Rainfall Effects on Transmission of Gypsy Moth (Lepidoptera: Lymantriidae) Nuclear Polyhedrosis Virus

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ABSTRACT We explored the action of rain on the translocation of the gypsy moth nuclear polyhedrosis virus (LdNPV) at tree, branch, and leaf level. Artificial rainfall was used to simulate naturally occurring rainfall on leaves contaminated with 1st-instar gypsy moths, Lymantria dispar (L.), which had died of LdNPV. Bioassays using larvae in mesh bags on originally contaminated branches and branches below indicated that rainfall is effective in moving virus from branch to branch. This result was confirmed by an experiment using similar methods and naturally occurring rainfall. To examine this effect at the level of the leaf, we bioassayed leaf disks extracted from leaves with and without cadavers. Mortality of test larvae in leaf disk bioassays was strongly correlated with the presence of a cadaver on a leaf, but not confined to disks with cadavers. Mortality caused by non-LdNPV factors was also associated with consumption of a cadaver.

KEYWORDS Lymantria dispar, rainfall nuclear polyhedrosis virus, virus transmission

Gypsy moth nuclear polyhedrosis virus (LdNPV) is the pathogen responsible for the collapse of high density gypsy moth, Lymantria dispar (L.), populations in the northeastern United States (Campbell 1963, Doane 1969). Epizootics typically develop among later instars that become infected by eating foliage contaminated with the cadavers of larvae previously killed by the virus (Doane 1969, Woods and Elkinton 1987).

Most studies of rainfall and virus transmission are concerned with artificial virus formulations. Many researchers have shown that artificial preparations of virus are more or less easily washed off foliage, depending on formulation used (Burgerjon and Grison 1965, Cunningham and Entwistle 1981, Mohamed et al. 1982). The role of rainfall in naturally occurring epizootics has not been well documented. Murray and Elkinton (1989) showed that rainfall running down tree trunks did not contaminate gypsy moth egg masses. However, Thompson (1978) used bioassays to measure the incidence of the Douglas-fir tussock moth Orgyia pseudotsugata (McD.), NPV on Douglas-fir, Pseudotsuga menziesii (Beissn.), branches before and after a light rain, and found that contamination of tested shoots increased from 12% at 1 d before a light rain to 100% at 2 d after. In experiments with the polyhedrosis virus of the velvetbean caterpillar, Anticarsia gemmatalis, on soybeans, Young and Yearian (1986, 1989) emphasize the role of splash contamination in moving inoculum from the soil onto plants, and from upper canopy to lower canopies on the same plant. Young and Yearian (1989) have also speculated about the importance of rain in moving virus from insect cadavers from higher to lower, and presumably more shaded, parts of a plant. Because UV light is known to inactivate insect viruses (Ignoffo et al. 1977, Jacques 1977), rainfall may do more than simply move virus; it may help to prolong its period of activity by bringing it to protected areas.

Other studies have related incidence of LdNPV to humidity. These studies do not identify a mechanism whereby humidity can influence virus mortality; we believe the answer may include rainfall effects, because periods of rainfall coincide with high humidity levels. A positive effect of relative humidity on LdNPV transmission has been suggested by Glaser (1915) and Wallis (1957, 1960). Wallis (1957) reported an increase in both humidity and virus caused mortality toward the end of the larval stage in a population; although work by Woods and Elkinton (1987) indicates that this increase may be part of the bimodal temporal pattern of LdNPV-caused mortality that occurs regardless of the weather conditions.

A number of researchers have included rainfall as a component of mathematical models relating defoliation by gypsy moth or trends in population levels to a variety of environmental factors. Campbell (1967) and Campbell and Sloane (1978) included precipitation in their models of gypsy moth population dynamics, partly on the strength of Wallis' (1957, 1960) previous work. Miller et al. (1989) analyzed gypsy moth defoliation records and weather data in Massachusetts and Connecticut. They found that rainfall in the previous year was negatively correlated with observed defolia-
tion. There are, of course, many possible causes for this effect.

It is logical to assume that the physical action of water droplets striking infected insects remains could spread LdNPV polyhedra to leaves of branches beneath a contaminated branch. On the other hand, Podgwaite et al. (1979) speculate that heavy rains may wash LdNPV off tree trunks and into the soil, whereas David and Gardner (1966) have shown that the granulosis virus of Pieris brassicae (Lepidoptera: Pieridae) is difficult to wash off cabbage leaves. We hypothesize that rain may affect virus transmission and subsequent levels of virus infection in 2 opposing ways: (1) transmission may be reduced when rain washes LdNPV off the foliage, or (2) transmission may be enhanced when rainfall spreads LdNPV concentrated in cadavers more evenly over the available foliage.

Recently, there has been increasing interest in using wild-type or genetically engineered insect pathogens, including viruses, for the control of pests in agricultural, silvicultural, and recreational forest settings. The extent to which rainfall is able to move insect pathogens within a forest setting may become an important question as these materials are considered for use. In this study we investigate the possible influence of rain in spreading LdNPV on foliage, washing LdNPV off of foliage, or doing both.

Materials and Methods

Artificial Rainfall Field Studies. We selected 2 locations in Amherst, MA, for our artificial rainfall studies. The 1st was in a hardwood plantation on the edge of the University of Massachusetts campus. Eight red oak trees, Quercus rubra L., were used at this site from 2 to 12 June 1992. The 2nd location was at the top of a hill on University property near an abandoned apple orchard. Eight red oak trees were used at this site from 9 to 19 June 1992.

To assess the ability of rainfall to move LdNPV off a branch contaminated with cadavers, and onto other branches or the ground, we selected 2 pairs of branches on opposite sides of each tree, with 1 branch of each pair directly above the other (Fig. 1). Forty LdNPV-infected 1st instar gypsy moth larvae were confined to the upper branch using spun polyester mesh bags (Kleen Test Products, Brown Deer, WI). Larvae were hatched from egg masses supplied by the USDA Otis Methods Development Laboratory, and were the standard New Jersey laboratory strain. These larvae had been given a 100- to 200-μl droplet of a virus solution containing 2.5 × 10^4 polyhedral inclusion bodies (PIBS) per milliliter, using a droplet dose technique (Hughes et al. 1986). Such a dose has been determined to cause death to all larvae within 4 d at 27°C (G. Dwyer, unpublished data).

All 1st instars were dead 1 wk after being deployed, at which time mesh bags were removed from the 2 up-down pairs of branches on each tree, and water was applied to 1 of the branch pairs in the following manner (Fig. 1). A gasoline-powered generator (Honda, Osaka, Japan) was used to run a water pump (Simer Pump, Minneapolis, MN) placed in a 113-liter container of water. A flexible hose leading from the pump was used with a brass spray head to aim the water and adjust droplet size to simulate a moderately heavy rain. The spray head was held ~1 m below the level of upper branch of the pair being treated, and the water spray aimed to arc and then fall onto the upper branch (Fig. 1). Approximately 58 liters of water were applied to the foliage over a 5-min period. By positioning a rain gauge under the branches, we determined this application to be equivalent to ~2.4 cm of naturally occurring rainfall.

When foliage had dried, 25 healthy 3rd instars were enclosed inside mesh bags to bioassay for the presence of LdNPV. Plastic bags were rolled up and placed at the base of the branches, to be unrolled over the mesh bags in case of naturally occurring rainfall, which did not occur during the experiment. One week later, branches with bags were removed from trees with pruning shears and returned to the laboratory. Insects were removed and reared individually for 2 wk in 58.8-ml cups containing artificial diet (Bell et al. 1981) at 27°C and a photoperiod of 24:0 (L:D) h. Larvae were examined for mortality weekly, and each dead insect was autopsied under the light microscope at 400X to verify virus as the cause of death (Woods and Elkinton 1987).

We transformed proportions (p) of larvae dying of LdNPV using the arcsine √p, and compared proportions of larvae dying in each treatment with an analysis of variance to determine the significance of tree effects, rain versus dry effects, up versus down (or branch position) effects, and the interaction of rain versus dry and up versus down effects (Analytical Software, 1992). To clarify the

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Fig. 1. Method used to apply simulated rain to branch contaminated with LdNPV-killed gypsy moth 1st instars: (a) dry treatment, upper branch with cadavers; (b) dry treatment, lower branch without cadavers; (c) simulated rain treatment, upper branch with cadavers; (d) simulated rain treatment, lower branch without cadavers.
effect of rain on branches in the same position, we compared proportions of larvae dying of LdNPV within the 2 upper branch treatments (rain up and dry up) and within the 2 lower branch treatments (rain down and dry down) with a Wilcoxon signed rank test (Sokal and Rohlf 1981).

Natural Rainfall Field Test. We performed an experiment from 15 to 28 July 1993 to study the effects of a natural, rather than artificial, rainfall on virus transmission. On Cape Cod, MA, at Otis Air National Guard Base, we set up 8 black oak trees, Quercus velutina L., with infected 1st instars in exactly the manner described in the artificial rainfall experiment. After all of the infected 1st instars died, 1 up-down pair of branches on each tree was left completely uncovered; the other pair was covered with a white plastic garbage bag (over the mesh bag) to protect the branch from rain. Because we were concerned about possible water condensation inside the plastic bags, the bags were opened along the bottom side to allow air flow and leaf respiration. Although some condensation sometimes formed on the inner surface of the plastic bag, repeated inspection of leaves within the mesh bags indicated that they remained dry.

A 1.44-cm rainfall occurred on 20 July 1993, 1 d after removal of the bags from the up-down pair of branches that served as the rain treatment. On 21 July 1993, we placed 25 uninfected 3rd instars in mesh bags on all branches, and then covered all mesh bags with opened plastic bags to prevent further exposure to rain. It did not rain during the remainder of the experiment. Although the precautionary measure of covering the mesh bags with plastic bags is likely to have had an effect on humidity inside the bags, we felt that this was acceptable because all treatments were treated the same. We cut down all branches on 28 July 1993, and reared larvae individually in the same manner as in the artificial rainfall field test. Statistical analyses were identical to those used for the artificial rainfall experiment.

Leaf Disk Bioassay. To more closely explore the ability of rain to spread LdNPV between leaves on the same branch, we set up an additional 18 trees with infected 1st instars confined for 1 wk in mesh bags as in the artificial rainfall test above. In this experiment, branches without 1st instars were not placed below infected branches. The dry treatments of these controls were covered with plastic bags until after the simulated rain treatments had been applied, to avoid contamination. After all the infected 1st instars had died (1 wk), we applied water to half of the cadaver-contaminated branches and half of the control branches as in the simulated rain experiment above, except that 12 liters of water was applied over a 1-min period instead of 58 liters over a 5-min period. We thought that this reduction in the amount of water would maximize LdNPV spreading effects and minimize washing-off effects. After the branches had dried (=2 h), they were cut down and brought into the laboratory. Disks 3.6 cm in diameter were immediately removed from leaves (Fig. 2). Those branches that had been bagged with infected 1st instars had leaves with 1st-instar cadavers, and leaves without cadavers. Two types of disk were removed from leaves with cadavers; a disk containing a cadaver (leaf with cadaver, disk with cadaver) and a disk with no cadaver (leaf with cadaver, disk no cadaver). Disks were also taken from leaves without a cadaver (leaf no cadaver, disk no cadaver), and from leaves on control branches that had been bagged without infected 1st instars. These 4 types of disks were taken from branches in both the rain treatment and the dry treatment, for a total of 8 treatments (Fig. 2). A minimum of 6 and a maximum of 8 disks of each of the treatment types were taken from each tree, depending on the number of cadavers that we found.

Each leaf disk was folded in half, and placed tent fashion in a 175 milliliter plastic cup containing 1 cm of agar (Keating et al. 1988). The agar was used to keep the leaf disk turgid and upright. One healthy, 3rd-instar test larva was placed in each cup for 96 h to eat the disk, then removed and reared on diet for 2 wk as above.

Because a substantial number of larvae died from non-LdNPV causes within a few days of eating a disk, they were not considered as part of the pool of virus-susceptible insects, and were not used to calculate proportions of larvae dying of LdNPV (Elkinton et al. 1992). The proportion of larvae in each treatment dying from LdNPV was calculated using the Abbott (1925) formula, by dividing the number of LdNPV deaths by the total number of larvae in each treatment group minus the number of larvae in the treatment dying of non-LdNPV causes. The causal agent of the non-LdNPV mortality appeared to act first, so the proportion of larvae dying from non-LdNPV causes was calculated without first subtracting the LdNPV mortal-
Results and Discussion

Artificial Rainfall Field Study. The results of the ANOVA indicate significant position effects, and a significant interaction between rain versus dry and position (Table 1). Further analysis with the Wilcoxon signed rank test showed that simulated rain significantly reduced mortality on upper branches, which were contaminated with the infected 1st-instar cadavers (Table 2) (SAS Institute 1985). The weighting used in these analyses was the number of leaf disks used to calculate the proportions dying as described above. We further analyzed the treatment effects with planned pairwise comparisons of the means, adjusting the alpha error by dividing 0.05 by the number of comparisons in each group (6), so that the null hypothesis was rejected when $P < 0.008$ (Sokal and Rohlf 1981).

![Fig. 3. Mean proportion LdNPV-caused mortality of 3rd-instar gypsy moth larvae confined for 1 wk on upper branches (contaminated with LdNPV-killed gypsy moth 1st instars) or lower branches of red oak trees; branches were treated with simulated rain or left dry. $P$ values are given for planned comparisons between the rain and dry treatments.](https://academic.oup.com/ee/article-2451/141/2480882/guest)

![Fig. 4. Mean proportion LdNPV-caused mortality of 3rd-instar gypsy moth larvae confined for 1 wk on upper branches (contaminated with LdNPV-killed gypsy moth 1st instars) or lower branches of red oak trees; branches were exposed to naturally occurring rain or kept protected from rain. $P$ values are given for planned comparisons between the rain and dry treatments.](https://academic.oup.com/ee/article-2451/141/2480882/guest)

Table 1. ANOVA of transformed proportions of 3rd-instar gypsy moth larvae dying of LdNPV

<table>
<thead>
<tr>
<th>Source</th>
<th>Simulated rain</th>
<th>Natural rain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$df$</td>
<td>$F$</td>
</tr>
<tr>
<td>Trees (A)</td>
<td>15</td>
<td>0.73</td>
</tr>
<tr>
<td>Rain vs Dry (B)</td>
<td>1</td>
<td>3.93</td>
</tr>
<tr>
<td>Up vs Down (C)</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>B x C</td>
<td>1</td>
<td>11.27</td>
</tr>
</tbody>
</table>

Larvae were confined with LdNPV-killed 1st-instar cadavers on red oak branches in mesh bags. Two pairs of branches were used on each tree, with 1 branch of each pair directly above the other. One of these up-down pairs on each tree was subjected to simulated or naturally occurring rain. Proportions transformed by arcsine square root before analysis (Sokal and Rohlf 1981).

Proportions transformed by arcsine square root before analysis (Sokal and Rohlf 1981) and weighted with the number of leaf disks used to calculate proportions in each treatment.

Table 2. Weighted ANOVA of transformed proportions of 3rd-instar gypsy moth fed contaminated leaf disks dying of non-LdNPV and LdNPV causes

<table>
<thead>
<tr>
<th>Source</th>
<th>Non-LdNPV mortality</th>
<th>LdNPV mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$df$</td>
<td>$F$</td>
</tr>
<tr>
<td>Rain vs Dry (A)</td>
<td>3</td>
<td>36.13</td>
</tr>
<tr>
<td>Treatment (B)</td>
<td>17</td>
<td>0.00</td>
</tr>
<tr>
<td>Trees (C)</td>
<td>3</td>
<td>3.20</td>
</tr>
<tr>
<td>A x B</td>
<td>3</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Proportions transformed by arcsine square root before analysis (Sokal and Rohlf 1981) and weighted with the number of leaf disks used to calculate proportions in each treatment.
These results support our hypothesis that rainfall acts to wash LdNPV off foliage and to spread LdNPV from contaminated to uncontaminated foliage. This explains the reason for the significant position versus rain–dry interactions in the ANOVA (Table 1); rainfall had the opposite effect on upper versus lower branches (Fig. 4).

Leaf Disk Bioassay. The weighted ANOVA results showed significant treatment effects (distance from cadaver) on LdNPV mortality (Table 2). In both rain and dry treatments, nearly all larvae consuming a leaf disk with a cadaver died of virus, if they did not first die from other causes (Table 3). Mortality was less in those larvae eating a disk without a cadaver from a leaf that had one, and less still when the disk consumed was from a leaf with no appreciable virus mortality in the controls. Although clear and significant differences were seen between treatments of varying distances from cadavers, no significant overall differences were seen between rain and dry treatments, and there were no significant rain versus dry by treatment interactions (Table 2). However, in the rain treatment, there was significantly higher LdNPV mortality among larvae feeding on leaves adjacent to cadavers (leaf no cadaver, disk no cadaver) than on disks from control leaves (Table 3). This effect was not seen in the dry treatment, possibly because the simulated rain spread virus from leaves with cadavers to nearby leaves without cadavers. The absence of overall rain effects may have been caused by the lower amount of water we used in this test as compared with the 1st experiment.

Insects in the leaf disk bioassay experienced some mortality not connected with virus, which nonetheless followed roughly the same pattern as the virus mortality (Table 3). Microscopic examination of the cadavers revealed large concentrations of bacteria. These may be associated with decaying gypsy moth cadavers present on the leaf surface (J. Podgwaite, personal communication). No mortality of this type was seen in larvae held in the mesh bags in the first 2 experiments, or in other experiments of this type we have performed (unpublished data). Thus, we suspect that this mortality is not a significant factor in field populations; an insect confined with contaminated foliage and agar is likely at greatly increased risk for bacterial infection, because agar is an excellent growth medium for bacteria. The interaction between these bacteria and LdNPV is unknown, although it appears that bacteria are capable of killing larvae more quickly. It is possible that mortality caused by bacteria may have obscured subtle effects of rainfall on virus transmission by decreasing the number of larvae available for viral infection.

Our experiments support the idea that rain can wash virus from branches contaminated with LdNPV-killed gypsy moth cadavers, and can spread this virus to branches beneath. We cannot tell from our results whether this would have the net effect of increasing or decreasing mortality from LdNPV in a population of gypsy moth, although this would probably depend on the amount of rainfall. Because many infected gypsy moth larvae die in the upper canopy of trees (Murray and Elkinton 1992), the spreading effect may become a significant factor by exposing insects in lower foliage to virus. These findings may thus explain earlier reports (Glaser 1915; Wallis 1957, 1960) relating humidity to the incidence of LdNPV mortality.

Acknowledgments

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