Transmission of Nuclear Polyhedrosis Virus to Gypsy Moth (Lepidoptera: Lymantriidae) Eggs via Contaminated Substrates

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ABSTRACT

Higher mortality from nuclear polyhedrosis virus (NPV) was observed among gypsy moth larvae hatched from egg masses laid on virus-treated bark substrates than larvae hatched from egg masses laid on bleach-treated or untreated bark. Eggs from the inner portions of the egg mass (proximal to the substrate) were more heavily contaminated than those in the outer portions. These results indicate that gypsy moth egg masses can become contaminated with infectious NPV during oviposition on contaminated substrates. This mechanism may be an important mode of transmission in high-density populations or in years following an epizootic.

KEY WORDS

Insecta, Lymantria dispar, nuclear polyhedrosis virus, transmission

NUCLEAR POLYHEDROSIS VIRUS (NPV) plays an important role in the population dynamics of the gypsy moth, Lymantria dispar (L.) (Campbell 1963). Infection of first instars is instrumental in the development of NPV epizootics (Doane 1969, 1970, 1976; Woods & Elkinton 1987). Doane (1969, 1970, 1975) showed that a major source of infection for newly hatched larvae is the overwintering stage, the eggs, which are deposited in masses in late summer. Leonard & Doane (1966) and Doane (1969, 1975) demonstrated that chemical disinfection of the surface of gypsy moth eggs almost entirely eliminated NPV-caused mortality among larvae hatched from the eggs, indicating that virus is carried on the surface of the egg. Upon hatching, first instars ingest NPV along with pieces of the egg chorion and egg mass setae (Doane 1969, 1975, 1976). But the mechanisms by which eggs become contaminated with NPV are not well understood.

Theoretically, eggs can become infected or contaminated with virus by two means: by vertical transmission, virus transmitted by infected or externally contaminated moths directly to their progeny, or by horizontal transmission, by environmental contamination of the egg mass.

Doane (1976) and Podgwaite et al. (1981) suggested that NPV is transmitted transovum (i.e., on the surface of the egg) by infected females to the eggs. Shapiro & Robertson (1987) reported in a laboratory study that infected female moths may transmit virus to offspring. They found 11% NPV-induced mortality among progeny of gypsy moths surviving an LD<sub>90</sub> dose of NPV. It was not determined whether transmission was transovum or transovarial (within the egg). However, in another study (Murray & Elkington 1989), we were unable to demonstrate transmission of NPV from sublethally dosed females to their progeny under field conditions, although we used lower doses of NPV to infect the parental stock than did Shapiro and Robertson.

Evans (1986) has argued that conclusive evidence for transovarial transmission of NPV in Lepidoptera is lacking and suggests that in this group, maternal transmission of NPV to eggs is the result of environmental contamination of the female rather than internal infection. We found that females surviving an NPV epizootic produced progeny that suffered a relatively low rate of NPV-induced mortality (10%) when oviposited into a clean environment (Murray & Elkinton 1989). However, mortality rates were high (20–55%) among progeny of wild and laboratory-strain moths when eggs were laid on trees located in sites where an epizootic had occurred, suggesting that eggs acquired most NPV directly from environmental sources rather than maternal ones. Furthermore, contamination of the eggs occurred during or shortly after oviposition. Eggs sampled within 3 d of oviposition had the same amount of inoculum as those that overwintered on forest trees (Murray & Elkinton 1989).

We proposed that in NPV-contaminated environments (e.g., after an epizootic), gypsy moth eggs become contaminated with NPV from the substrate on which they are deposited. In the study reported here, we examined the transfer of NPV from artificially contaminated substrates to eggs and the subsequent infection and NPV mortality among first instars.

Materials and Methods

In August 1987, after adult emergence, we felled three black oak (Quercus velutina Lam.) trees from a site on Cape Cod, Mass., where a resident gypsy moth population suffered a moderate level of NPV infection. Peak prevalence of NPV during the lar-
val period was 20%. The bole of each tree was cut into four 1-m long bolts, and each of the 12 bolts was nailed upright onto a square fiberboard base. Each of three treatments was randomly assigned to a group of four bolts. Three treatments were applied: (1) the entire bark surface was sprayed with an NPV solution containing 5 × 10^8 polyhedral inclusion bodies (PIB) per ml water (Gyepchek provided by the U.S. Forest Service, Hamden, Conn.), or (3) bolt remained untreated (control). Newly eclosed New Jersey strain gypsy moths were obtained from a laboratory colony (provided by the USDA Otis Methods Development Center, Otis Air National Guard Base, Mass.). Male and female moths were caged in pairs in inverted 355-ml waxed paper cups stapled onto the bark surface of each bolt. Females mated and subsequently laid 12–18 egg masses onto each bolt for a total of 63, 50, and 58 egg masses in the bleach-treated, NPV-treated, and untreated control treatments, respectively. The bolts with the egg masses on them remained in an outdoor screened insectary until the following spring.

In April 1988, each egg mass was gently scraped from the bolt into a clean 30-ml covered plastic cup using a knife blade disinfected with 10% chlorine bleach. Approximately half of the egg masses within each treatment were divided into an inner half (containing those eggs proximal to the underlying bark surface) and an outer half (eggs distal to the bark surface). All eggs were refrigerated at 10°C for 20 d, after which they were placed in an incubator at 29°C to hatch.

We did bioassays of egg masses by the following procedure, which has been shown to be a good measure of the relative amount of inoculum associated with egg masses (Doane 1976, Woods & Elkinton 1987). Approximately 20 newly hatched first instars were randomly selected from each egg mass (whole egg masses) or each half egg mass (divided egg masses) and were transferred to 180-ml cups containing 85 ml of artificial diet (Bell et al. 1981). Larvae were reared for 14 d at 27°C (±1°C) and about 50% RH. The photoperiod was 16:8 (L:D). Larvae were checked after 7 and 14 d for NPV mortality. Secondary infection within cups before 14 d is minimal under these rearing conditions (Woods & Elkinton 1987). At each observation, the number of virus-killed larvae was recorded and dead larvae were removed. Cause of mortality was judged on the basis of gross morphological appearance, but questionable cadavers were examined under a compound microscope for the presence of PIBs. After 14 d, the total number of larvae in each cup which had died from NPV infection was recorded. The mean percentage of NPV-caused mortality was compared among larvae hatched from egg masses from the three bolt treatments and from eggs from the inner versus outer portions of the egg masses.

Percentages were converted to arcsine square roots and were analyzed by ANOVA using the computer statistical package SAS PROC GLM (SAS Institute 1987). Treatment mean separations at the α = 0.05 level were determined by Sidak's test for inequality (SAS Institute 1987).

**Results and Discussion**

The results are shown graphically in Fig. 1. Larvae that hatched from egg masses laid on the NPV-treated bolts showed significantly higher (P ≤ 0.05) NPV-caused mortality compared with those hatching from egg masses laid on either the bleach-treated or the untreated bolts. The mortality rate among larvae hatched from eggs from the bleach-treated bolts was not significantly different (P ≤ 0.05) than those hatched from eggs laid on the untreated bolts.

Among the untreated and bleach-treated groups the mean mortality rate did not vary significantly (untreated, F = 0.12, df = 1, P = 0.73; bleach-treated, F = 0.84, df = 1, P = 0.36) with position of eggs in the mass (inner versus outer). However, significantly greater (F = 8.14, df = 1, P = 0.01) NPV-induced mortality was recorded among larvae hatched from inner eggs compared with larvae hatched from outer eggs when egg masses were laid on NPV-treated bolts.

Environmental persistence of NPV may be critical for maintenance of the pathogen in low-density populations in the years following an epizootic (Doane 1976, Podgwaite et al. 1979, Weseloh & Andreadis 1986). First instars that traverse a virus-contaminated surface can become infected (Weseloh & Andreadis 1986, Woods et al. 1989), presumably by transfer of virus to food where it is ingested.
However, the importance of this mechanism to transmission of NPV across generations in natural populations is not known. Alternatively, there is much evidence that environmental contamination of egg masses is an important mode of transmission of the virus from one generation to the next for gypsy moth (Doane 1970, 1975; Woods & Elkinton 1987; Murray & Elkinton 1989) as well as for other Lepidoptera (Wigley 1976, Thompson 1978). The results of this study indicate that egg masses can become contaminated upon oviposition on contaminated substrates. This is supported by a similar finding that egg masses laid on tree trunks have significantly higher amounts of NPV inoculum than do egg masses deposited on other substrates such as rocks, understory vegetation, and branches in the same site (Woods et al. in press).

Acquisition of NPV from contaminated substrates by egg masses could help explain why gypsy moth populations often remain at low density after an epizootic (Doane 1976). During an epizootic, NPV-infected larvae die in large numbers on tree trunks, thereby contaminating the bark with PIBs. Bark flaps and crevices on tree boles are among favored oviposition sites (Bess 1961, Campbell et al. 1975). Because NPV can persist on bark for at least 1 yr (Doane 1975, Podgwaite et al. 1979, Weseloh & Andreaids 1986), many oviposition sites are likely to be heavily contaminated with NPV following an epizootic. It is apparent from this study that egg masses oviposited on NPV-contaminated bark substrates readily become contaminated with the virus.

We found that the innermost eggs (those in direct contact with the substrate) produced larvae that were about twice as likely to die from NPV compared with larvae hatched from eggs in the outermost part of the egg mass. Doane (1975) found that the setae deposited into the egg mass from the abdomen of the female during oviposition are a good source of NPV inoculum for newly hatched larvae. It is likely that virus from the substrate is incorporated into the egg mass, especially onto those eggs deposited into the first (inner) layers, as the female repeatedly moves her abdomen back and forth on the substrate while depositing eggs and her abdominal setae into the egg mass. The outer layers would not become contaminated with as much inoculum because neither the eggs nor the female's abdomen are in direct contact with the substrate during oviposition.

It is also possible that NPV was initially dispersed throughout the egg mass but was inactivated by sunlight and other environmental factors in the outer exposed portion of the egg mass. However, because the bolts were somewhat protected from sunlight and wind and were almost completely protected from precipitation in the screened insectary, we believe it is unlikely that much environmental degradation of NPV in the outer portions of the egg mass occurred. This is supported by our earlier finding that the amount of inoculum associated with egg masses 3 d after oviposition was not significantly altered after overwintering on forest trees (Murray & Elkinton 1989).

Perhaps the most effective location for virus to overwinter is on the surface of eggs in the inner portions of the egg mass. As pointed out by Evans (1986), persistence of viruses outside the host, which is the most common means of transmission from one period to another, is dependent on the quantity of virus remaining viable and the probability of the inoculum being encountered by susceptible hosts. Inner eggs and the virus associated with them are somewhat protected from predation, parasitism, and environmental damage by the thick layer of outer eggs and setae overlaying them. Therefore, it is highly probable that inner eggs and the virus present on them will both remain viable over the winter, thereby increasing the likelihood that larvae hatching from these eggs will become infected the next season.

It has been suggested that the primary source of inoculum for eggs and first instars is maternal (Doane 1976, Podgwaite et al. 1981). Although we cannot rule out the possibility that maternal transmission occurs, the results of this study, in conjunction with our earlier findings (Murray & Elkinton 1989), indicate that environmental contamination of the oviposition substrate may also be an important route of transmission to eggs, at least in contaminated environments.

This mechanism of transgenerational transmission may also have important implications for management of the gypsy moth. Dispersal of infected first instars from contaminated egg masses can serve to introduce NPV from a contaminated area into uninfected populations or to augment the disease level in a moderately diseased population (Bogenschutz et al. 1989). Current suppression strategies that use insecticidal formulations of NPV are aimed at spraying early instars and the results of these applications have often been disappointing (Lewis & Yendol 1981, Podgwaite 1985). However, because the virus appears to be protected from environmental degradation within the egg mass, perhaps trees can be sprayed with NPV before oviposition in the late summer. Such a practice might augment the prevalence of naturally occurring disease in the early instars the following spring, thereby taking advantage of secondary spread of the virus through the population to initiate an epizootic.

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