Virus and Insect Parasite Interaction in the Lawn Armyworm, Spodoptera mauritia acronyctoides (Guenée)[1,2]

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With increased utilization of microbial agents for control of noxious insects, information on the effects of these microbes to other agents such as insect parasites become of prime importance. An interaction between parasites and pathogens undoubtedly would occur when their activities overlap or otherwise come in contact, as when they occupy the same host.

There has been no report of a nuclear polyhedrosis virus of a given insect directly infecting a parasite of that insect. Indirectly, however, the pathogen may affect the parasite adversely by rendering the host unsuitable as food or by depleting host material. Moreover, the premature death of the host has been cited as a primary cause of parasite loss (Kelsey, 1960, Steinhaus, 1954).

Another parasite-pathogen interaction is the important role of parasites in the dissemination of microbial pathogens. Parasites have been found to serve as mechanical vectors of viruses when they feed on, or oviposit in their common host (Bird, 1961; Lower, 1954; Thompson & Steinhaus, 1950).

Material and Methods

The hosts utilized were first to third instar caterpillars of the noctuid *Spodoptera mauritia acronyctoides* (Guenée), commonly known as the lawn armyworm in Hawaii (Tanada & Beardsley, 1958). The hosts were reared in sterilized half-pint ice cream cartons covered with sterile petri dish halves. All test larvae were provided with bouquets of young shoots of napier grass, *Pennisetum purpureum* Schumach.

The pathogen used was a virus described as *Borrelinavirus* sp. (Tanada, 1960). This virus could infect all 7 or 8 larval instars of the host but was more pathogenic to the younger than the older larvae. The inoculum was obtained from existing laboratory stock fed to susceptible hosts for fresh stock concentrations. A fixed concentration of this stock, 2.0×10^6 polyhedra/ml, was used in all tests. This concentration was sufficient to cause

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100% mortality in most of the treated hosts.

The parasite was a braconid, *Apanteles marginiventris* (Cresson), a solitary internal larval parasite. This parasite preferred to oviposit on the minute first instar caterpillars although it could also parasitize second and third instar larvae.

EXPERIMENTAL PROCEDURES AND RESULTS

Mortality of Larval Parasites

Significant parasite larval mortality in infected but living hosts was determined as follows: larvae from egg masses of uninfected adults were subdivided into two lots; 4 to 8 days after hatching the first group was fed virus on foliage and immediately exposed to the parasites; the second lot, the control, was exposed only to the parasites.

Then 20 larvae were picked from each lot four days after exposure to the parasite and every two days thereafter and dissected in Ringers solution. When possible, hosts showing symptoms of disease but still alive were selected for dissection. The number and condition of the parasite larvae (dead or alive) were recorded. Table 1 summarizes these observations.

Table 1. Mortality of *Apanteles marginiventris* larvae in virus-diseased and uninfected *Spodoptera mauritia acronyctoides* caterpillars

Treatment	No. of Hosts Dissected	No. of Parasite Larve Found	No. of Dead Parasite Larvae	% Mortality
Virus	180 live hosts	75	18	24.00
	60 dead hosts	46	39	84.78
Control	160	166	17	10.24

Although parasite mortality was apparently higher in infected hosts than in the controls, the difference was not significant statistically. Parasite mortality in dead hosts, however, was significantly higher than in the live infected hosts or in the controls. This mortality, however, was not attributable to a frank infection of the parasite larva by the virus. Live, apparently normal, parasite larvae were found in hosts that just died from an infection. Moreover, the parasites generally completed development in severely infected hosts that remained alive until the parasite pupated. Appropriate micro-sections were made of severely infected hosts with the parasite larva *in situ* to substantiate histologically the resistance of the parasite to infection by the virus. These sections were used to detect any pathological changes in the parasite due either to an infection or to feeding on an infected, physiologically abnormal host. All specimens were fixed in Duboscq-Brasil's modification of Bouin's fixative, dehydrated and cleared in a methyl benzoate-benzene series and embedded in paraplast (Biological Research Inc.). The sections were cut longitudinally at 6μ and stained. There were no observable pathological changes in the tissues of these parasite larvae. Histologically, there was no evidence of an infection. Parasite larvae mortality, therefore, was attributable directly to

the host's death. The parasite larvae, even later instars, could not complete development as saprophytes as found for some tachinids (Bird, 1961).

Parasite Adult Emergence

In a subsequent test, hosts (2–8 days old) were exposed to parasites two days before or two days after the virus treatment and were held for emergence of adult parasites. Only 11 parasites were reared from 360 infected hosts while 183 were reared from an equivalent number of uninfected hosts (Table 2). Again this difference was attributable to premature host mortality. The young caterpillars were highly susceptible to the virus. None of the hosts treated with virus two or four days after hatching survived long enough to permit parasite development. Adults were obtained only from those hosts that were treated with virus six and eight days after hatching. These older hosts lived longer than the young hosts, thus permitting more parasites to complete development.

The 11 parasites reared from diseased hosts in the previous experiment were fed a honey-water solution and allowed to mate. Four days later, the females were offered 2-day old hosts. The percentage of parasitization by the females did not differ from the controls (76.7 *vs.* 83.4%) and their progeny included both sexes, indicating that the parasites from the diseased hosts were still sexually functional. The parasites, although reared from diseased hosts, did not carry a sufficient charge of virus to infect new hosts.

Table 2. Number of adult *Apanteles marginiventris* reared in virus-diseased and in non-diseased hosts

Treatment	Tested (Days after hatching)	No. of hosts used	No. of adult parasites emerged	
			Host exposed to parasite before pathogen	Host exposed to pathogen before parasite
Virus	2		0	0
	4	360	4	0
	6		5	2
Control	2		26	36
	4	360	22	40
	6		24	55

Mechanical Transmission of the Virus

The parasites were able to transmit the virus to uninfected hosts if first allowed to oviposit in diseased hosts. To determine quantitatively the vector efficiency, 8-day-old parasites were confined for one hour with 20 severely infected hosts. The time was sufficient for the parasites to have stung the diseased caterpillars. Each female parasite then was transferred to a sterilized glass vial containing a single 2-day old uninfected host. After stinging host number 1, the parasite was transferred immed-

iately to a new vial containing host number 2, and so on until 20 hosts were parasitized. Approximately 30 to 45 minutes were required for the parasite to oviposit in all 20 hosts.

All test caterpillars then were fed and cared for individually. Dead insects were dissected to ascertain parasitization and smears were prepared to check for the pathogen.

Under these conditions, the virus was transmitted very successfully (Table 3). All of the parasites tested transmitted the pathogen. The least efficient vector transmitted the virus to 4 hosts while the most efficient effected transfer to 10 out of 20 hosts. Overall, 35% of the exposed hosts died from a virus infection, showing symptoms four to six days after oviposition.

Table 3. Mechanical transmission of the nuclear polyhedrosis-virus by *Apanteles marginiventris* to the lawn armyworm during oviposition

Trial	No. of Test Insects	No. remaining healthy[a]	No. infected	No. dead from other causes	Sequence of infection[b]
1	20	9	10	1	1-2-3-4-6-7 8-14-15-16
2	20	11	9	0	1-4-5-8-11 15-18-19-20
3	20	14	5	1	3-4-7-10-12
4	20	15	4	1	4-7-11-12

a Uninfected caterpillar that eventually died upon parasite pupation.
b The host number in the 20 serially exposed test caterpillars that succumbed to disease.

There was no apparent logical sequence in the transmission of the pathogen by the parasite. Since all of the hosts were parasitized, indicating penetration of the host integument, transmission in sequence until the exhaustion of the viral inoculum on the ovipositor was expected. However, transmission was apparently random, probably depending upon the depth, speed, and site of penetration. Individual variations in host susceptibility to the virus undoubtedly also played an important role.

DISCUSSION

That the virus only indirectly affects the parasite by causing the premature death of the host was demonstrated quantitatively by the studies involving dissections, micro-sections, and adult emergence of the parasites. Not only were the parasites able to complete development in severely infected hosts but also seemed to be unaffected by the changes caused by the disease. Adult parasites reared from diseased hosts were apparently normal.

Interactions of these two biological control agents, therefore, need not necessarily be detrimental. Indeed, when coupled with the fact that parasites were efficient vectors of the virus, the interaction could result in the

better over-all control of the pest. The parasite could materially aid in initiation of epizootics by establishing new foci of infection and effecting a rapid spread of the pathogen within a given population.

SUMMARY

A quantitative study of some interactions was conducted between a nuclear-polyhedrosis virus and the parasite, *Apanteles marginiventris* (Cresson), when both occurred simultaneously within the same host, the lawn armyworm, *Spodoptera mauritia acronyctoides* (Guenée).

Mortality of parasite larvae was due primarily to the premature death of the diseased host caterpillar rather than to the direct effect of the pathogen. Although infected, hosts that survived long enough enabled parasite larvae to mature, pupate, and emerge as adults. Viral infection apparently did not cause sufficient changes to render the host nutritionally inadequate for parasite development. Adult parasites thus reared were apparently normal and still sexually functional. They did not transmit the virus to other hosts upon oviposition. However, parasites, allowed to oviposit in diseased hosts prior to exposure to uninfected hosts, successfully transmitted infective doses of the virus at random rather than logical order. Histological examinations of sections of parasite larvae reared from infected hosts indicated that the parasite was refractile to viral infection.

REFERENCES

BIRD, F. T. 1961. Transmission of some insect viruses with particular reference to ovarial transmission and its importance in the development of epizootics. JOUR. INSECT PATHOL., 3: 352–80.

KELSEY, J. M. 1960. Interaction of virus and insect parasites of *Pieris rapae* L. PROC. 11TH INTERN. CONGR. ENT., 2: 790–96.

LOWER, H. F. 1954. A granulosis virus attacking the larvae of *Persectania ewingii* Wests. (Lepidoptera: Agrotidae) in South Australia. AUST. JOUR. BIOL. SCI., 7: 161–67.

STEINHAUS, E. A. 1954. The effects of disease on insect populations. HILGARDIA, 23: 197–261.

TANADA Y. 1960. A nuclear polyhedrosis virus of the lawn armyworm *Spodoptera mauritia* (Boisd.)(Lepidoptera: Noctuidae). PROC. HAW. ENT. SOC., 17: 304–08.

TANADA Y. AND. J. W. BEARDSLEY. 1958. A biological study of the lawn armyworm, *Spodoptera mauritia* (Boisduval) in Hawaii (Lepidoptera; Phalaenidae). IBID., 15: 411–36.

THOMPSON, C. G. AND E. A. STEINHAUS. 1950. Further tests using a polyhedrosis virus to control the alfalfa capterpillar. HILGARDIA, 19:412–45.