Susceptibility of Pupae of Two Cocoon-forming Lepidopterous Species to the Entomogenous Nematode, Neoaplectana carpocapsae (Rhabditida: Steinernematidae)

Patricia L. Moyle and Harry K. Kaya[1]

The entomogenous nematode, *Neoaplectana carpocapsae,* and its associated bacterium, *Xenorhabdus nematophilus,* infect a number of insect species in different orders. Infectivity tests have been conducted primarily against larvae of pest species with little information available on the infectivity of *N. carpocapsae* to lepidopterous pupae. Schmiege (8) demonstrated that pupae of the spruce budworm, *Choristoneura fumiferana,* were slightly susceptible to *N. carpocapsae,* and Lewis and Raun (5) reported that 30% of the pupae of the European corn borer, *Ostrinia nubilalis,* were infected by the nematode compared to 100% infection of larvae and adults. Pupae of the sphingid, *Herse convolvuli,* were moderately susceptible to *N. carpocapsae* (4). Exposure of pupae of the beet armyworm, *Spodoptera exigua,* and of the armyworm, *Pseudaletia unipuncta,* to *N. carpocapsae* resulted in ca 75 and 50% mortality, respectively (2). Naked pupae (without silken cocoons) of *Galleria mellonella* exposed to similar conditions as the armyworms resulted in 100% mortality. These results confirmed earlier studies by Sandner and Stanuszek (7) with pupae of *G. mellonella.* This paper reports the ability of *N. carpocapsae* to infect pupae within silken cocoons of *G. mellonella* and *Bombyx mori.*

Stock suspensions of infective juveniles of *N. carpocapsae* (DD–136 strain) were obtained by infecting *G. mellonella* larvae as described by Dutky et al. (1) and maintained at 10 C in 0.1% formalin at a concentration of 1,000 nematodes per ml. New nematode stocks were obtained every 3–4 wk.

To obtain intact silken cocoons containing pupae of *G. mellonella,* late last instar larvae were placed in a plastic petri dish (15 × 100 mm). About 3–4 d after cocoon formation, cocoons containing prepupae were transferred to a clean petri dish. Pupae in 3–5-d-old silken cocoons were used in all tests. Five pupae were placed in a petri dish containing two pieces of filter paper (Whatman no. 1) to which ca 1,000

Received for publication 8 December 1980.

[1]Division of Nematology, University of California Davis, CA 95616.

nematodes in 2 ml of 0.1% formalin were added. Cocoons were maintained at 25 ± 2 C. After 7 d, pupae were removed from the cocoons, dissected, and examined for the presence of nematodes.

Silkworm eggs were obtained from Dr. H. Watanabe, Laboratory of Sericulture, University of Tokyo, Japan. After the eggs hatched, larvae were fed mulberry leaves until pupation. Silkworm pupae in cocoons which were ca 24-h old were treated as described for *G. mellonella*. For both *G. mellonella* and *B. mori*, naked pupae (without cocoons) were also treated with nematodes. There were three replicates for each species with 10 cocoons or pupae in each replicate.

Scanning electron microscope micrographs (SEM) of the outer and inner surfaces of silk cocoons of *G. mellonella* and *B. mori* were made and the diameters of the pores in the inner layers of the cocoons were measured. The means and standard deviations of the pore openings were calculated for both species.

G. mellonella and *B. mori* pupae in silken cocoons were highly susceptible to *N. carpocapsae*. Infection rate of pupae (n = 30) with or without cocoons was 100% for both species. SEM photographs of the outer and inner surfaces of *G. mellonella* and *B. mori* showed that the cocoons were porous. Cocoons of *G. mellonella* are thin with visible pores (Fig. 1A). Cocoons of *B. mori* are multilayered (Fig. 1B) with large spaces (pores) among the silk strands (Fig 1C). The mean diameter of the 10 largest inner pores was 63.1 ± 8.8 μm for *G. mellonella* and 151.1 ± 11.5 μm for *B. mori*. The greatest mean width of infective juveniles of *N. carpocapsae* is 24 μm with a range of 22–28 μm (6). Thus, the silken cocoons of these lepidopterous species are sufficiently porous to allow infective juveniles to pass through the silk layers. Once the infective nematodes are inside the cocoon, the nematodes probably enter the host's hemocoel through the spiracles. These results are in contrast to those obtained with the silken cocoons of hymenopterous insects (certain parasitic braconids and ichneumonids) which have a nonporous inner layer (3). This nonporous layer serves as a mechanical barrier to prevent nematodes from invading the cocoons and infecting the developing pupae. If the nonporous layer is damaged, then the nematodes can invade the cocoon and infect the pupae. The differences in cocoon construction of *G. mellonella* and *B. mori*, and of certain hymenopterous insects, may account in part for the difference in infection rates by *N. carpocapsae*.

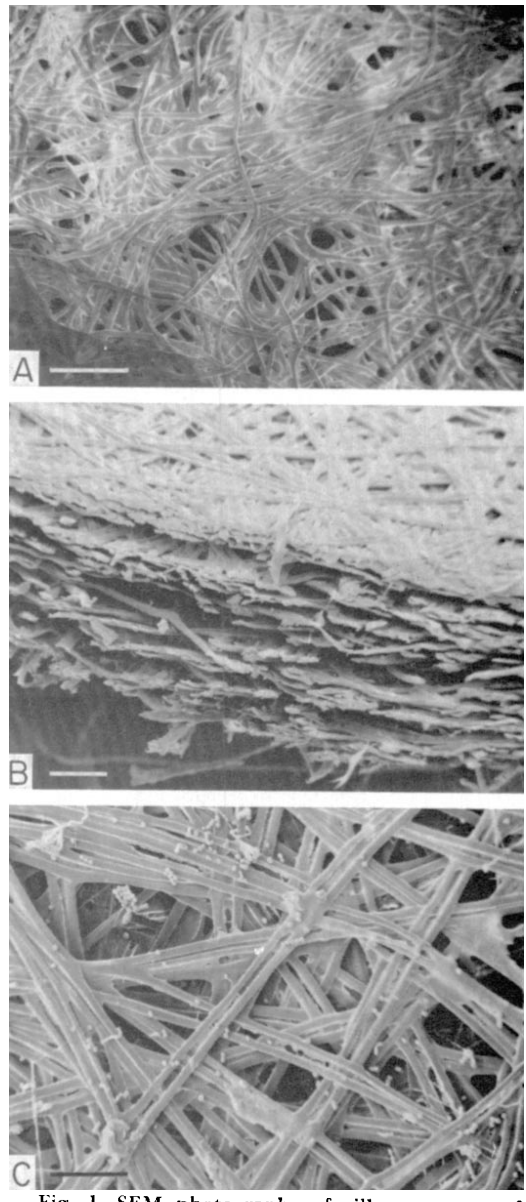

Fig. 1. SEM photographs of silken cocoons of *Galleria mellonella* and *Bombyx mori*. A) Inner surface of *G. mellonella* cocoon. B) Multilayer cocoon of *B. mori*. C) Inner surface of *B. mori*. Bar = 100 μm.

LITERATURE CITED

1. Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1964. A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol. 6: 417-422.

2. Kaya, H. K., and A. H. Hara. 1980. Differential susceptibility of lepidopterous pupae to infection by the nematode Neoaplectana carpocapsae. J. Invertebr. Pathol. 36:389-393.

3. Kaya, H. K., and P. G. Hotchkin. 1981. The nematode Neoaplectana carpocapsae Weiser and its effect on selected ichneumonid and braconid parasites. Environ. Entomol., in press.

4. Laumond, C., H. Mauléon, and A. Kermarrec. 1979. Données nouvelles sur le spectre d'hôtes et le parasitisme du nématode entomophage Neoaplectana carpocapsae. Entomophaga. 24:13-27.

5. Lewis, L. C., and E. S. Raun. 1978. Laboratory and field evaluation of the DD-136 strain of Neoaplectana carpocapsae for control of the European corn borer, Ostrinia nubilalis. Iowa State J. Res. 52:391-396.

6. Poinar, G. O., Jr. 1967. Description and taxonomic position of the DD-136 nematode (Steinernematidae, Rhabditoidea) and its relationship to Neoaplectana carpocapsae Weiser. Proc. Helminth. Soc. Wash. 24:199-209.

7. Sandner, H., and S. Stanuszek. 1971. Comparative research on the effectiveness and production of Neoaplectana carpocapsae s. l. Zesz. probl. Postep. Nauk roln. 121:209-226.

8. Schmiege, D. C. 1963. The feasibility of using a neoaplectanid nematode for control of some forest pests. J. Econ. Entomol. 56:427-431.