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VECTORING GYPSY MOTH NUCLEAR POLYHEDROSIS VIRUS BY *APANTELES MELANOSCELUS* [*HYM.* : *BRACONIDAE*] (1)

B. RAIMO (2), R. C. REARDON (3) & J. D. PODGWAITE (4)

(2) Dept. of Entomology, University of New Hampshire, Durham, NH 03824, U.S.A.

(3) Gypsy Moth Program, Northeast Area State & Private Forestry, Forest Service, Hamden, CT 06514, U.S.A.

(4) Northeastern Forest Experiment Station, Forest Service, Hamden, CT 06514, U.S.A.

Gypsy moth *Lymantria dispar* L. larvae were exposed to *Apanteles melanoscelus* (RATZEBURG) females contaminated with nuclear polyhedrosis virus. Three methods of contamination (ovipositor, total body surface, and exposure to infected hosts) and two exposure periods (2 and 24 hours) were tested. A significantly greater incidence of larval mortality caused by virus occurred among larvae exposed to contaminated than among larvae exposed to uncontaminated parasites for 2 and 24 hours. No significant differences occurred in larval mortality caused by virus for the 3 methods of contamination for the 2- and 24-hour tests or in parasite emergence from larvae parasitized by contaminated or uncontaminated parasites.

In the northeastern United States, epizootics of nuclear polyhedrosis virus (NPV) have been associated with reduction of dense populations of gypsy moth, *Lymantria dispar* LINNEAUS (*Lep.* : *Lymantriidae*). The U.S. Department of Agriculture is currently collecting safety, efficacy, and environmental impact data necessary to register the virus for aerial application to suppress the gypsy moth.

Current methods of applying microbial insecticides for gypsy moth suppression are similar to those used in chemical control programs : topical applications of sprays and dusts (ORLOVSKAJA, 1961; GRIGOROVA, 1962; ROLLINSON *et al.*, 1965; MAGNOLER, 1968, 1974; CARDINAL & SMIRNOFF, 1973). These methods when used alone may not be feasible for disseminating virus over large areas because of the current cost of producing virus. Augmenting these traditional methods of virus dissemination, therefore, with an effective means of transmitting the pathogen from a focal point, is desirable.

The objectives of this study were to : (1) show that an established parasite of the gypsy moth has the ability to transmit NPV and successfully infect gypsy moth larvae, and (2) develop methods of contaminating parasites which could augment efforts to initiate epizootics artificially in populations of the gypsy moth.

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Parasites and predators have often been cited as important vectors of insect pathogens (METALNIKOV & METALNIKOV, 1935; THOMPSON & STEINHAUS, 1950; SMITH *et al.*, 1956; SMIRNOFF, 1959, 1961; TANADA, 1964; MAGNOLER, 1968). In at least one instance, parasites are considered to have been responsible for introducing an NPV into North America from Europe (BALCH & BIRD, 1944; BALCH, 1958). One method of transmitting the disease from one host to another is by the contaminated ovipositor of a parasite. The ovipositor can act as an inoculating needle, injecting pathogens into susceptible hosts (PAYNE, 1933; BILIOTTI, 1956). THOMPSON & STEINHAUS (1950) showed that the ovipositor of *Apanteles medicaginis* MUESEBECK, after it was contaminated by stinging a virus-infected larva, could infect a series of 2 or 3 larvae of *Colias eurytheme* BOISDUVAL. BELL *et al.* (1974) demonstrated the transmission of *Serratia marcescens* — from infected *Heliothis zea* (BODDIE) larvae to healthy larvae —, by the ovipositor of *Micropilitis croceips* CRESSON.

MATERIALS AND METHODS

The parasite used in this study was *Apanteles melanoscelus* (RATZEBURG) (*Hym.* : *Braconidae*), an oligophagous larval parasite introduced into New England from Europe in 1911 (CROSSMAN, 1922). *A. melanoscelus* has 2 generations per year in New England. First generation females emerge from overwintering cocoons in May and parasitize 1st and 2nd stage gypsy moth larvae. Females of the 2nd generation parasitize 3rd and 4th stage larvae.

The New Jersey Department of Agriculture supplied colonies of *A. melanoscelus* which were maintained in the laboratory at 20°C and 50 % relative humidity, and supplied with a honey-water solution as a food source. We used gypsy moth larvae which were reared from field-collected eggs, depilated and surface-sterilized by methods described by LEONARD & DOANE (1966). Larvae were fed artificial diet (5) obtained from Bioserv ®, Inc., Frenchtown, New Jersey. To prevent diapause of the parasites, parasitized larvae were maintained at 20°C, 50 % relative humidity and 24-h photoperiod. Larvae were reared individually in 30 ml plastic jelly cups containing diet. Parasite cocoons were removed as soon as they were produced, but host larvae were kept in the jelly cups for 10 days after removal of cocoons, or until death.

Three methods of contaminating female parasites with NPV were tested : (1) manually contaminating the ovipositor of the parasite with virus suspension of a known concentration, (2) applying virus suspension to the total body surface of the parasite, and (3) exposing the parasite to infected larvae. In addition, one test group of parasites was not contaminated. The control group consisted of larvae that were not exposed to parasites. Host larvae were exposed to parasites for both 2 and 24 hours, and we made 5 replicates of each test. Each test was continued for 30 days after the hosts were initially exposed to parasites.

MANUAL CONTAMINATION OF THE OVIPOSITOR

After female parasites were cold-anesthetized (— 8°C for 2 minutes), their ovipositors were brushed with a cotton swab dipped in a virus suspension containing 1×10^9 polyhedral inclusions bodies (PIB's) per ml. Three contaminated females were placed in a 5 l. paper container with 10 2nd and 10 3rd stage gypsy moth larvae. The open end of the container was covered with organdy fabric held in place by an

(5) Mention of a particular product should not be taken as endorsement by the Forest Service or the U.S. Department of Agriculture.

elastic band. After being exposed to these parasites, larvae were removed from the container, placed in 30 ml plastic cups (one larva per cup), and reared on artificial diet 30 days — or until 10 days after parasite emergence, or until death. Larvae were examined daily and, when they died, we made smears of the cadavers stained with Giemsa (diluted 1 : 20). The smears were viewed under oil emersion at 1,000×. If more than 10 PIB's were viewed within the microscopic field virus was recorded as the cause of mortality.

Contamination of total body surface

To contaminate the total body surface with virus, an atomizer was used. The atomizer was held 10 cm from a Petri dish containing anesthetized female parasites. The mist contained 1 X 10^9 PIB's/ml.

Contamination by exposure to infected hosts

Three contaminated females were placed in a 5 l. container with 10 2nd and 10 3rd stage gypsy moth larvae. First and 2nd stage larvae were fed artificial diet containing 1×10^7 PIB's/ml for a period of 48 hours. Previous study showed that this dosage had an LT50 of 9 days. After 48 hours, this diet was removed and the larvae were fed uncontaminated diet. Seven days after the larvae had been infected, 10 2nd and 10 3rd stage infected larvae were placed in 5 l. paper container with 3 uncontaminated female *A. melanoscelus* (we assumed that, after 7 days, virus rods and PIB's would occur in the infected larva's hemolymph). Next, we exposed parasites to infected larvae for a maximum of 24 hours.

After exposure to infected larvae, 3 female parasites in each group were transferred to 5 l. paper containers containing 10 2nd and 10 3rd stage non-infected larvae. One group of parasites was exposed to non-infected larvae 2 hours, and a second group was exposed to non-infected larvae 24 hours. After exposure to the contaminated parasites, larvae were removed and placed in 30 ml plastic cups (one larva per cup) and were handled exactly as those in previous tests.

RESULTS

Virus mortality

Analysis of variance was used to determine significant differences in incidence of larval mortality caused by virus between treatment and control groups (table 1). The incidence of mortality caused by virus in larvae exposed to contaminated and uncontaminated parasites for 2 and 24 hours was significantly higher than that in larvae not exposed. The incidence of mortality caused by virus in larvae exposed to uncontaminated was compared to that in larvae exposed to contaminated parasites. When larvae were exposed to contaminated parasites for 2 hours, a significantly greater number of larvae died of virus. When larvae were exposed to contaminated parasites for 24 hours, however, there was no significant difference at the 5 % level. The incidence of virus in all larvae exposed to contaminated parasites (both 2 and 24 hours) was significantly higher than it was in all larvae exposed to uncontaminated parasites.

The mean percentages of larvae dying of virus in each of the 5 replicates for the 2 h tests were : 29.0 % of larvae exposed to parasites contaminated topically; 38.0 % of those exposed to parasites with contaminated ovipositors; and 16.0 % of those

TABLE 1

Gypsy moth larval mortality (in percent) for all tests

Replicate	Control	Treatments							
	larvae not exposed to *A. melanoscelus*	larvae exposed to uncontaminated *A. melanoscelus*		larvae exposed to totally contaminated *A. melanoscelus*		larvae exposed to *A. melanoscelus* with contaminated ovipositor		larvae exposed to *A. melanoscelus* exposed to infected host	
		2 hour	24 hour	2 hour	24 hour	2 hour	24 hour	2 hour	24 hour
1	1.25	2.5	5.0	25.0	20.0	10.0	15.0	12.5	20.0
2	0.0	5.0	15.0	15.0	10.0	15.0	65.0	10.0	22.5
3	2.5	7.5	20.0	45.0	45.0	60.0	45.0	12.5	25.0
4	1.25	32.5	5.0	25.0	80.0	55.0	15.0	37.5	17.5
5	13.75	0.0	5.0	35.0	20.0	50.0	65.0	7.5	30.0
Sign. 5% (*) level	ab	ac	bd	ac	bd	ac	bd	ac	bd

(*) a, b, c = sign. 5 % level; d = not sign. (F = 2.4893)

exposed to parasites previously exposed to infected larvae. The incidences of mortality caused by virus were higher for each of the 3 groups for the 24 h test : 35.0 %, 41.0 %, and 23.0 % respectively. Analysis of variance showed that, however, these percentages were not significant at the 5 % level.

Natural mortality caused by virus in larvae not exposed to parasites was 3.8 %. Of larvae exposed to uncontaminated parasites for 2 hours and 24 hours, 9.5 % and 10.0 %, respectively, died of virus.

Parasite emergence

The percentage of host larvae producing *A. melanoscelus* cocoons is shown in table 2. Analysis of variance indicated no significant differences in parasite emergence between larvae exposed to uncontaminated or contaminated parasites for 2 and 24 hours.

Total mortality

The percentage of total mortality among host larvae are shown in table 3. In the 2 hr exposure experiments, a significantly greater number of host larvae died when exposed to contaminated than when exposed to uncontaminated parasites. Larvae exposed to parasites for 24 hours produced the same results.

DISCUSSION

Apanteles melanoscelus was chosen in this study for several reasons : (1) it is well established and abundant throughout the range of the gypsy moth, (2) It is easily reared in the laboratory, (3) It can reproduce parthenogenetically, and (4) has high reproductive capacity (200 to 300 ovipositions per female). (5) First-generation females parasitize small larvae (instars I - III); therefore, the probability of maturation immunity to the virus infection in the host is greatly reduced. (6) The mechanics of oviposition, whereby the parasite's ovipositor enters the host's body cavity, is conducive to the successful transmission of a pathogen. Last (7), a positive correlation between the incidence of NPV and adult *A. melanoscelus* in gypsy moth populations has been demonstrated by Reardon & Podgwaite (1976).

Our tests demonstrated that *A. melanoscelus* was capable of vectoring lethal doses of NPV to gypsy moth larvae. Three modes of infection were possible : (1) intrahemocoelic inoculation of NPV inclusion bodies, by means of the contaminated ovipositor, (2) intrahemocoelic inoculation of NPV rods, by means of the contaminated ovipositor, and (3) the external contamination of gypsy moth larvae and/or gypsy moth diet by contaminated parasites.

Bergold (1947) identified the virus rod as the infectious agent of nuclear polyhedrosis viruses. To liberate the infectious virus particles the polyhedra must dissolve. Bergold stated that a solution with a pH less than 5 or more than 8.5 is needed to dissolve the polyhedra. Since the pH of the hemolymph of gypsy moth larvae is ca 6.5 (Lewis & Rollinson, 1961), some investigators believe that infection does not take place by the injection of polyhedra into the insect body cavity. Little is known about the physicochemical properties of polyhedra or of insect hemolymph, however, and despite speculation, it is not definite that polyhedra cannot dissolve in larval gypsy moth hemolymph. Lewis & Rollinson (1961) have shown that the pH of gypsy moth larvae-hemolymph is dependent not only on the larval age, but also on the larval condition (resting or feeding). The first possible mode of infection, the injection of PIB's into the body cavity, therefore, cannot be discounted.

TABLE 2

Parasite emergence (in percent) for all tests

Replicate	larvae exposed to uncontaminated *A. melanoscelus*		larvae exposed to totally contaminated *A. melanoscelus*		larvae exposed to *A. melanoscelus* with contaminated ovipositor		larvae exposed to *A. melanoscelus* exposed to infected host	
	2 hour	24 hour	2 hour	24 hour	2 hour	24 hour	2 hour	24 hour
1	27.5	52.5	35.0	20.0	30.0	80.0	20.0	22.5
2	32.5	45.0	50.0	90.0	65.0	35.0	25.0	32.5
3	67.5	65.0	30.0	10.0	10.0	60.0	19.0	30.0
4	32.5	77.5	40.0	30.0	15.0	75.0	15.0	22.5
5	55.0	55.0	40.0	75.0	45.0	10.0	20.0	40.0
Signif.(*)	a	b	a	b	a	b	a	b

(*) a = not sign. (F : 2.3829); b = not sign. (F : 1.7861)

TABLE 3

Total gypsy moth larval mortality (in percent) for all tests

Replicate	Control	Treatments							
	larvae not exposed to *A. melanoscelus*	larvae exposed to uncontaminated *A. melanoscelus*		larvae exposed to totally contaminated *A. melanoscelus*		larvae exposed to *A. melanoscelus* with contaminated ovipositor		larvae exposed to *A. melanoscelus* exposed to infected host	
		2 hour	24 hour	2 hour	24 hour	2 hour	24 hour	2 hour	24 hour
1	18.75	75.0	75.0	95.0	95.0	80.0	90.0	62.5	75.0
2	7.5	85.0	87.5	75.0	90.0	85.0	90.0	40.0	57.5
3	11.25	82.5	92.5	70.0	55.0	70.0	95.0	35.0	72.5
4	10.0	82.5	87.5	75.0	90.0	80.0	85.0	65.0	50.0
5	18.75	65.0	65.0	90.0	95.0	90.0	90.0	45.0	72.5
Sign. 5% (*) level		a	b	a	b	a	b	a	b

(*) a, b = sign. 5% level

If the mode of infection was the intrahemocoelic inoculation of NPV rods by the ovipositor, then viable rods must have been present either in the virus suspension (contaminated ovipositor and totally contaminated treatments) or in the infected larva's hemolymph (contamination by exposure to infected host treatment). Since the presence of some viable virus rods even in the most carefully purified suspension of NPV polyhedra is likely, this second mode of infection is quite possible.

Because PIB's may have been transferred to the gypsy moth larvae and/or diet by contaminated parasites, a third mode of infection is also possible. Since the purpose of this study was only to determine the capacity of the parasite to vector virus, further tests will be necessary to determine which of the possible modes of infection exist.

TANADA (1964) noted that the capacity of pathogens to spread throughout the host population or host environment is very important in the occurrence of epizootics among insects. Dispersal of pathogens can occur by movement of healthy carriers, infected hosts, parasites, predators, and by weather (wind, rain, snow, etc.). MAGNOLER (1974) observed the dispersal of virus after initial establishment and suggested that epizootics could occur over large areas by treating scattered plots with high concentrations. Releases of *A. melanoscelus* in such scattered treatment areas may be helpful not only in augmenting the dispersal of the disease but also in establishing centers of infection from which the disease may spread by other means.

WESELOH & ANDERSON (1975) have determined that *A. melanoscelus* is an effective parasite in low gypsy moth populations. They state that inundative releases of this parasite (6,000 individuals/ha) into areas where gypsy moth numbers are low can achieve immediate parasitism of 40 %. If the parasites were topically contaminated prior to such an inundation, a focal point of virus could be established in sub-epidemic populations of the gypsy moth.

WOLLAM & YENDOL (1976) have shown that, when *A. melanoscelus* was released in conjunction with *Bacillus thuringiensis* BERLINER, greater foliage protection plus lower gypsy moth larval - and pupal - counts result than with either *B. thuringiensis* or *A. melanoscelus* alone. They attributed lower larval - and pupal - counts to the parasitization of the residual host population after mortality due to *B. thuringiensis*. The possible benefit of inundation by this parasite in conjunction with an application of virus is obvious.

We conclude, on the basis of our own and previous studies, that *A. melanoscelus* can be used in several ways to augment efforts to initiate virus epizootics artificially in gypsy moth populations. Further tests to determine its ability to vector the virus will be made in the 1976 field season, and laboratory tests will be conducted to determine the mode(s) of transmission involved in vectoring.

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RÉSUMÉ

La transmission de la polyédrose nucléaire du bombyx disparate au moyen d'*Apanteles melanoscelus* [*Hym. : Braconidae*]

Des larves de *Lymantria dispar* L. furent exposées à des femelles d'*Apanteles melanoscelus* (RATZEBURG) contaminées par le virus de la polyédrose nucléaire. Trois méthodes de contamination (ovipositeur, surface totale du corps et exposition à des hôtes infectés) et deux délais (2 et 24 heures) furent comparés. Une mortalité significativement plus grande par le virus eut lieu chez les larves exposées aux parasites contaminés que chez les larves exposées aux parasites non contaminés pendant 2 et 24 heures. Il n'y eut pas de différences significatives dans la mortalité larvaire pour les trois méthodes de contamination selon la durée du contact : 2 et 24 heures et pas de différences significatives dans les sorties de parasites à partir des larves parasitées par des insectes contaminés et non-contaminés.

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