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NATURAL OCCURRENCE OF THE NUCLEOPOLYHEDROSIS VIRUS OF THE GYPSY MOTH, *LYMANTRIA DISPAR* [*LEP.* : *LYMANTRIIDAE*] IN WILD BIRDS AND MAMMALS (1)

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Three species of birds and 5 species of mammals were captured in the wild from 2 plots in which mortality from naturally occurring nucleopolyhedrosis virus (NPV) among gypsy moth, *Lymantria dispar* (L.), larvae was 15 % and 70 %. Bioassays of intestinal contents showed that blue jays, *Cyanocitta cristata* (L.), towhees, *Pipilo erythrophthalmus* (L.), white-footed mice, *Peromyscus leucopus* (RAFINESQUE), redback voles, *Clethrionomys gapperi* (VIGERS), raccoons, *Procyon lotor* (L.), and a chipmunk, *Tamias striatus* (L.), contained infectious NPV (polyhedra) in their alimentary tracts, whereas robins, *Turdus migratorius* (L.), and masked shrews, *Sorex cinereus* (KERR), did not. Comparisons among mice and voles indicated that those collected from the plot in which the NPV mortality was greatest (70 %) contained the most virus. We concluded that birds and mammals can passively transport infectious gypsy moth NPV in the wild.

Nucleopolyhedrosis viruses (NPVs) are among the primary natural regulators in dense populations of many insect species, and some appear promising as applied control agents in pest management programs. Understanding the epidemiology of these viruses is critical to predicting population trends and implementing appropriate pest management strategies. Recent studies have emphasized the mechanisms of NPV transmission within and between insect generations. Physical factors, such as wind and rain, have been implicated (BIRD, 1961; LONGWORTH, 1973; JAQUES, 1975). NPVs are also transported by parasitic and predacious insects (VAGO *et al.*, 1966; BERGOIN, 1966; SMIRNOFF, 1975; CAPINERA & BARBOSA, 1975; RAIMO *et al.*, 1977) as well as by the migration of infected larvae and adults (BIRD, 1961; SMIRNOFF, 1962; NEILSON & ELGÉE, 1968; LONGWORTH, 1973; BUSE, 1977). Vertical vectoring of NPV is effected by transovum and possibly transovarial transmission (DOANE, 1969, 1970; HAMM & YOUNG, 1974; NEELGOND &

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MATHAD, 1978), although the possibility of transovarial transmission has been seriously questioned (DAVID & TAYLOR, 1976).

Vertebrates can be passive vectors of insect viruses. Birds have been identified as having the potential for transporting NPV within contaminated ecosystems (BIRD, 1955; FRANZ *et al.*, 1955; HOSTETTER & BIEVER, 1970; SMIRNOFF, 1975) and recently have been shown to transport NPV of *Gilpinia hercyniae* (HARTIG) up to 6 km away from populations experiencing NPV epizootics (ENTWISTLE *et al.*, 1977). Until recently, the role of mammals in NPV epidemiology has been virtually ignored. LAUTENSCHLAGER & PODGWAITE (1977) demonstrated that 2 small mammal predators of the gypsy moth, *Lymantria dispar* (L.),.passed virulent gypsy moth NPV through their alimentary tracts, suggesting that small mammals could distribute infectious NPV in the wild. The amount of gypsy moth NPV that 5 species of wild mammals and 3 species of birds passed and the period of passage were reported later (LAUTENSCHLAGER & PODGWAITE, 1979). They concluded in that paper that mammals and birds pass significant amounts of NPV and that both have features that contribute to their ability to transport NPV. However, no evidence that birds and mammals actually carry gypsy moth NPV in the wild has been reported.

This is a report on the incidence of infectious gypsy moth NPV in wild birds and mammals and the relative potential of these vertebrates to transport the virus in the wild.

MATERIALS AND METHODS

COLLECTION

Animals were collected from 2 untreated 14-ha plots in the Bald Eagle State Forest, Mifflinburg, Pennsylvania, and examined for the presence of gypsy moth NPV. These animals also served as controls in studies of the safety of NPV for mammals and birds (LAUTENSCHLAGER *et al.*, 1978, 1979). Although the 2 study plots were not treated with NPV, gypsy moth populations therein suffered varying degrees of natural NPV mortality reaching epizootic proportions in 1 plot.

Birds and mammals were collected during the latter half of July when gypsy moth pupae and adults were predominant, and when few NPV-infected gypsy moth larvae were available as a food source. Robins, *Turdus migratorius* (L.); blue jays, *Cyanocitta cristata* (L.); and towhees, *Pipilo erythrophthalmus* (L.), were collected by shotgun and transported within 5 h of death to the veterinary pathology laboratory of Pennsylvania State University. White-footed mice, *Peromyscus leucopus* (RAFINESQUE); redback voles, *Clethrionomys gapperi* (VIGERS); chipmunks, *Tamias striatus* (L.); masked shrews, *Sorex cinereus* (KERR); and raccoons, *Procyon lotor* (L.), were live-trapped and transported to the laboratory within 24 h of capture. Following necropsy, the stomachs and lower gastrointestinal tracts of study animals were excised, packaged, and frozen at —15°C.

BIOASSAYS

Organs were thawed and $\leq$ 2-g portions were individually homogenized for 2 mn in 2 ml of sterile distilled water in sterile tissue grinders. The intestinal contents were homogenized with the tissue. Each homogenate was bioassayed against 5 replicates of 10 newly molted 2nd-stage laboratory stock gypsy moth larvae (Pennsylvania strain) reared in sterile plastic Petri dishes. For bioassay, 0.03 ml aliquots of test homogenates were applied to the 5 exposed surfaces of each of two 1.25 cm^3 artificial diet blocks (ODELL & ROLLINSON, 1966) placed on the inside top of each plastic Petri dish. The treated

blocks were left undisturbed for 1 h to allow absorption of the test material. Test larvae were allowed to feed on the treated diet for 48 h, after which the treated diet was removed and replaced with untreated diet. Control larvae were fed untreated diet. Other groups of 50 larvae were fed diet containing known concentrations (LC_{25}-LC_{75}) of gypsy moth NPV (Hamden Standard K) (LEWIS & ROLLINSON, 1978). All larvae were reared for 16 days at 23 ± 2°C and a 16-h photoperiod. Larvae were checked daily for mortality, and deaths from NPV were confirmed microscopically.

A tissue sample was considered to show NPV activity if its homogenate elicited significantly more mortality ($p < 0.05$, logit chi-square analysis) than that measured in control larvae. The number of polyhedral inclusion bodies (PIB)/g of tissue that this activity represented was estimated by extrapolation from dose-mortality curves generated from Hamden Strain K NPV bioassay data (LEWIS & ROLLINSON, 1978).

RESULTS AND DISCUSSION

Bioassays showed that the large and small intestines of both of the blue jays examined contained active NPV (table 1). The estimate of 2.37×10^7 PIB/g of large intestine was the highest level of PIB in any bird or mammal. Only the large intestine of 1 of the 2 towhees examined contained the virus and NPV activity was not observed in the bioassay of robin intestines.

TABLE 1

Incidence and estimated amounts of gypsy moth NPV in the alimentary tracts of 3 bird species collected from areas of natural NPV outbreaks

Species	No. examined	Stomach		Small intestine		Large intestine	
		No. with NPV	PIB/g [a]	No. with NPV	PIB/g [a]	No. with NPV	PIB/g [a]
Blue jay	2	--	--	2	$5.15 \pm 3.44 \times 10^5$	2	$2.17 \pm 2.37 \times 10^7$
Robin	2	--	--	0	0.0	0	0.0
Towhee	2	0 [b]	0.0	0	0.0	1	2.43×10^5

(a) Polyhedral inclusion bodies/g tissue as estimated from bioassays (mean ± standard error)
(b) Only one stomach bioassayed.

Only 1 of the 3 raccoons tested contained NPV in its small intestine but all 3 harbored the virus at relatively low levels (4×10^4 PIB/g) in their large intestines (table 2). The intestines of the 2 masked shrews examined contained no active NPV. This was surprising since shrews, *Blarina brevicauda* (SAY) and *Sorex* spp., are known to be important predators of the gypsy moth (SMITH & LAUTENSCHLAGER, 1978). It is likely that shrews do eat infected gypsy moths in the wild, but the sample size did not allow a clear interpretations of the shrew's role in this ecosystem. The large intestine of the 1 chipmunk tested contained active NPV.

NPV was detected in the alimentary tracts of the majority of the white-footed mice and redback voles examined (table 3). The virus was found in the small intestines of 40 % of the mice and 71 % of the voles, while 73 % of the mice and all of the voles harbored NPV in their large intestines. The samples of mice and voles were large enough to allow

TABLE 2

Incidence and estimated amounts of gypsy moth NPV in the lower alimentary tracts of three mammal species collected from areas of natural NPV outbreaks

Species	No. examined	Small intestine		Large intestine	
		No. with NPV	PIB/g [a]	No. with NPV	PIB/g [a]
Raccoon	3	1	2.50×10^4	3	$2.66 \pm 1.20 \times 10^4$
Shrew	2	0	0.0	0	0.0
Chipmunk	1	0	0.0	1	4.29×10^5

(a) Polyhedral inclusion bodies/g tissue as estimated from bioassay (mean ± standard error).

comparison of the estimated PIB levels between animals taken from plot 1 and those taken from plot 14 (table 3). Plot 1 harbored a low-density gypsy moth population and only 15 % of the natural larval mortality in this plot was caused by NPV. The gypsy moth population in plot 14 was of intermediate density and natural NPV mortality in this plot reached 70 % (WOLLAM *et al.*, 1978). Although the likelihood of animals coming in contact with and digesting NPV appeared to be greater in plot 14 than in plot 1, t-tests of mean PIB estimates revealed no significant differences ($p > 0.05$) between animals collected from the 2 plots. However, t-tests did reveal differences in large intestine PIB levels of significance, $p < 0.08$ and $p < 0.17$, for mice and voles respectively, indicating that animals from plot 14 may have ingested more NPV than those from plot 1.

TABLE 3

Incidence and estimated amounts of gypsy moth NPV in the alimentary tracts of two small mammal species collected from areas of natural NPV outbreaks

Species	Plot	Total no.	Stomach		Small intestine		Large intestine	
			No. with NPV / No. examined	PIB/g [a]	No. with NPV / No. examined	PIB/g [a]	No. with NPV / No. examined	PIB/g [a]
White-footed mouse	1	22	1/2	2.37×10^3	10/22	$2.00 \pm 1.40 \times 10^5$	14/22	$6.95 \pm 4.22 \times 10^5$
	14	8	1/1	1.20×10^6	2/8	$2.59 \pm 2.54 \times 10^5$	8/8	$3.31 \pm 2.75 \times 10^6$
Redback vole	1	4	—	—	3/4	$1.48 \pm 0.94 \times 10^5$	4/4	$7.61 \pm 2.80 \times 10^5$
	14	11	3/3	$1.67 \pm 0.84 \times 10^7$	7/10	$3.58 \pm 2.94 \times 10^5$	11/11	$9.61 \pm 4.27 \times 10^6$

(a) Polyhedral inclusion bodies/g tissue as estimated from bioassays (mean ± standard error).

The data presented here verify the suggestion from an earlier laboratory study (LAUTENSCHLAGER & PODGWAITE, 1979) that wild birds and mammals ingest infectious NPV

and maintain it within their alimentary tracts. Since the animals in this study were collected when NPV-infected gypsy moth larvae were not available as food, it is probable that the majority of the NPV ingested and maintained in these animals was from alternate food sources (berries, seeds or other plant materials) that had been contaminated with NPV from larval cadavers. This could account for the high incidence and amounts of NPV in redback voles, animals that are primarily herbivorous.

Following an NPV epizootic, most of the virus is deposited in the forest litter and soil, where it remains infectious for at least a year (PODGWAITE *et al.*, 1979). The litter is a reservoir of NPV and it is probable that birds and mammals feeding there not only ingest the virus but also carry it externally on hair, feathers, bills, and feet, from which it is washed or shaken loose later. Although there are no field data supporting this hypothesis, other biotypes, notably algal species, have been shown to be transported in this manner (PROCTOR, 1959; SCHARF, 1978).

The potential of mammals to transport gypsy moth NPV should not be underestimated. Since the litter is the primary habitat of small mammals, they are likely candidates in NPV dispersal. Also, in northeastern North America, mammals are normally much more numerous than birds in most areas (SMITH & LAUTENSCHLAGER, 1978) and they are year-round residents, available at all times to passively transport NPV.

Although the evidence we have presented indicates that birds and mammals can transport infectious NPV in the wild, it should be emphasized that this is not the only way NPV is transported from one area to another, or within an ecosystem. Obviously, the other potential means of transport : rain and wind, parasites, predacious insects, dispersal of infected larvae and adults, transovum and possibly transovarial transmission-may all be important, and any reasonable hypothesis of NPV epidemiology will account for these mechanisms. The movement of infectious NPV in the wild likely results from a combination of all these mechanisms, with the prime sources of movement varying with the biological and physical conditions in the ecosystem.

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RÉSUMÉ

Présence naturelle du virus de la nucléopolyhédrose de *Lymantria dispar* [*Lep.* : *Lymantriidae*] chez les oiseaux et les mammifères sauvages

Trois espèces d'oiseaux et 5 espèces de mammifères ont été capturées en 2 endroits où la mortalité due à la présence naturelle du virus de la nucléopolyhédrose (VNP) chez les larves de *Lymantria dispar* (L.), était de 15 % et de 70 %. Le système digestif des geais bleux, *Cyanocitta cristata* (L.), guiraca à poitrine rose, *Pipilo erythrophthalmus* (L.), souris-au-pied-blanc, *Peromyscus leucopus* (RAFINESQUE), campagnols-au-dos-rouge, *Clethrionomys gapperi* (VIGERS), ratons laveurs, *Procyon lotor* (L.) et d'un tamia, *Tamias striatus* (L.), était infecté par le virus alors que celui des rouges-gorges, *Turdus migratorius* (L.) et des musaraignes, *Sorex cinereus* (KERR), ne l'était pas. Une comparaison entre les campagnols et les souris a indiqué que les animaux provenant de l'endroit où la mortalité par virose était la plus forte (70 %) contenaient le plus de virus. Nous concluons que les oiseaux et les mammifères peuvent transporter passivement dans la nature les polyèdres nucléaires de *L. dispar*.

REFERENCES

BIRD, F. T. — 1955. Virus diseases of sawflies. — *Can. Entomol.*, 87, 124-127.

— 1961. Transmission of some insect viruses with particular reference to ovarial transmission and its importance in the development of epizootics. — *J. Insect Pathol.*, 3, 352-380.

BERGOIN, M. — 1966. Passage des corps d'inclusion de virus d'une polyédrie nucléaire dans le tube digestif d'un orthoptère détritivore, *Acheta domesticus* L. — *Entomophaga*, 11, 253-259.

BUSE, A. — 1977. The importance of birds in the dispersal of nuclear polyhedrosis virus of European spruce sawfly, *Gilpinia hercyniae* [*Hymenoptera* : *Diprionidae*] in mid-Wales.—*Entomol. Exp. Appl.*, 22, 191-199.

CAPINERA, J. L. & BARBOSA, P. — 1975. Transmission of nuclear-polyhedrosis virus to gypsy moth larvae by *Calosoma sycophanta*. — *Ann. Entomol. Soc. Am.*, 68, 593-594.

DAVID, W. A. L. & TAYLOR, C. E. — 1976. Transmission of a granulosis virus in the eggs of a virus-free stock of *Pieris brassicae*. — *J. Invertebr. Pathol.*, 27, 71-75.

DOANE, C. C. — 1969. Trans-ovum transmission of a nuclear-polyhedrosis virus in the gypsy moth and the inducement of virus susceptibility. — *J. Invertebr. Pathol.*, 14, 199-210.

— 1970. Primary pathogens and their role in the development of an epizootic in the gypsy moth. — *J. Invertebr. Pathol.*, 15, 21-33.

ENTWISTLE, P. F., ADAMS, P. H. W. & EVANS, H. F. — 1977. Epizootiology of a nuclear-polyhedrosis virus in European spruce sawfly, *Gilpinia hercyniae* : birds as dispersal agents of the virus during winter. — *J. Invertebr. Pathol.*, 30, 15-19.

FRANZ, J., KRIEG, A. & LANGENBUCH, R. — 1955. Untersuchungen über den Einfluss der Passage durch den Darm von Raubinsekten und Vögeln auf die infektiosität insektenpathogener Viren. — *Z. Pflanzenkr. Pflanzenpathol. Pflanzenschutz.*, 62, 721-726.

HAMM, J. J. & YOUNG, J. R. — 1974. Mode of transmission of nuclear-polyhedrosis virus to progeny of adult *Heliothis zea*. — *J. Invertebr. Pathol.*, 24, 70-81.

HOSTETTER, D. L. & BIEVER, K. D. — 1970. The recovery of virulent nuclear-polyhedrosis virus of the cabbage looper, *Trichoplusia ni*, from the feces of birds. — *J. Invertebr. Pathol.*, 15, 173-176.

JAQUES, R. P. — 1975. Persistence, accumulation, and denaturation of nuclear polyhedrosis and granulosis viruses. In : Baculoviruses for Insect Pest Control : Safety Considerations (M. SUMMERS, R. ENGLER, L. A. FALCON, & P. V. VAIL, eds). — *Am. Soc. Microbiol.*, Washington, D. C., 90-99.

LAUTENSCHLAGER, R. A. & PODGWAITE, J. D. — 1977. Passage of infectious nuclear polyhedrosis virus through the alimentary tracts of two small mammal predators of the gypsy moth, *Lymantria dispar*. — *Environ. Entomol.*, 6, 737-738.

— 1979. Passage of nucleopolyhedrosis virus by avian and mammalian predators of the gypsy moth, *Lymantria dispar* L. — *Environ. Entomol.*, 8, 210-214.

LAUTENSCHLAGER, R. A., ROTHENBACHER, H. & PODGWAITE, J. D. — 1978. Response of small mammals to aerial applications of the nucleopolyhedrosis virus of the gypsy moth, *Lymantria dispar*. — *Environ. Entomol.*, 7, 676-684.

— 1979. Response of birds to aerial applications of nucleopolyhedrosis virus of the gypsy moth. — *Environ. Entomol.*, 8, 760-764.

LEWIS, F. B. & ROLLINSON, W. D. — 1978. Effect of storage on the virulence of gypsy moth nucleopolyhedrosis inclusion bodies. — *J. Econ. Entomol.*, 71, 719-722.

LONGWORTH, J. F. — 1973. Viruses and invertebrates. In : Viruses and Lepidoptera (North-Holland Research Monographs, Frontiers of Biology). — *North-Holland Publ.*, Amsterdam, vol. 31, 429-441.

NEELGOND, Y. F. & MATHAD, S. B. — 1978. Transmission of nuclear polyhedrosis virus in laboratory populations of the armyworm, *Mythimma (Pseudaletia) separata*. — *J. Invertebr. Pathol.*, 31, 143-147.

NEILSON, M. M. & ELGEE, D. E. — 1968. The method and role of vertical transmission of a nucleopolyhedrosis virus in the European spruce sawfly, *Diprion hercyniae*. — *J. Invertebr. Pathol.*, 12, 132-139.

ODELL, T. M. & ROLLINSON, W. D. — 1966. A technique for rearing the gypsy moth, *Porthetria dispar* (L.), on an artificial diet. — *J. Econ. Entomol.*, 59, 741-742.

PODGWAITE, J. D., SHIELDS, K. S., ZERILLO, R. T. & BRUEN, R. B. — 1979. Environmental persistence of the nucleopolyhedrosis virus of the gypsy moth, *Lymantria dispar* L. — *Environ. Entomol.*, 8, 528-536.

PROCTOR, V. W. — 1959. Dispersal of freshwater algae by migratory water birds. — *Science,* 130, 623-624.

RAIMO, B. J., REARDON, R. C. & PODGWAITE, J. D. — 1977. Vectoring gypsy moth nuclear polyhedrosis virus by *Apanteles melanoscelus* [*Hym.* : *Braconidae*]. — *Entomophaga,* 22, 207-215.

SCHARF, C. S. — 1978. Birds and mammals as passive transporters for algae found in lichens. — *Can. Field Nat.*, 92, 70-71.

SMIRNOFF, W. A. — 1962. Trans-ovum transmission of virus of *Neodiprion swainei* MIDDLETOWN [*Hymenoptera : Tenthredinidae*]. — *J. Insect Pathol.*, 4, 192-200.

— 1975. Parasites and predators as vectors of insect viruses. In : Baculoviruses for Insect Pest Control : Safety Considerations (M. SUMMERS, R. ENGLER, L. A. FALCON & P. V. VAIL, eds). — *Am. Soc. Microbiol.*, 131-133.

SMITH, H. R. & LAUTENSCHLAGER, R. A. — 1978. The predators of the gypsy moth. — *U. S. Dep. Agric. Inf. Bull.*, 534, 72 pp.

VAGO, C., FOSSET, J. & BERGOIN, M. — 1966. Dissemination des virus de polyédries par les éphippigères predateurs d'insectes. — *Entomophaga,* 11, 177-181.

WOLLAM, J. D., YENDOL, W. G. & LEWIS, F. B. — 1978. Evaluation of aerially applied nuclear polyhedrosis virus for suppression of the gypsy moth, *Lymantria dispar* L. — *U. S. Dep. Agric. For. Serv. Res. Pap.* NE-396, 8 pp.