sampling in the virus-spread plots.

DISCUSSION

Ballooning and virus spread

Comparison of the larval dispersal data with the wind data (Fig. 4) suggests that the direction of initial larval dispersal is determined by the direction of the wind, a comparison facilitated by the strong directionality of the winds during larval dispersal in our experiment. Understanding how wind determines the distance that larvae disperse is more difficult (Mason and McManus 1981). In general, although the effects of weather on ballooning are undoubtedly substantial, beyond the rather obvious effects of wind direction on ballooning direction, weather effects are beyond the scope of this paper. Although density may have affected ballooning as well, such effects are again beyond our province, since our interest is largely in the effects of ballooning rather than in the determinants of ballooning (see Hunter and Lechowicz 1992). We note in passing, however, that the density of larvae on the release trees in the ballooning plot was within about a factor of 2 of the density of larvae on the release trees in the spread plots, suggesting that the effects of density on ballooning may have been similar in the two different types of plot.

We instead focus on the extent to which ballooning affected virus spread. The dispersal data suggest that the initial density of virus declined with distance in the virus-spread plots, and the initial decline in percentage infection with distance in Fig. 5b and c supports this interpretation. Fig. 8, however, shows that any such effect was transitory in plot 1, since the spatial distribution of the fraction infected flattened out by the end of the experiment. Fig. 9 shows that the spatial distribution in plot 2 was even less predictable, first flattening out and then returning to a decline with distance. Figs. 6 and 7 similarly show that any directionality to virus infection rapidly disappeared. The distance and direction that the virus spread in our experimental plots were thus, for the most part, not predictable from our measurements of larval ballooning. Although in the later weeks of the experiment the sample sizes were low in some sectors, there is no obvious pattern in the

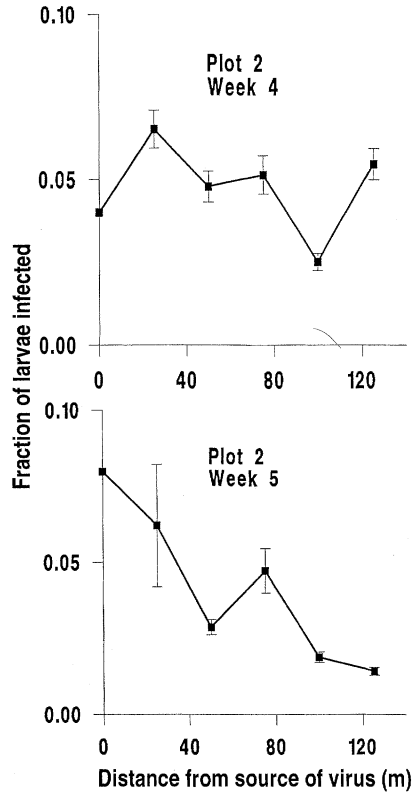

FIG. 9. Results of the last 2 wk of the virus spread experiment in plot 2, summarized with respect to direction. Details as in Fig. 8. Here, the distribution of the fraction infected perhaps begins to flatten out in week 4, but then returns to a decline with distance in week 5.

sample sizes that might be causing this lack of predictability. Moreover, sample size is less of an issue for the distance data, since samples at the same distance but different directions were pooled. It is possible that some of the infections far from the source of the virus that we released in distance or direction were due to "wild" virus. The lack of infections in the control plots, however, suggests that this possibility is indeed remote.

It is possible that some of the differences between the ballooning data and the spread data may have been due to small differences between the ballooning and spread plots either in terms of tree species composition or tree density, or in terms of extremely localized weather effects. The spread data, however, are so grossly different from the ballooning data that it is likely that such small differences played only a small part. Similarly, by collecting from the ground it is possible that we have introduced biases into the virus-spread data. Previous work in our laboratory, however, has shown that although NPV (nuclear polyhedrosis virus)-infected late-instar gypsy moth larvae tend to be found somewhat higher in trees than do uninfected larvae (Murray and Elkinton 1992), the difference in height is typically <1 m. Any such bias is therefore likely to be extremely slight. Moreover, our interest lies in the spatial distribution of infections; our assumption is thus that, if there is some small bias in our data towards lower infection rates, this bias at least did not increase with distance from the source of the virus.

The virus-spread data thus leave us with the question of how the virus was able to spread beyond what we would expect from larval ballooning. The instar data in Fig. 10 demonstrate that essentially the entire population in the spread plots was past the first instar when we began collecting larvae from the spread plots. In fact, NPVs, including gypsy moth NPV, are well known for their ability to prevent larval molting by glycosylating the molting hormone ecdysteroid (O'Reilly and Miller 1988, Park et al. 1993). Because there were so few first instars at the time that we began collecting in the spread plots, and since the NPV would prevent initially infected larvae from making it to the second instar, it thus appears likely that the initially infected larvae in those plots were all dead. Because only first instars balloon, and since even first instars that have eaten are too heavy to balloon (M. L. McManus, *personal communication*), we therefore suspect that additional mechanisms of dispersal were operating to drive the spatial spread of the virus.

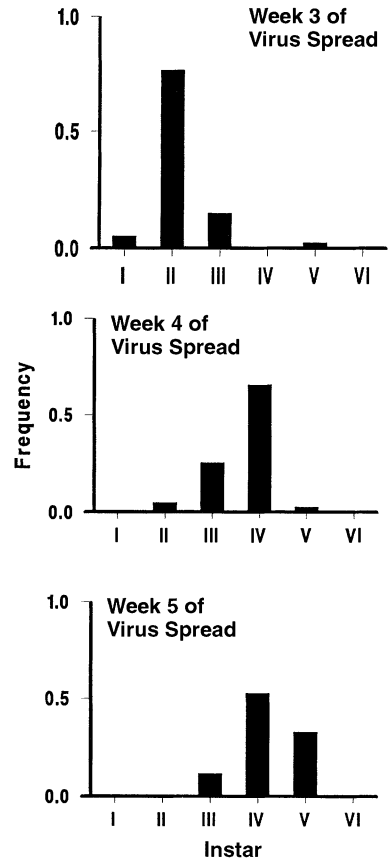

FIG. 10. The stage distribution of larvae in the virus-spread plots, pooled over the two plots. These data show that by the 3rd wk of virus spread, essentially all of the larvae in the plots were beyond the first instar.

Ballooning and crawling: a mathematical theory of virus spread

One possible candidate for an additional dispersal mechanism is larvae crawling between trees after ballooning ended. Previous work in our laboratory has shown that this kind of movement does occur, although the net distance moved is usually small ($\approx$5 m/d, Liebhold et al. 1986). Unlike ballooning, however, crawling occurs simultaneously with secondary transmission, which could dramatically increase its effect upon the spatial spread of the virus. That is, as overall levels of infection near the source of the virus rise, an increasing fraction of larvae that are dispersing outward will be infected, so that the effect of dispersal will be greater even though the distance dispersed is short. Ideally, one would hope to test this kind of mechanism experimentally. A cheaper and more immediate method of exploring this particular mechanism is with a mathematical model of disease spread that incorporates simultaneous dispersal and transmission (Murray et al. 1986, Holmes et al. 1994). Work with these models has shown that they can be adapted to make predictions of short-term spatial spread of insect NPVs (Dwyer 1992). In an earlier paper (Dwyer and Elkinton 1993) we showed that a mathematical model of temporal disease dynamics provides a good description of the dynamics of NPV in natural gypsy moth populations, for which the distribution of the NPV is often roughly spatially uniform so that dispersal is largely not an issue in virus dynamics. Here we use what is virtually the same mod-

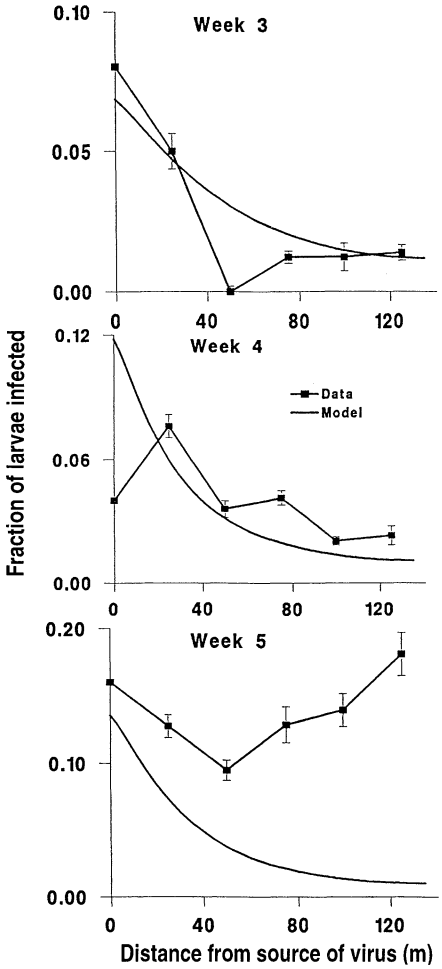

FIG. 11. Comparison of the fit of the model equations A.1–A.3 (see *Appendix*) to the plot 1 data from the virus-spread experiment, using the parameter estimates in Table 1. The model begins with a spatial distribution of virus that was adjusted so that the model output matched the data for week 3. The model represents the hypothesis that the virus spread after ballooning was a result of virus transmission plus inter-tree dispersal. Given that each of the model's parameters was estimated independently of the virus-spread data, the poor fit of the model prediction to the data in week 5 suggests that this hypothesis is incorrect.

el, with the additions of larval dispersal and a non-uniform initial distribution of the virus. In this section, we give only a brief description of the model equations and the parameter estimates, with the details relegated to the Appendix.

The model is from Dwyer (1992), and was derived from Anderson and May (1981), whose work in turn grew out of the long tradition of mathematical modelling in human epidemiology (Bailey 1975). The basis of this modelling approach is an accounting of the changes in the populations of healthy and infected hosts, including for insect pathogens the population of infectious pathogen particles outside of the host. The critical assumption in such models is that horizontal transmission is directly proportional to the product of the densities of healthy insects and infectious pathogen particles; as we showed in our earlier work (Dwyer and Elkinton 1993), this assumption works reasonably well as a description of the dynamics of gypsy moth NPV within a season. The spatial model that we use here differs from that earlier work in adding host dispersal, in the form of larval crawling, to the dynamics of host and pathogen, so that the resulting model keeps track of host and pathogen dynamics in both space and time (Dwyer 1992). In particular, the model allows us to describe the spatial spread of NPV within a season, and thus to examine the influence of larval crawling upon NPV spread. To mimic the initial distribution of the virus at the end of ballooning, we fit the model to the virus-spread data from the 3rd wk of the experiment, and then compared its subsequent predictions to the data from the 4th and 5th wk of virus spread.

The model parameter estimates are from our earlier work (Dwyer and Elkinton 1993), with the exception of the parameter for larval dispersal between trees, which came from published mark–recapture data from our laboratory (Liebhold et al. 1986). The model thus allows us to quantitatively investigate the hypothesis that the spread of the virus in the later weeks of the experiment was due to a combination of secondary transmission and small-scale larval movement. Figs. 11 and 12 show the fit of the model to the data from the spread plots. It is important to remember that the distribution of virus was fit to the data in week 3, so that the interesting comparison between model and data is for weeks 4 and 5. This comparison suggests that the cases of extreme spread cannot be explained by simultaneous crawling and secondary transmission after ballooning. That is, the extremely high percentage of infection in the last week in plot 1 in the far reaches of the plot may have been due to some additional, as yet unknown, mechanism of dispersal. The high levels of infection at long distances in plot 2, week 4 are a little closer to the model than are the data for plot 1 week 5, but also suggest that there was some additional mode of dispersal. An additional puzzle is why the infection rate at long distances in plot 2 declined from week 4 to week 5. Otherwise, in plot 1 week 4 and plot 2 week 5, the model is reasonably close to the data, although in both cases it overestimates the infection rate at the zero point.

Alternative explanations for the extent of virus spread

Obviously, in the absence of an experimental test, a mathematical model cannot prove or disprove whether or not a particular mechanism of dispersal is of importance. The model does suggest, however, that crawling alone cannot account for the observed rate of spread. One candidate for an additional mechanism is mechanical vectoring by parasitoid insects; in fact, the parasitoid *Cotesia melanoscelus* has been shown to

vector gypsy moth NPV (Raimo et al. 1977). The most abundant parasitoid among our collected larvae was the tachinid fly *Compsilura concinnata,* which reached levels of 16% parasitism in each plot in the last week of sampling. This parasitoid can disperse long distances quite rapidly, and recent work in our laboratory has demonstrated that it is capable of transmitting the NPV on its ovipositor from infected to healthy insects (R. Parker, *personal communication*). The absence of any quantitative relationship between levels of parasitism and disease transmission, however, makes it difficult to assess this alternative hypothesis.

Another possibility is that what is missing from the model is not an additional mechanism of dispersal, but rather a more accurate description of the effects of density on horizontal transmission. As we explained in our earlier paper, the value of per capita horizontal transmission in the model underestimates per capita transmission in natural populations at low host and pathogen densities. This effect may partially explain the high rates of infection at long distances in our data as compared with the model. In other words, if per capita transmission at low virus densities is actually higher than the model assumes, then since virus densities declined with distance from the source of the virus, the unexpectedly high infection rates at long distances may have been due to higher per capita transmission rates than would be predicted from the model. We are currently pursuing modifications to the original model to test this hypothesis (G. Dwyer and J. S. Elkinton, *personal observations*).

A final possibility is that NPV occlusion bodies themselves were able to disperse on the wind. Although there is no obvious reason to eliminate this hypothesis, we have some circumstantial evidence that this does not happen. Specifically, we have performed a number of small-scale transmission experiments, with both gypsy moth and the closely related Douglas-fir tussock moth, in which groups of uninfected and infected larvae have been maintained on foliage that is adjacent (≈1 m separation) but not touching (Dwyer 1991, Dwyer and Elkinton 1993). The lack of cross-contamination in these experiments suggests that wind dispersal of NPV is very slight.

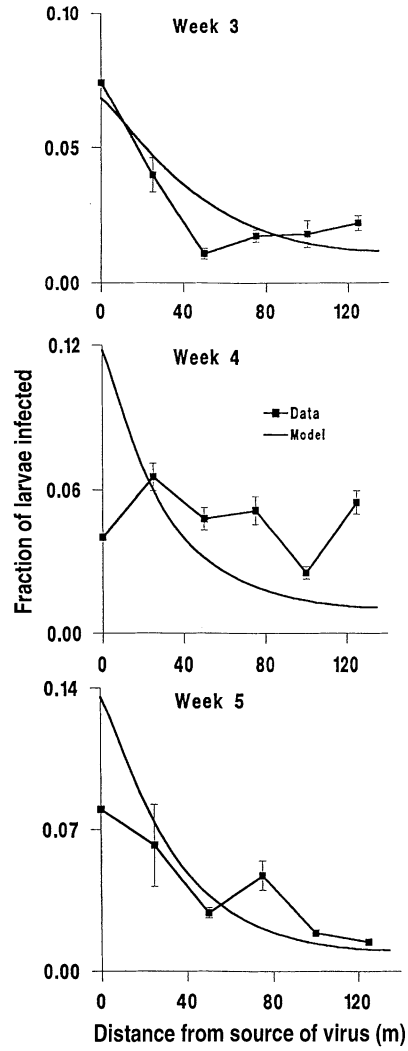

FIG. 12. Comparison of the fit of the model equations A.1–A.3 to the plot 2 data from the virus-spread experiment. Details as in Fig. 11. Here, the model does poorly in week 4, again suggesting an additional dispersal mechanism beyond ballooning and inter-tree crawling.

CONCLUSIONS

Irrespective of what the missing additional dispersal mechanism is, our experiment suggests that larval ballooning alone is not a sufficient explanation for the spatial spread of gypsy moth NPV. The model further suggests that larval crawling following ballooning is not sufficient either. Although our best guess is that parasitoid vectoring is somehow involved, we do not yet really know what additional dispersal mechanism is operating. Instead, what we hope to have accomplished is first to have demonstrated the feasibility and usefulness of creating experimental epizootics in insect populations. Both the lack of infections in the control plots and the initial drop-off in infection in the spread plots strongly suggest that there were few if any wild infections in our experimental plots. Second, the design of the experiment enabled us to critically examine the notion that ballooning drives virus spatial spread, an hypothesis that so far has been without an experimental test.

Finally, the comparison between our data and the model suggests that further development of the theory is needed. It may well be that the mechanisms of dispersal in the model, ballooning followed by larval crawling, accurately represent dispersal in other systems; for gypsy moth NPV, however, the poor fit of the model to the data indicates that some additional mechanism of dispersal is operating as well. A model that includes parasitoid vectoring would, among other

things, give us some intuition as to the extent to which such vectoring would increase the extent of virus spread. An additional difficulty is that our two spread plots differed substantially from each other in their pattern of virus spread, and it is not clear how a conventional deterministic differential-equation model could deal with such variability. Indeed, the similarity of the plots in terms of both setup and stand characteristics prevents any easy explanation for these differences. In any case, we hope to have made clear that a comparison between models and experiments can test the assumptions of both theoreticians and field biologists. In particular, using a theory adapted to the short-term dynamics of the virus has suggested a gap in our understanding of spatial spread, in a way that would not be possible with more conventional models that describe long-term dynamics (Anderson and May 1992, Dwyer 1994). Moreover, the large spatial scale and complex habitat over which gypsy moth NPV spreads make it extremely difficult to experimentally test the importance of either parasitoids or larval crawling in dispersing the NPV. With this kind of system, and perhaps for many others as well, spatial spread models can thus allow for exploration of otherwise untestable hypotheses.

Apart from these larger conceptual issues, our experiment has demonstrated that gypsy moth NPV can travel long distances even in a single season. Although our plots were among the largest used in ecological field experiments (Kareiva and Andersen 1988), they still did not indicate the maximum extent of virus spread. This long-distance spread has interesting consequences for the spatial scale of gypsy moth outbreaks. Previous work in our laboratory has shown that experimental outbreaks on the spatial scale of a hectare are controlled by parasitoids (Gould et al. 1990); perhaps as a result, natural gypsy moth outbreaks tend to be on a much larger scale, on the order of square kilometres (Liebhold et al. 1991), and are typically terminated by virus epizootics. The rapid spatial spread of the virus that we observed suggests that such large-scale outbreaks could indeed be controlled by the virus, at least within a few seasons, even if the virus were originating at a single point. Finally, the long-distance spread of the virus has consequences for the release of genetically engineered organisms, within which NPVs are among the most popular (O'Reilly and Miller 1989, Wood and Granados 1991). An issue of concern for any such release is whether the area that the novel organism invades can be limited (Manasse and Kareiva 1991). The rapid spread that we observed with a wild-type virus indicates that initial releases of engineered viruses should focus on restricting the dispersal of the virus.

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APPENDIX

Modelling the Spatial Spread of the Virus

The model that we use was first presented by Dwyer (1992), as a modification of an earlier model of Anderson and May (1981). The model is:

$$\frac{\partial S}{\partial t} = -\nu PS + D\frac{\partial^2 S}{\partial x^2} \quad \text{(A.1)}$$

$$\frac{\partial I}{\partial t} = \nu PS - \alpha I + D\frac{\partial^2 I}{\partial x^2} \quad \text{(A.2)}$$

$$\frac{\partial P}{\partial t} = \lambda I - \mu P. \quad \text{(A.3)}$$

Here S is susceptible (uninfected) larvae, I is infected larvae, P is the pathogen population outside of the host, ν is the transmission constant, D is the diffusion constant or diffusivity associated with larval crawling, α is the disease-induced mortality rate, λ is the rate of production of the pathogen by infected hosts, μ is the breakdown, or decay rate, of the pathogen, t is time, and x is distance from the central release point. In the field spatial spread obviously occurs in at least two dimensions, but for computational convenience in the model we confine disease spread to one spatial dimension, as a first approximation. Spatial spread in one-dimensional models is typically as rapid as in two-dimensional models (Murray et al. 1986, Mollison 1991), suggesting that the one-dimensional approximation may be sufficient.

As we mentioned in the *Discussion: Ballooning and crawling* . . . section, in an earlier paper we used a very similar model to describe the dynamics of virus in natural populations (Dwyer and Elkinton 1993). The major differences between the earlier model and Eqs. 1–3 are that in the earlier model there is a fixed delay of length τ between infection and death, and that there is no dispersal. For the natural populations to which we applied the earlier model, for all intents and purposes the virus was distributed roughly uniformly throughout the population, so that the earlier model considered only temporal dynamics; that is, there was no consideration of dispersal. Since in this paper we are concerned with spatial dynamics, dispersal is a critical process. Because spatially distributed partial-differential-equation models incorporating time delays are notoriously unstable numerically (G. M. Odell, *personal communication*), here we assume that there is a constant death rate of infected larvae. In fact, applying the same assumption to the earlier model only slightly affects its predictions, and the constant death rate of Eqs. 1–3 if anything allows for more rapid spread (Dwyer 1992). The similarities of the two models allow us to make direct use of Dwyer and Elkinton's (1993) estimates of the parameters that describe the interaction between the virus and the host: the transmission rate ν, the disease-induced death rate α, the pathogen production rate λ, and the pathogen decay rate μ. Here we briefly describe our method of estimating these parameters, because our methods have some bearing on the interpretation of the model results.

As described in Dwyer and Elkinton (1993), we estimated ν from small-scale epizootics. That is, we performed an experiment in which healthy and infected larvae were contained on the foliage of red oak, in mesh bags. All of the larvae were first reared in the laboratory; to mimic early season field conditions, the infected larvae were first instars and the healthy larvae were third instars. The infected larvae were infected several days before the experiment began, so that they died shortly after the experiment began. Nuclear polyhedrosis virus (NPV) occlusion bodies produced by the resulting infected cadavers infected some of the healthy larvae; the fraction of these larvae that were infected is an estimate of horizontal transmission, and so can be translated into an estimate of the transmission parameter ν (Dwyer 1991, 1992).

Table A1. Values of parameters from Eqs. 1–3. The pathogen production rate λ, the disease-induced death rate α, the disease decay rate μ, and the transmission constant ν were all either taken directly from or were modified from Dwyer and Elkinton (1993). The diffusion constant D was estimated from data of Liebhold et al. (1986).

Parameter	Value
λ: Pathogen production rate	1.43×10^8 d^{-1}
α: Disease-induced death rate	0.071 d^{-1}
μ: Disease decay rate	0.003 d^{-1}
ν: Transmission constant	1.45×10^{-12} m^2/d
D: Diffusion constant	4.0 m^2/d

Since the death rate of infected larvae and the dispersal rate of larvae do not come into the calculation of ν, Dwyer and Elkinton's (1993) estimate of ν can be used directly in Eqs. 1–3. Similarly, the death rate of infected larvae does not affect Dwyer and Elkinton's estimate of the pathogen decay rate μ, which also can be used as is. Next, the disease-induced death rate α in the present model is just the inverse of the disease incubation time τ in Dwyer and Elkinton's model. Finally, the pathogen production rate λ in Eqs. 1–3 is equal to $\Lambda\alpha$, where Λ is the number of virus particles produced by an infected larva (Anderson and May 1981); since Dwyer and Elkinton (1993) estimate Λ, we also have an estimate of λ.

The success of the earlier model when we used these parameter estimates gave us some confidence that we could extend our approach to look at not just temporal dynamics but spatial dynamics as well. Specifically, the model did an excellent job of predicting the dynamics of the virus in natural populations at high densities, in fact at densities close to the densities of our experimental virus-spread plots (Dwyer and Elkinton 1993). Moreover, our estimate of the transmission rate ν was, if anything, an overestimate, as the model underestimated levels of infection at some lower densities.

The remaining parameter is the diffusion constant D, which describes the rate of larval crawling. The assumption in Eqs. 1–3 is that larvae move randomly between trees, a close approximation to the movement of a variety of insects (Kareiva 1982), including the closely related Douglas-fir tussock moth, *Orgyia pseudotsugata* (Dwyer 1992). Liebhold et al. (1986) present the mean dispersal distances of crawling gypsy moth larvae, data derived from a set of mark–recapture experiments. The diffusion approximation implies that the distribution of larvae in a point release, such as in Liebhold et al.'s (1986) data, will be approximately normal (Okubo 1980), with a probability distribution in polar coordinates of

$$f(r, \theta) = \frac{1}{2\pi Dt} re^{-r^2/4Dt},$$

where r is the radial distance from the origin, and θ is the angle. The mean dispersal distance is then the expectation of r, $E[r]$. The usual methods (Ross 1984, Cain 1990) can be used to show that

$$E[r] = \sqrt{\pi Dt}.$$

Applying this formula to Liebhold et al.'s (1986) data gives a mean value of $D = 4.0$ m^2/d, with a range of from 1.8 to 7.6 m^2/d. This fairly low value reflects the low, but non-zero, rate at which Liebhold et al.'s larvae dispersed between trees.

Given estimates of the model parameters (summarized in Table A1), we can use the model to generate predictions of the rate at which the virus will spread spatially in our virus-spread plots. The question that we use the model to ask is whether the combination of larval crawling between trees and secondary virus transmission between larvae can explain the

extent to which the virus spread after ballooning. Our intent is thus to begin with ballooning, and then add to it the additional dispersal mechanism of inter-tree crawling. As we discuss in the main text (see *Discussion: Ballooning and virus spread*), extrapolating from our ballooning data to an initial distribution of the virus is not trivial, and our main interest is in how the initially rapid drop-off in the fraction infected with distance in some cases leveled out. We therefore adjusted the initial distribution of the pathogen in the model until the model roughly fit the distribution of the fraction infected in each plot in the 3rd wk. We then ran the model to generate predictions of the spatial distribution of the fraction infected in the 4th and 5th wk. To mimic the ballooning and death process in the model, we began the simulations at the end of larval ballooning, on the assumption that the infected larvae finished ballooning and died 2 wk after release, 1 wk before the first collection date. Knowing the initial distributions of healthy and infected larvae is thus equivalent to knowing the respective larval distributions at the end of ballooning. In the interests of simplicity, we assumed that the initially healthy larvae ballooned in such a way as to disperse themselves uniformly over the sampling grid. A final detail is that we assumed that each initially infected larva produced 4×10^8 polyhedral inclusion bodies at the time of death, a number taken from measurements of infected, newly hatched larvae (Shapiro et al. 1987).