Transmission of Nuclear Polyhedrosis Virus Prior to Death of Infected Loblolly Pine Sawfly, *Neodiprion taedae linearis* Ross, on Loblolly Pine[1]

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Abstract Nuclear polyhedrosis virus (NPV) was transmitted by infected loblolly pine sawfly, *Neodiprion taedae linearis* Ross, larvae prior to death on loblolly pine. Third instars reared on pine foliage previously fed upon for 24 h by NPV-infected larvae at densities of 4 and 16 per terminal resulted in up to 69.9 and 93.1% mortality, respectively, in previously unexposed cohorts. Third instars reared on pine foliage wetted with washings from frass of infected larvae also resulted in a high level of mortality. Results suggest that a high level of secondary transmission of NPV occurs before death of primary-infected larvae. This transmission appears to be the result of NPV in the digestive tract released either through regurgitation or in the feces.

Key Words Loblolly pine sawfly, *Neodiprion taedae linearis,* nuclear polyhedrosis virus, loblolly pine, virus transmission.

The nuclear polyhedrosis virus (NtNPV) of the loblolly pine sawfly, *Neodiprion taedae linearis* Ross (Hymenoptera: Neodiprionidae), is a major mortality factor of larval populations on loblolly pine in southern Arkansas and northern Louisiana (Young et al. 1972). The disease is usually evident during the late instars. In years of high sawfly density, NtNPV epizootics typically occur, removing much of the population.

The virus is easily transmitted within a colony with a single virus-infected larva responsible for the death of a high percentage of members of a colony (Young and Yearian 1987). Transmission between colonies is much less than that among members of a colony and is related to distance between colonies and height of colonies in a tree (Young and Yearian 1989a). Spread of virus between trees also occurs and is related to distance between trees and prevailing winds (Young and Yearian 1989a). This rapid transmission of sawfly NPV and epizootics that remove much of the population is typical of NPV of other sawfly species as well (Bird 1955, 1961, Smirnoff 1962). Several factors have been shown to promote spread of sawfly NPV disease in the population. The sawfly NPV's are spread by beneficial arthropods, birds and small mammals (Bird and Elgee 1957, Entwistle et al. 1977, Entwistle 1982, Olofsson 1989a), and these viruses survive well, such as from one year to the next on virus-contaminated trees (Neilson and Elgee 1968, Kaupp 1983). These viruses appear also to be transmitted by female sawfly that have either survived infection or have become externally contaminated with the viruses (Bird 1961, Smirnoff 1962, Olofsson

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1989b). Transmission of sawfly NPV appears to be quite rapid in comparison to that of most NPV's of Lepidoptera. In most Lepidoptera only low levels of NPV transmission occur before death of the primary-infected larvae (Ali et al. 1987, Young and Yearian 1988).

Sawfly larvae regurgitate digestive fluids as a defensive mechanism (Entwistle et al. 1983). Because sawfly NPV's replicate in the midgut cells, they are liberated into the digestive fluids when contents of infected cells are disrupted and regurgitated (Entwistle et al. 1983). Viral polyhedra also have been observed in regurgitant of loblolly pine sawfly larvae in late stages of disease (Young, unpubl. data). Reported herein are results of a study to examine the potential of transmission (in loblolly pine sawfly) of NtNPV from infected larvae prior to their death to healthy cohorts.

Materials and Methods

Test 1: Transmission by infected larvae. Test 1 was conducted on loblolly pine foliage collected in Washington Co., AR, an area that typically contained low population levels of the sawfly. Larvae were collected as second instars from loblolly pine in Calhoun Co., AR, an area of typically high population levels, and transported to the test at the Main Agricultural Experiment Station, Fayetteville, AR. Colonies were held on bouquets of pine foliage in the laboratory at 22 ± 1°C until the test was initiated.

The NtNPV used in this study was produced in a natural population of sawfly larvae in Calhoun Co., AR, in 1992. Larvae were treated in the field, collected prior to death, brought to the laboratory, and, after death, partially purified and quantified as described by Yearian et al. (1973).

The test was a 4 × 2 factorial arrangement of treatments with days and density as factors. Larvae infected as a virus source (primary infected larvae) were treated as early third instars with NtNPV by holding them for 24 h on a terminal of pine needles (25 per terminal) in a 700-ml wax-coated paper cup. Untreated larvae were handled in a similar manner as in untreated control. The terminal stem was inserted through the bottom of the holding cup into a 270-ml plastic cup containing water. Terminals were covered with plastic bags, and the bags were affixed to the cups with rubber bands. The terminals were treated by dipping in a 2×10^7 PIB/ml suspension of the virus and air dried. At this dose, 100% of the primary-infected larvae died from virus infection. The primary-infected larvae were transferred to an untreated pine terminal (4 or 16 larvae per terminal), and held in a 700-ml wax-coated paper cup for 24 h. These primary-infected larvae were transferred to a new untreated pine terminal every 24 h through the fifth day of infection (near death). A few larvae were near death from disease after the fourth day of infection and were removed from the colony to prevent death from occurring in the colony. The primary-infected larvae were removed and replaced by 25 untreated early third instars per terminal, which were reared until death or pupation when mortality was recorded. The test was replicated five times.

Data were corrected for control mortality with Abbott's (1925) formula and analyzed using analysis of variance (GLM). Means were separated by LSD ($P < 0.05$) (SAS Institute 1988).

Test 2: Transmission from frass of infected larvae. The primary-infected larvae were exposed to the virus on contaminated foliage (25/terminal) as in Test 1. After 24 h, frass from the bottom of the holding cup was collected. The larvae in each cup were transferred to a similar holding cup containing uncontaminated foliage and transferred

every 24 h thereafter through 5 days. A few larvae appeared to be in the latter stages of disease after 4 d and were removed to prevent dead larvae from contaminating frass in the bottom of the holding cup. All frass in each cup from each day was collected and frozen.

The bioassay was conducted in a 10-year-old pine plantation in Calhoun Co., AR. A single colony of larvae on a tree (only one colony per tree) was labeled with colored plastic flagging. Mean colony size was 69.9 ± 6.8 larvae. Treatments were frass from NtNPV exposed and unexposed (control) larvae collected on each day of days two through five. Each day's sample of frass from 25 primary-infected larvae or control was wetted with 50 ml of 2% KCl, the frass was allowed to settle, and the supernatant was poured into a 100-ml plastic screw-cap bottle. The flagged cluster of pine needles was then wetted with supernatant by holding the cluster of needles together and pouring the supernatant over it. Colonies were observed daily, and when larvae in the day-5 treatment were observed to be in the late stages of disease (after 8 d), all colonies were collected, and placed individually in 2.3-kg paper bags with additional terminals added for food. The bag was sealed and returned to the laboratory. These bagged colonies were held under high humidity which would minimize drying of the pine needles. Larvae were transferred to the laboratory and held at 22 ± 1°C under high humidity. Mortality was recorded 7 d later when larvae in controls were predominantly fifth and sixth instars. Each treatment consisted of a terminal of 25 larvae, and the test was replicated five times.

Differences within a treatment between days were analyzed using GLMP, and means were separated with LSD ($P < 0.05$). Differences between treatments were analyzed by the paired *t*-test.

Results and Discussion

Test 1: Transmission by infected larvae. Corrected mortality of sawfly larvae reared on pine was less than 30% when the terminal had previously harbored larvae (4 or 16) infected with NtNPV for 3 d or less (Table 1). Mortality of previously unex-

Table 1. Corrected mortality (%) of loblolly pine sawfly larvae reared from the third instar on loblolly pine foliage on which NtNPV-infected larvae had previously fed for 24 hours

Days primary larvae infected NtNPV	Infected larval density/terminal*,**	
	4	16
2	13.7 ± 5.5cA	28.3 ± 9.4bcA
3	14.5 ± 5.6cA	20.1 ± 7.9cA
4[t]	35.2 ± 5.9bA	46.7 ± 7.6bA
5	69.9 ± 4.6aB	93.1 ± 4.6aA

* Means in a row (capital letters) or columns (small letters) not followed by the same letter are significantly different (PROC GLM, 5% level [SAS Institute 1988]). Data corrected by Abbott's formula (Abbott 1925).

** One pine bouquet per treatment, replicated 5 times.

[t] Some larvae were in late stages of disease and removed on this day.

posed larvae increased significantly when the virus exposure period of primary infected larvae was increased from 3 to 4 d ($P < 0.05$). Transmission was greatest when primary-infected larvae had been exposed to virus for 5 d. Transmission differed with density (4 or 16) only when primary-infected larvae had been exposed to virus for 5 d when mortality was 69.9 and 93.1%, respectively. There was not a significant interaction with days of virus exposure of primary-infected larvae and their density ($F = 1.38$, df = 3, $P = 0.2685$). Significant group effects were observed for days of exposure of primary-infected larvae ($F = 33.82$, df = 3, $P = 0.0001$) and their density ($F = 9.62$, df = 1, $P = 0.0044$) (Table 1).

Some transmission of virus in a colony occurred when primary-infected larvae had been exposed to it for 2 d. High levels of transmission occurred, however, only when the primary infected larvae were in the latter stages of disease. The levels of transmission of NtNPV prior to death of infected host were much greater than observed in NPV-infected lepidopterous larvae (Young and Yearian 1989b). The level of secondary infection in *Pseudoplusia includens* Walker (>30%) or *Anticarsia gemmatalis* (Hübner) (>10%) prior to death was quite low and was only observed when primary-infected larvae were within 24 h of death. Jacques (1962) found a low level of NPV transmission prior to death of *Trichoplusia ni* (Hübner) larvae and thought that this could be due to disruption of fore- or hindgut or integument in larvae near death. The rapid spread of NtNPV by primary-infected cohorts in our test may explain the high level of intracolony transmission and lower levels of intercolony transmission observed in sawfly colonies on trees (Young and Yearian, 1987).

Test 2: Transmission from frass of infected larvae. Mortality in sawfly colonies on terminals wetted with washings from frass collected from 2-day infected larvae was only 3% (Table 2). However, when the frass donors had been infected for 3 d, mortality was 53.7%. The percentage of virus-killed larvae increased to 90.8% when frass was from larvae infected for 5 d. In contrast, mortality in colonies reared on foliage wetted with frass from untreated (control) larvae was <10%, except for the 5-day frass (26.7%) (Table 2). These results suggest that infectious virus from midgut of infected sawflies is discharged in feces. Virus in feces appears to be responsible for much of the rapid and high level of secondary transmission in loblolly pine sawfly. Virus regurgitated as a defensive measure may also be important in secondary virus

Table 2. Mortality (%) in larvae reared on loblolly pine branch wetted with washings from frass from NtNPV-infected larvae

Frass donors days infected	Control larvae*	NtNPV exposed larvae*
2	0.3 ± 0.2bA	2.8 ± 1.5cA
3	1.0 ± 0.5bB	53.7 ± 7.1bA
4**	8.0 ± 4.6bB	54.8 ± 8.6bA
5	26.7 ± 8.4aB	90.8 ± 4.3aA

* Means in a row (capital letters) or column (small letters) not followed by the same letter are significantly different (PROC GLM, 5% level; LSD [SAS Institute 1988]).

** A few larvae were near death and removed from the container on this date to prevent dead larvae in day-5 frass.

transmission (Entwistle et al. 1983). Most intracolony disease spread appears to take place before death of primary-infected larvae. Movement of infected individuals among colonies and dropping of feces could also account for some of the transmission among colonies within a tree (Young and Yearian 1989a). These results and those of previous studies suggest that NtNPV epizootics could occur from low numbers of primary-infected individuals in the population.

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