Interaction Between Neoaplectana carpocapsae and a Granulosis Virus of the Armyworm Pseudaletia unipuncta

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Abstract: Neoaplectana carpocapsae developed and reproduced in armyworm hosts infected with a granulosis virus (GV). Macerated tissues of dauer juveniles from GV-infected hosts had sufficient GV to infect 1st and 2nd instar armyworms. Electron-microscope examination of dauer juveniles and adult female nematodes confirmed the presence of GV in the lumen of the intestine. No GV was observed in other tissues of the nematode. *Key Words:* DD-136 nematode, nematode-insect virus interaction, insect virus, *Baculovirus*.

The mutualistic relationship of the DD-136 strain of *Neoaplectana carpocapsae* and the associated bacterium, *Achromobacten nematophilus,* has been clearly established (1, 6). Very little is known, however, about the interactions between other insect pathogens and this nematode. Lysenko and Weiser (4) examined the microflora associated with *N. carpocapsae* and its host, *Galleria mellonella,* and found several bacterial species other than *A. nematophilus* in the gut of the nematode. Veremtchuk and Issi (9) reported that the nematode, *N. agriotos* (= *N. carpocapsae*), which developed in *Pieris brassicae* larvae infected with the protozoan *Nosema mesnili* was also infected by the protozoan. Seryczyńska (8) studied the defense reactions of the Colorado potato beetle against the fungi *Paecilomyces farinosus* and *Beauveria bassiana,* and *N. carpocapsae.* She found that the simultaneous exposure to the spores of either fungi and the nematode increased the number of hemocytes in the hemolymph over that in untreated beetles. We are not aware of any studies of insect viruses in *N. carpocapsae.* Accordingly, a study was initiated to investigate the interaction between *N. carpocapsae* and a granulosis virus (GV) in the armyworm *Pseudaletia unipuncta.*

MATERIALS AND METHODS

GV and nematode infections: The Oregonian strain of GV, obtained from Dr. Y. Tanada, University of California, Berkeley, was used to infect newly molted 5th-stage larvae of the armyworm as described by Kaya and Tanada (3). Ten days after feeding on the virus, 6th-instar armyworms which showed typical signs and symptoms of a GV infection and an equal number of healthy 6th-instar armyworms were weighed. Each armyworm larva was placed in a petri dish (100 × 15 mm) containing ca 500 dauer juveniles of *N. carpocapsae* on moist filter paper. After

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48 h each dead armyworm was placed in a test tube containing 10 ml of sterilized distilled water (2). Fourteen days after placement in the test tube, the total number of dauer juveniles which emerged from the host was determined with a 1-ml Peters counting slide (Hawksley and Sons Ltd., Sussex, England), and the number of juveniles per mg of armyworm was calculated. All tests were conducted at 25 C.

Infectivity of dauer juveniles: Healthy 6th-instar armyworms were exposed individually for 48 h to ca 500 dauer juveniles obtained from GV-infected or from healthy hosts. Five days after exposure to the nematodes, the dead armyworms were dissected and examined for nematode adults.

Bioassay of GV: Healthy 6th-instar or 10-day-old GV-infected armyworms were exposed to dauer juveniles as described above. After 48 h, dead GV-infected and healthy armyworms were placed in separate nematode traps to collect the resulting dauer juvenile progeny as described by Poinar (5). These nematodes were collected in sterilized distilled water. After the dauer juveniles emerged from their hosts, they were processed through a Baermann funnel. The dauer juveniles were centrifuged at 5 *g* for 5 min, the supernatant discarded, and 20 ml of sterilized distilled water added to the nematode suspension. The juveniles were centrifuged and washed 5 times, and the final suspension was adjusted to 1000 nematodes per ml. The nematodes were stored at 5 C until used.

For bioassay, 10 ml of the stock suspension was centrifuged as above. Ten thousand nematodes were concentrated in 0.5 ml of water. The supernatant which contained the dauer juveniles from the GV-infected armyworms was saved. The juveniles were macerated in a tissue grinder, and 1.5 ml of sterile distilled water was added to the macerated tissues. In Trial 1, 0.1 ml of the tissue suspension from GV-infected hosts was placed on a piece of artificial diet (1.5 × 1.5 × 1.5 cm) in a petri dish, and 5 newly-molted 2nd stage armyworm larvae were added to each dish. One-tenth ml of the supernatant which had contained dauer juveniles from GV-infected host was tested as above. Macerated nematode tissues of dauer juveniles from healthy armyworms served as control. Fresh food for the armyworms was added 3 days later and thereafter as needed. There were 8 replicates within each treatment. In Trials 2 and 3, 5 ml of artificial diet was placed in a 26-ml snap-cap vial, and 0.1 ml of macerated tissues of nematodes from GV-infected hosts and supernatant which had contained dauer juveniles from GV-infected hosts or of macerated tissues of nematodes from healthy hosts were added to each vial. After the surface of the diet had dried, a newly hatched 1st-instar armyworm was placed into each vial. Five days later, each larva was transferred to a new vial with fresh food. In Trial 2 there were 3 replicates of 12 larvae, and in Trial 3 there were 2 replicates of 10 larvae in each treatment. In all trials 12 days after initiation of the tests, the armyworm larvae were dissected and their fat tissues examined for GV infection with a compound microscope.

Electron microscopy: Dauer juveniles which emerged from GV-infected hosts or adult female nematodes dissected from GV-infected hosts were washed in Millonig's phosphate buffer (pH 7.4) and fixed in 4% gluteraldehyde in the phosphate buffer. The adults were cut into sections to facilitate the penetration of the fixative. The dauer juveniles were fixed without cutting. The nematodes were postfixed in 1% solution of osmium tetroxide in the phosphate buffer for 2 h at 4 C. Following fixation, the nematodes were rinsed in buffer, embedded in 2% block agar, and dehydrated. After dehydration, they were embedded in Epon-Araldite mixture and sectioned with diamond knives mounted on a Porter-Blum M7-2 ultramicrotome. The sections were stained with saturated aqueous uranyl acetate followed by lead citrate and examined with a Zeiss EM 9A electron microscope.

RESULTS AND DISCUSSION

N. carpocapsae developed and reproduced in healthy and GV-infected hosts (Table 1). There were no significant differences between healthy and GV-infected hosts in the number of dauer juveniles produced per armyworm. On the basis of body weight, however, the healthy armyworms (which were lighter than the GV-infected armyworms) produced significantly more juveniles per mg than the

TABLE 1. Production of dauer juveniles of *Neoaplectana carpocapsae* in healthy and granulosis-virus-infected (GV) armyworm larvae.

Host	No. armyworms	x̄ larval weight (mg)[a]	x̄ no. dauer juveniles/ armyworm ± SE	Dauer juveniles/ (mg)[a]
Healthy	24	377 a	98,290 ± 13,840	262 a
GV-infected	24	739 b	109,300 ± 16,497	149 b

[a]Numbers followed by different letters within the same column are significantly different at the 5% level (*t*-test).

GV-infected hosts. Aside from this difference, the GV-infected hosts did not inhibit the growth, development, or reproduction of *N. carpocapsae*. In support of this conclusion, Sandner and Stanuszek (8) found that dauer juvenile production of *N. carpocapsae* per mg of *Galleria* larvae and pupae was highest in the lightest hosts. Accordingly, dauer juvenile production was not proportional to weight of the host.

Dauer juveniles from GV-infected hosts were not adversely affected in their ability to infect armyworms and develop to adults. Dissection of 20 armyworms infected with dauer juveniles from GV-infected hosts revealed adult *N. carpocapsae*. An equal number of armyworms exposed to dauer juveniles from healthy hosts showed similar results. Additionally, dauer juveniles from GV-infected or healthy hosts stored at 5 C for 3 months were still alive and active.

Macerated tissues of dauer juveniles from GV-infected hosts had sufficient GV to infect 1st- and 2nd-instar armyworms (Table 2). In Trial 1, 22.2% (8/36) of the 2nd instars were infected and in Trials 2 and 3, 94.2% (49/52) of the 1st instars were infected. No armyworm became infected that was fed macerated tissues of dauer juveniles from healthy hosts or the supernatant in which the dauer juveniles were suspended before centrifugation.

Electron-microscope examination of dauer juveniles and adult females confirmed the presence of GV in the lumen of the intestine (Figs. 1A, 1B). The GV inclusion bodies appeared to be intact and undamaged. No GV was observed in other tissues. The dauer juveniles acquired the GV either from the host or the adult female nematode. Younger female adults deposited eggs into the host which, upon hatching, acquired the GV through the buccal cavity, whereas in older females the young hatched within the mother and fed on tissues of the female (matricidal), acquiring the GV which was in the lumen of the intestine.

The presence of GV in the lumen of the intestine of dauer juveniles does not mean that the nematode can vector the virus. In fact, that will not occur because the nematode will kill its host long before the GV has an opportunity to replicate. On

TABLE 2. Infectivity of granulosis virus (GV) from macerated tissues of dauer juveniles of *Neoaplectana carpocapsae* isolated from GV-infected armyworms.

Source of inoculum	Number		
	Healthy	Infected with GV	Died unknown causes
Trial 1[a]			
1. Dauer juveniles—Healthy hosts	38	0	2
2. Dauer juveniles—GV-infected hosts	28	8	4
3. Supernatant[b]	40	0	0
Trial 2[c]			
1. Dauer juveniles—Healthy hosts	31	0	5
2. Dauer juveniles—GV-infected hosts	2	31	3
3. Supernatant[b]	32	0	4
Trial 3[c]			
1. Dauer juveniles—Healthy hosts	17	0	3
2. Dauer juveniles—GV-infected hosts	1	18	1
3. Supernatant[b]	18	0	2

[a]Insects were in the 2nd instar when test initiated.
[b]Supernatant in which dauer juveniles from GV-infected armyworms were suspended before centrifugation.
[c]Insects were in the 1st instar when test initiated.

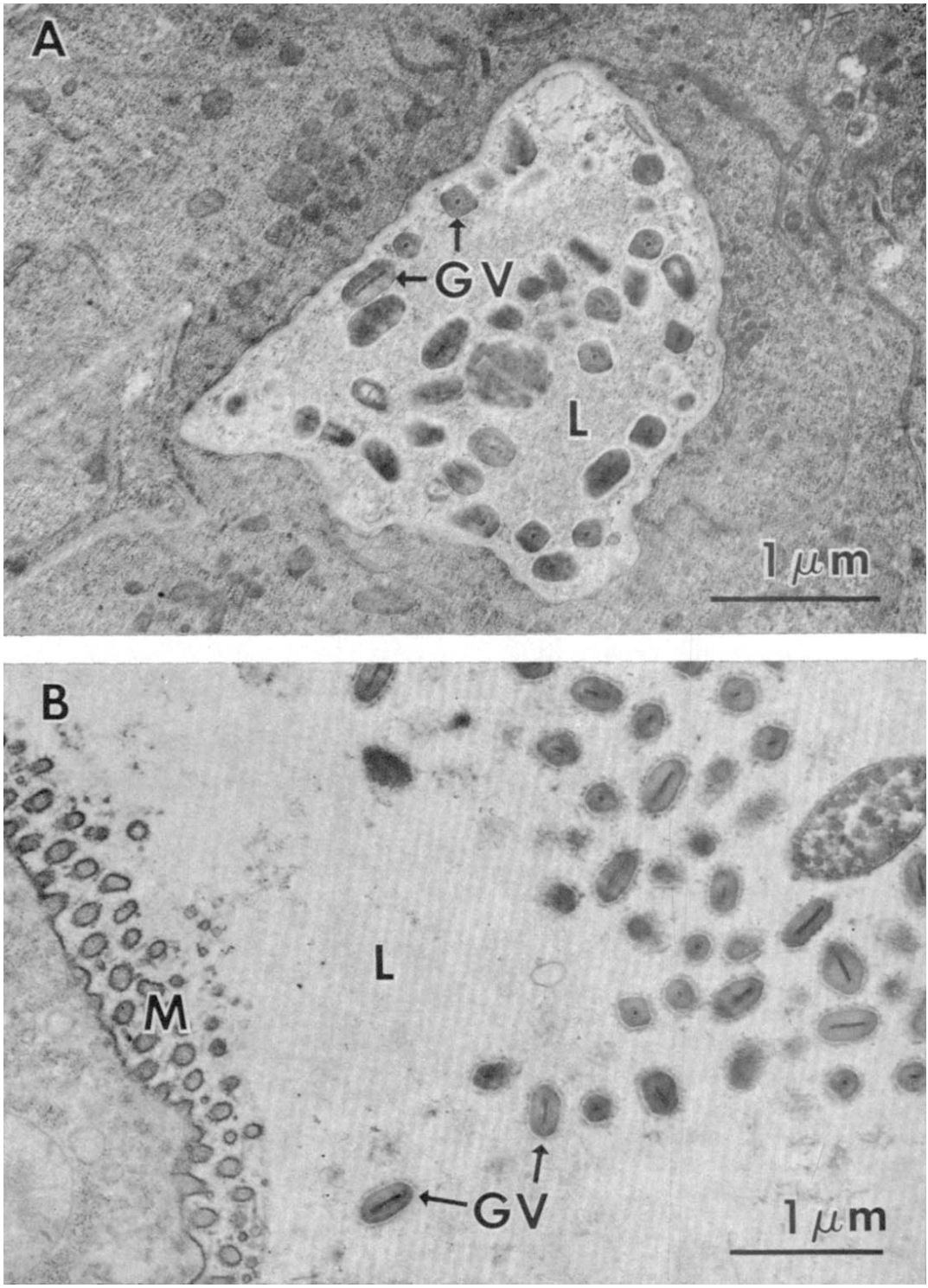

FIG. 1. A) Granulosis virus (GV) in intestinal lumen of dauer juveniles of *Neoaplectana carpocapsae* from GV-infected armyworms. B) Granulosis virus (GV) in intestinal lumen of adult *Neoaplectana carpocapsae* female from GV-infected armyworm. Note inclusion bodies (capsules) of GV appear to be intact and undamaged. (L = lumen of intestine, M = microvilli.)

the other hand, the presence of GV in the intestine of the juveniles may have practical significance. First, the nematodes may serve as a means of dispersing the GV to other locations by their lateral movement in the soil. Second, the GV is not inactivated in the intestinal lumen and therefore may be protected from the external environment, with its longevity thereby extended. Third, it may be possible to integrate GV (and perhaps other insect viruses) and nematode in pest-management programs. The last needs further research, but it is a plausible program because of the ease of nematode production and the strides made in the production of insect viruses in tissue culture. The addition of viruses to the nematode cultures may result in sufficient acquisition of the virus by the nematode in the lumen of its intestine. Because *N. carpocapsae* has a wide host range, it can be used in situations where there are two or more insect pests and where one of the pests is susceptible to the virus.

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