Infectivity of Neoaplectana carpocapsae and Heterorhabditis heliothidis to pupae of the parasite Apanteles militaris

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Abstract: The infectivity of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* to *Apanteles militaris*, a gregarious parasite of the armyworm, was determined at 100, 1,000, 5,000, and 10,000 nematodes per petri dish. For both nematode species, the percentage of infected *A. militaris* within a cocoon cluster decreased as inoculum levels decreased. At the highest inoculum level, *N. carpocapsae* infected an average of 32% of the parasite pupae within a cocoon cluster, whereas *H. heliothidis* infected an average of 22%. Covariance analysis indicated, however, that *N. carpocapsae* had significantly greater infectivity than did *H. heliothidis*. Some of the dauer juveniles of *N. carpocapsae* on the body of the armyworm contacted the emerging parasites and eventually became enveloped within the silken cocoons. Dauer juveniles produced by *N. carpocapsae* in parasite pupae could not penetrate and escape from silken cocoons even when the cocoons were placed in a moist environment. *Key Words:* nematode-insect parasite interaction.

Neoaplectana carpocapsae Weiser has been applied as a biological control agent against a number of pestiferous insects (1). The use of this nematode should augment rather than be antagonistic to the insect parasites of these pestiferous insects. Since little information is available on the impact of entomophilic nematodes upon insect parasites, such studies have been initiated. Kaya (5) demonstrated the susceptibility of various stages of an internal gregarious parasite, *Apanteles militaris* (Walsh), to infection by the DD-136 strain of *N. carpocapsae*. Since *N. carpocapsae* cannot penetrate the silken cocoons of *A. militaris*, the pupae developing within the cocoons are immune to infection. As *A. militaris* larvae emerge from their armyworm host and spin their cocoons, however, they become susceptible to infection. When these emerging parasites (usually 20-70 parasites emerge from a single host) are exposed to ca 1500 dauer juveniles of *N. carpocapsae* in a petri dish, 9-10% become infected. This paper presents information on the effect of different densities of dauer juveniles of *N. carpocapsae* and *Heterorhabditis heliothidis* (Kahn, Brooks and Hirschmann) (= *Chromonema heliothidis*) on the infection rate of *A. militaris* as the parasites are spinning their cocoons. Also reported are observations on how the dauer juveniles of *N. carpocapsae* contacted *A. militaris* larvae as they spin their cocoons, and the fate of dauer juveniles produced within the cocoons.

MATERIALS AND METHODS

A stock suspension of dauer juveniles of *N. carpocapsae* or *H. heliothidis* was obtained by infecting *Galleria mellonella* (L.) by the method described by Dutky *et al.* (3). Each suspension was adjusted to 2,000 dauer juveniles per ml and stored at 10 C.

Colonies of the armyworm, *Pseudaletia unipuncta* (Haworth), and the parasite, *A. militaris*, were established and maintained as described previously (6). Colonies and all tests were maintained at 25 C.

Plastic petri dishes (100 × 15 mm) containing a 9-cm filter paper (no. 1 Whatman) were infested with dauer juveniles of *N. carpocapsae* or *H. heliothidis* at 4 different inoculum levels: 100, 1,000, 5,000, and 10,000 dauer juveniles per petri dish. To obtain these inoculum levels, the stock suspension was diluted so that there were 100 or 1000 dauer juveniles per 2 ml or was concentrated by centrifugation of 5 *g* for 2 min and adjusted to 5,000 or 10,000 dauer juveniles per 2 ml. Controls were dishes with 2 ml of sterile water containing 0.1% formalin. Armyworms containing 10-day-old *A. militaris* were added to each dish.

Five days after the parasites emerged from the armyworm, the infected and uninfected pupae were counted. Cocoons

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containing healthy parasites appeared black, whereas cocoons infected with *N. carpocapsae* were white or creamy and cocoons infected with *H. heliothidis* were red. Only fully formed cocoons were counted. Cocoons containing infected parasites were dissected, and the adult nematodes within each were counted. Dissections were made of all infected parasites except for 5000 nematodes/dish in the *H. heliothidis* series. There were three or four replicates with 10 armyworms in each replicate. Armyworms which did not produce parasites were discarded.

Observations were made to determine the process by which *N. carpocapsae* gained access to the parasites as they spun their cocoons. Armyworms containing 10-day-old *A. militaris* were placed individually in petri dishes containing 10,000 dauer juveniles. Just before and during parasite emergence from the host, the movement of the nematode was observed with a stereomicroscope. Five such observations were made.

The possibility that dauer juveniles of *N. carpocapsae* emerged from fully formed parasite cocoons was investigated as follows. All infected parasites within the cocoons from a single host were placed in a test tube (12 × 75 mm) containing 1 ml of sterile water. Cocoons were supported above the water by a method described by Kaya (4). Cocoons were placed into the test tube 5 days after emerging from the host, and 16 days after infection the test tubes and cocoons were examined for juveniles. There were six replicates with 17-43 infected parasites within a replicate.

RESULTS AND DISCUSSION

Linear-regression analyses were made with percent infection plotted against the different inoculum levels for each replicate. Because there were no significant differences among the replicates, the data from that experiment were combined. Fig. 1 shows the relation between number of nematodes/dish and percentage of pupae of *A. militaris* infected within a cocoon

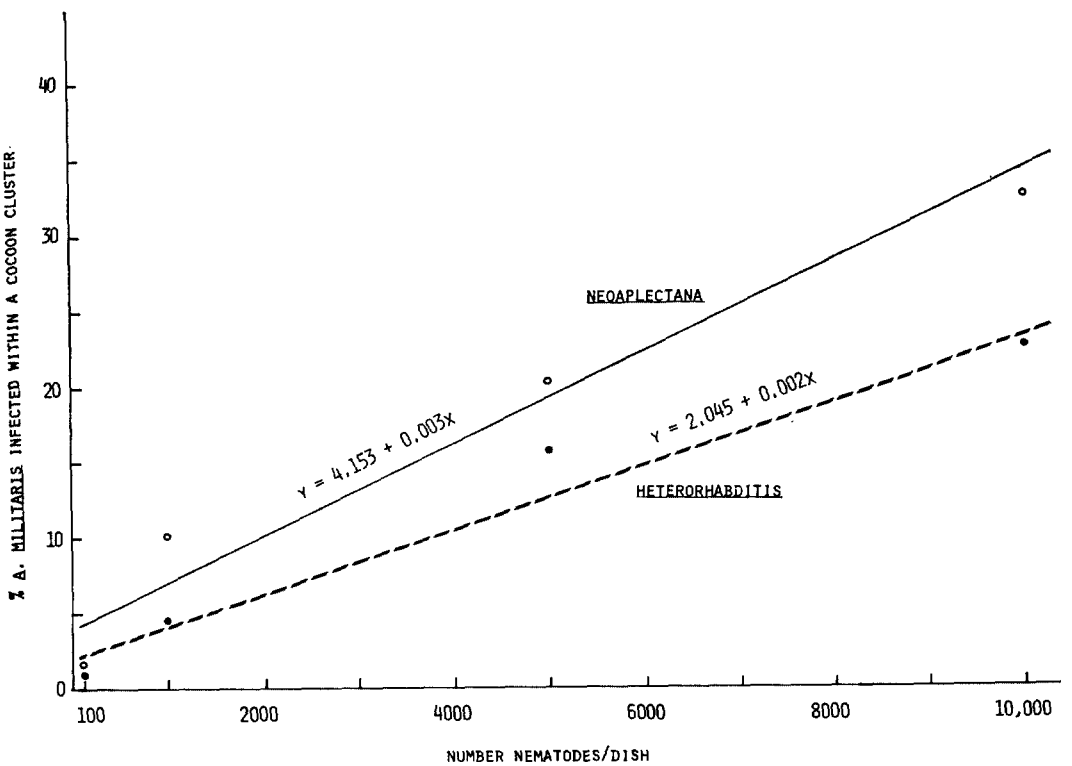

FIG. 1. The relationship between percent *Apanteles militaris* infected within a cocoon cluster and the number of nematodes of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis*. Covariance analysis showed that *Neoaplectana* was significantly different ($P < 0.05$) from *Heterorhabditis*.

cluster for *N. carpocapsae* and *H. heliothidis*. Covariance analysis between the slopes of *N. carpocapsae* and *H. heliothidis* revealed that they were significantly different from each other ($F = 5.34$; $P < 0.05$). Accordingly, *N. carpocapsae* and *H. heliothidis* differed in infectivity to *A. militaris* larvae as they were spinning their cocoons. The reason for the difference is not known. A possible explanation is that *N. carpocapsae* may be more active than *H. heliothidis*, and hence more apt to contact the parasite.

Percent infection for *N. carpocapsae* or *H. heliothidis* was not significantly correlated with the number of parasites emerging from a host. For example, at 10,000 nematodes/dish the correlation coefficient was 0.23 ($F = 2.24$; df 1,39) for *N. carpocapsae*, and 0.32 ($F = 2.93$; df 1,25) for *H. heliothidis*.

When an armyworm larva was placed in a petri dish containing 10,000 *N. carpocapsae*, few dauer juveniles (10-20) were observed on various parts of the armyworm body. As the parasites emerged from the armyworm and began spinning their cocoons, some of the dauer juveniles on the body came into contact with the parasites, apparently fortuitously. The parasite continued to spin the cocoon and the juvenile eventually became enveloped within it. Since the cocoon spinning process took about 1.5 hr to complete (2), the actual mechanism of nematode penetration into the parasite's hemocoel was not observed. In a given cocoon cluster, infected pupae appeared to be distributed at random.

Fig. 2 shows the number of *N. carpocapsae* adults found in pupae exposed to different densities. At the lowest density (100 nematodes/dish), the infected parasites contained either one male or one female. At the higher densities, it was not unusual to find more than one adult nematode per

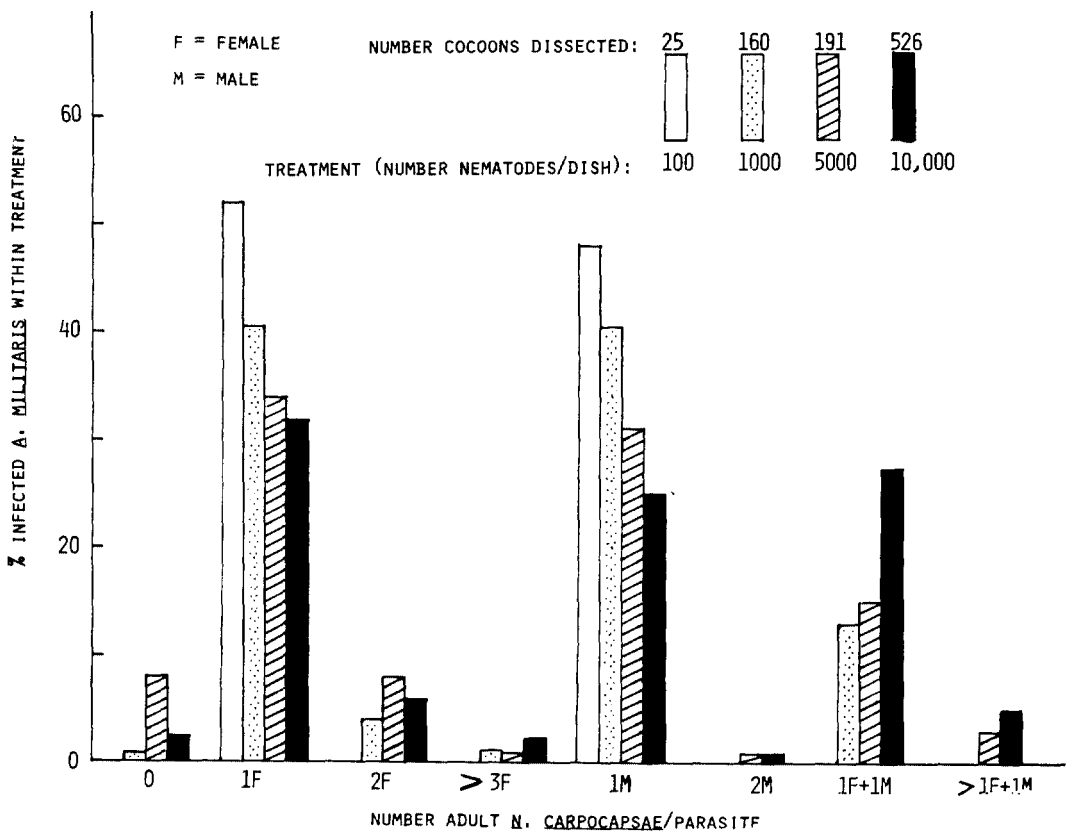

FIG. 2. The number of *Neoaplectana carpocapsae* adults found in pupae of *Apanteles militaris* exposed to different densities of dauer juveniles.

parasite. Interestingly, when more than one adult nematode was found in the host, it usually had one male and one female. These data confirm earlier findings reported by Kaya (5).

Unlike *N. carpocapsae, H. heliothidis* is hermaphroditic in the first generation (7). Consequently, all parasites with *H. heliothidis* contained progeny. At 10,000 nematodes/dish, 282 parasites were infected. Of these, 5% had no nematodes, 61.7% had one adult nematode, 23.4% contained two adults, and 9.9% had three or more adults. Of 53 parasites infected at 1,000 nematodes/dish, 13.2% had no nematode adult, 83% had one adult and 3.8% had two adults. At 100 nematodes/dish, seven of seven infected parasites had one nematode adult.

Dauer juveniles produced by *N. carpocapsae* in parasite pupae could not penetrate and escape from cocoons held for 11 days in a moist environment. When the cocoons were dissected, dauer juveniles were found in all six replicates at the following percentages: a, 37% (14/38); b, 55% (11/20); c, 68% (19/28); d, 6% (1/17); e, 30% (13/43); and f, 45% (15/33). These data demonstrate that the parasites formed normal cocoons and were not harmed by the nematode during cocoon formation.

Even when inoculated with 10,000 *N. carpocapsae* or *H. heliothidis,* many cocoon-spinning *A. militaris* escaped infection. The likelihood is remote that emerging parasites will encounter such numbers in the field. It is conceivable, however, that the parasites could encounter 1,000 to 5,000 dauer juveniles in a given area if the nematodes were applied as a biological insecticide against the armyworm. The degree of infection of *A. militaris* by the two nematode species in the laboratory suggests that the nematode would not limit *A. militaris* when exposure occurred in the cocoon-spinning or pupal stages. That is because *A. militaris* is a gregarious parasite. Even so, further study is needed of the combined effects of the entomophilic nematode and *A. militaris* on the armyworm under field conditions.

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