# INTRA- AND INTER-COLONY TRANSMISSION OF A NUCLEAR POLYHEDROSIS VIRUS OF THE LOBLOLLY PINE SAWFLY, *NEODIPRION TAEDAE LINEARIS* ROSS, ON PINE [1,2]

S. Y. Young and W. C. Yearian
Department of Entomology
University of Arkansas
Fayetteville, AR 72701
(Accepted for publication October 2, 1986)

## ABSTRACT

A high level (37 - 98%) of secondary transmission of a nuclear polyhedrosis virus from primary infected larvae (PIL) of the loblolly pine sawfly, *Neodiprion taedae linearis* Ross (Hymenoptera: Diprionidae), to other members of the same colony occurred on loblolly pine, *Pine taeda* L., in Arkansas. Intra-colony transmission was significantly ($P < 0.05$) related to PIL density (No. of PIL/colony) but not to PIL instar at death. Inter-colony secondary transmission from PIL-infected colonies was lower (2 - 50%) than intra-colony transmission. Inter-colony transmission was greater when the PIL colony was placed in the lower canopy than in the upper canopy. Regardless of placement of the PIL colony (upper or lower canopy), mortality in other non-PIL colonies, was highest in the upper canopy. Migration of infected larvae is discussed relative to these findings.

Key Words: *Neodiprion taedae linearis* Ross, nuclear polyhedrosis virus, loblolly pine, loblolly pine sawfly, virus transmission, *Pinus taeda*.

J. Entomol. Sci. 22(1): 29-34 (January 1987)

## INTRODUCTION

Nuclear polyhedrosis viruses (NPV) (Baculoviridae) have been isolated from several diprionid (Hymenoptera) sawfly species. These viruses are highly virulent and have been tested extensively for sawfly control (Cunningham 1982). Formulations of redheaded pine sawfly, *Neodiprion lecontei* (Fitch) NPV, and European pine sawfly, *Neodiprion sertifer* (Geoff.) NPV, have been registered as microbial insecticides in Canada and the United States, respectively.

The NPV of sawflies are usually endemic in the host population, with epidemics of disease occurring at high sawfly densities. Although these viruses are major natural mortality factors for sawflies, their epizootiology is poorly understood. With a few exceptions (Entwistle et al. 1983; Kaupp 1983; Smirnoff 1961), most epizootic studies have been largely qualitative in nature. We have been conducting research on an NPV infecting larvae of the loblolly pine sawfly, *Neodiprion taedae linearis* Ross, the major defoliator of pine in Arkansas. In previous studies the virus has proven efficacious when sprayed from the ground or air (Yearian and Young 1971; Yearian et al. 1973; Young and Yearian 1984).

The loblolly pine sawfly has a single generation each year. The females lay eggs in clusters on foliage in the fall and eggs hatch in the following spring. Larvae feed

[1] Published with the approval of the Director, Arkansas Agriculture Experiment Station.
[2] Use of a trade name does not imply endorsement or guarantee of the product or the exclusion of other products of similar nature.

in groups, primarily on older foliage, for 30 - 40 days before crawling to the ground to pupate (Anonymous 1972). The males have five and the females have six larval instars. We recently initiated studies on the epizootiology of the loblolly pine sawfly NPV, reporting here results of transmission studies of the NPV within and between loblolly pine sawfly colonies on loblolly pine, *Pinus taeda* L.

## MATERIALS AND METHODS

The tests were conducted in the spring of 1984 and 1985 in Calhoun County, Arkansas. The area is predominantly loblolly pine forests with historically high loblolly pine sawfly populations in most years. Sawfly populations in the study area were much higher in 1984 than in 1985. The NPV was endemic in sawfly populations in this area in 1984 and 1985.

Test 1 (1984) — Intra-colony transmission of NPV was examined via release of primary infected larvae (PIL) previously treated with NPV to die predominantly as first instars (PIL1) or predominantly as third instars (PIL3). The PIL larvae released had been treated with NPV as newly enclosed first instars for PIL1 or second instars for PIL3 by tagging and spraying selected colonies and surrounding foliage with a dosage of $10^3$ polyhedral inclusion bodies (PIB)/ml, using a 7.6-1 compressed air sprayer. Previous studies have shown this dosage of virus to result in 100% mortality from disease with infected larvae completing no more than one molt prior to death. The NPV sprayed had been produced and purified as described by Yearian and Young (1971). Three days after spraying the PIL-larvae, most of the terminals containing these colonies were clipped and transferred to the test site. The remaining colonies were observed to determine if the larvae died at the desired size.

Individual PIL were transferred to untreated colonies of the same age, using 00 size camel's hair brushes (PIL1) or soft-touch forceps (PIL3). The PIL were released at densities of 0, 1, 4, or 16 PIL/colony for each of the two PIL age classes (PIL1 or PIL3); 10 colonies per treatment were used. Colonies were observed in the field until virus symptoms were detected in the non-PIL members. Symptoms were inactivity and a yellow discoloration of the abdominal segments (Aizawa 1963). All colonies were then collected by clipping the branch and placing the colony in a brown paper bag. Additional foliage was added after which the bag was stapled closed and taken to the laboratory. The colonies were held in the bags at room temperature until all larvae had either died or formed cocoons. When needed, additional fresh foliage was collected from a loblolly forest in which sawflies were not present. The number of larvae per colony and larval instar at death were determined for each colony.

Test 2 (1985) — Inter-colony transmission of NPV was examined in an open stand (trees not touching) of loblolly pine (ca. 8 m in height). The procedures were generally as given for Test 1. Trees selected for the test contained sawfly colonies in both the upper and lower canopy. Colony density was sufficiently low, however, so that colonies remained isolated until larvae were large. Colonies selected in the upper canopy were in the terminal leaders and those in the lower canopy were ca. 4 m above ground level. PIL, previously infected as in Test 1 so that death occurred as either PIL1,2 or PIL3,4 were inserted in colonies, at densities of 0, 1, 4, or 16 PIL/colony for each of the two PIL classes in either the upper or lower canopy. When most larvae were early fifth instars, the colony with PIL as well as

two colonies without PIL (one each from the upper and lower canopies) were collected. Colonies selected without PIL for high colonies were on terminals no more than 2 m below the leader terminal and those selected for low colonies were generally no more than 2 m above the lowest terminal. The collected colonies were handled and data taken as in Test 1. Data were analyzed by ANOVA and Duncan's Multiple Range Test.

## RESULTS

Test 1 (1984) — The presence of PIL1 or PIL3 in a sawfly colony resulted in transmission of NPV and death of other members of the colony at all PIL density levels. The mortality of non-PIL larvae in these colonies varied from 37% to 98% with a mean of 73% (Table 1). Mean percent mortality was related to PIL density, increasing from 45% at a density of 1 PIL/colony to 95% at 16 larvae/colony. Mean larval mortality was not significantly related to PIL size at death. The mortality in the treatment without PIL was 3.5%. The mean instar at death for larvae in all treatments with 0 PIL was 4.2 and did not differ significantly between treatments (data not shown).

Table 1. Mortality (%) from nuclear polyhedrosis virus in loblolly pine sawfly colonies* following introduction and subsequent death of primary infected larvae (PIL).

| No. of PIL introduced per colony | Colony mortality (%)† | | |
|---|---|---|---|
| | PIL1 | PIL3 | Combined |
| 1 | 52 c | 37 c | 45 C |
| 4 | 80 ab | 76 b | 78 B |
| 16 | 98 a | 93 a | 95 A |
| Combined (1 - 16 PIL) | 76 A‡ | 69 A | 73 |

* Mean number of larvae per colony collected was 66.9.

† Mortality of non-PIL in colonies after introduction of PIL1 and PIL3 which were predominantly first and third instar at death, respectively.

‡ Data were corrected (Abbott 1925) for 3.5% mortality that occurred in the treatment with 0 PIL. Means within column and row followed by the same lower case letter(s) are not significantly different ($P > 0.05$), and means within column or row followed by the same upper case letter are not significantly different ($P > 0.05$), Duncan's multiple range test, after Arcsin percentage transformation.

Test 2 (1985) — Most colonies in which PIL had been released contained at least some cadavers at the time of collection, with many containing no live larvae. The presence of one or more PIL in a single colony in a tree generally resulted in low levels of transmission to other colonies within that tree; treatment mortalities ranged from 2% to 50%, and averaged ca. 15% (Table 2). Percent mortality in all treated colonies did not differ significantly with canopy location (upper or lower) of the treated colony or with density of PIL in the treated colony. The mean percent mortality in treatments did differ with location (high or low) of the collected colony (Table 2). Mean percent mortality was higher when the PIL-treated colony was located in the lower canopy (18.9%, averaged across all densities) than in the upper canopy (11.3%) ($P < 0.05$).

Table 2. Inter-colony transmission of nuclear polyhedrosis virus from a loblolly pine sawfly colony containing primary infected larvae (PIL) to other colonies on the tree: Effect of PIL density and treated colony location on % larval mortality in the non-PIL colony collected.*

| Canopy location of the collected non-PIL colonies | Colony mortality (%) by canopy location in which PIL were released and PIL density | | | | | | |
|---|---|---|---|---|---|---|---|
| | Upper Canopy | | | Lower Canopy | | | Mean |
| | 1 PIL | 4 PIL | 16 PIL | 1 PIL | 4 PIL | 16 PIL | Upper-Lower |
| | *Percent mortality in the non-PIL colony collected* | | | | | | |
| PIL1,2 at death | | | | | | | |
| Upper | 8.7 cdef† | 24.4 bc | 15.1 cdef | 50.4 a | 7.4 cdef | 15.3 cdef | 20.6 A |
| Lower | 4.6 ef | 5.0 ef | 24.1 bcd | 7.2 cdef | 34.9 ab | 7.6 cdef | 13.2 B |
| PIL3,4 at death | | | | | | | |
| Upper | 17.6 cdef | 6.3 def | 2.2 f | 3.7 f | 40.3 a | 22.2 cdef | 15.4 AB |
| Lower | 17.2 cdef | 1.7 f | 6.0 def | 12.1 cdef | 23.5 bcd | 2.0 f | 10.4B |
| Mean (Instar - Location) | 12.1 A | 9.9 A | 8.6 A | 18.4 A | 26.6 A | 11.8 A | 14.9 |

* Mean number of larvae per colony: 39 PIL1,2 treatments and 32 PIL3,4 treatments.

† Data were corrected (Abbott 1925) for 2.2% mortality that occurred in the treatment with 0 PIL. Treatment means within columns and rows followed by the same lower case letter(s) are not significantly different ($P > 0.05$) and means within column or row followed by the same upper case letter(s) are not significantly different ($P > 0.05$); Duncan's multiple range test after Arcsin percentage transformation.

Averaging over the entire trees, mean percent was significantly higher in non-treated colonies collected from the upper canopy (20.6%) in PIL1,2 treatments than in those from the lower canopy in either PIL1,2 (13.2%) or PIL3,4 (10.4%) treatments. The mean percent mortality from non-treated colonies collected from the upper canopy in PIL3,4 treatments (15.4%) was intermediate (Table 2). Also, the percent mortality in non-treated colonies for all treatments (PIL1,2 and PIL3,4) collected from the upper canopy (18.0%) was significantly higher than for treatments collected from the lower canopy (11.8%) ($P < 0.05$). The mortality in the treatments with 0 PIL was 2.2%.

## DISCUSSION

Transmission of NPV within a host population is dependent on both primary transmission and secondary transmission, and with the exception of very early mortality, it is difficult to distinguish the two (Bird 1953). We examined secondary transmission of NPV of *N. taedae linearis* by artificial inoculation of colonies with virus using PIL. Results showed a high level of secondary transmission of NPV from the PIL to other members within a colony (intra-colony), with up to 50% of the larvae in the colony succumbing to the virus following the introduction of a single PIL. The percent mortality increased with the density of PIL introduced into the colony but did not differ when PIL died as first or third instar. These results suggest that infection of individuals within a colony at hatch is not necessary to obtain high levels of secondary transmission within a colony. Primary infection can be delayed until at least the second instar when PIL that die as PIL3 are treated. The high level of secondary transmission within a loblolly pine sawfly colony may be related to the gregarious feeding behavior of larvae in the colony, with larvae feeding together on needles until they approach maturity. Bird (1955) attributed the density-dependent nature of disease outbreaks in *N. sertifer* to the eggs being laid in clusters and the larvae feeding gregariously. In contrast, he noted that in *Gilpinia hercyniae* Htg., which lays eggs singly and larvae feed singly, the epizootics were not density dependent.

Entwistle et al. (1983) found that transmission between larvae of *G. hercyniae* was not necessarily delayed until death of the PIL, but that active virus, supposedly released from the infected midgut cells into the gut lumen, could be regurgitated. A regurgitating behavior is routinely used by sawfly larvae as a defense mechanism against predators. If regurgitant of the loblolly pine sawfly contains virulent NPV, then transmission should occur earlier and to higher levels within a colony than would occur if noninfected individuals were first exposed to the NPV following death of PIL.

Inter-colony transmission of NPV from larvae in the colony containing PIL to other non-treated colonies within the tree was much lower than intra-colony transmission. Inter-colony transmission was greater when the non-treated colony was located in the upper rather than in the lower canopy or when the treated colony was located in the lower rather than in the upper canopy. This higher level of inter-colony transmission from PIL colonies low in the tree may be explained by the behavioral patterns of larvae infected with baculovirus. Larvae during later stages of disease tend to move higher in the canopy of the host plant. This could lead to establishment of infected larvae in other colonies in the upper canopy. Bird (1953) reported that disease spread in sawfly populations that feed gregariously

is density dependent. If this is true for NPV infections of loblolly pine sawfly, then conditions in the present study in 1985 may have restricted inter-colony transmission. Sawfly colony size and population density were low in 1985. Hence, there was adequate foliage that allowed the colonies to remain intact and thus isolated during development.

## ACKNOWLEDGMENTS

We thank Gabrielle Boys, John Guerber, and Kathy Wood for their technical assistance.

## LITERATURE CITED

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-67.

Aizawa, K. 1963. The nature of infections caused by nuclear polyhedrosis viruses. *In* E. A. Steinhaus [ed.]. Insect Pathology, Vol. 1, Academic Press, N.Y. 661 pp.

Anonymous. 1972. Insects and diseases of insects of the south. USDA For. Serv. SE. 81 p.

Bird, F. T. 1953. The use of virus disease in the biological control of the European pine sawfly, *Neodiprion sertifer* (Geoffr). Can. Entomol. 85: 437-46.

Bird, F. T. 1955. Virus diseases of sawflies. Can. Entomol. 87: 124-27.

Cunningham, J. C. 1982. Field trials with baculoviruses: Control of forest insect pests. *In* Microbial and Viral Pesticides [ed.], E. Kurstak. Marcel Dekker, Inc. New York, pp. 335-86.

Entwistle, P. F., P. H. W. Adams, and H. F. Evans. 1983. Employing defensive regurgitation to estimate levels of nuclear polyhedrosis virus infection in *Gilpinia hercyniae* (Hymenoptera: Diprionidae) larvae. J. Invertebr. Pathol. 41: 262-64.

Kaupp, W. J. 1983. Persistence of *Neodiprion sertifer* (Hymenoptera: Diprionidae) nuclear polyhedrosis virus on *Pinus cortorta* foliage. Can. Entomol. 115: 869-73.

Smirnoff, W. A. 1961. A virus disease of *Neodiprion swainei* Middleton. J. Invertebr. Pathol. 3: 29-46.

Yearian, W. C., and S. Y. Young. 1971. Virus control of the Arkansas pine sawfly. Ark. Farm Res. No. 20, 3 p.

Yearian, W. C., S. Y. Young, and J. M. Livingston. 1973. Field evaluation of a nuclear polyhedrosis virus of *Neodiprion taedae linearis*. J. Invertebr. Pathol. 22: 34-37.

Young, S. Y., and W. C. Yearian. 1984. Effect of nuclear polyhedrosis virus application rate and timing on mortality of *Neodiprion taedae linearis* Ross on pine. J. Georgia Entomol. Soc. 19: 265-71.