

Scanning Electron Microscopic Observations of *Duboscqia penetrans* Parasitizing Root-knot Larvae

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Mankau (2) published a literature review on *Duboscqia penetrans* Thorne, 1940. Mankau and Imbriani (3), and Mankau (4) renamed this nematode parasite *Bacillus penetrans*.

We found mature spores of *D. penetrans* attached to the cuticles of root-knot larvae, *Meloidogyne incognita* (Kofoid and White) Chitwood. The parasite reduced a population of 2,000 root-knot nematodes/500 cm³ soil to a population of 20 in 3 weeks. Three to 12 parasite spores were observed on the body of the nematode larvae. With the aid of a scanning electron microscope, it was possible to observe the morphology of mature spores of *D. penetrans*. A description and spore measurements are herein reported.

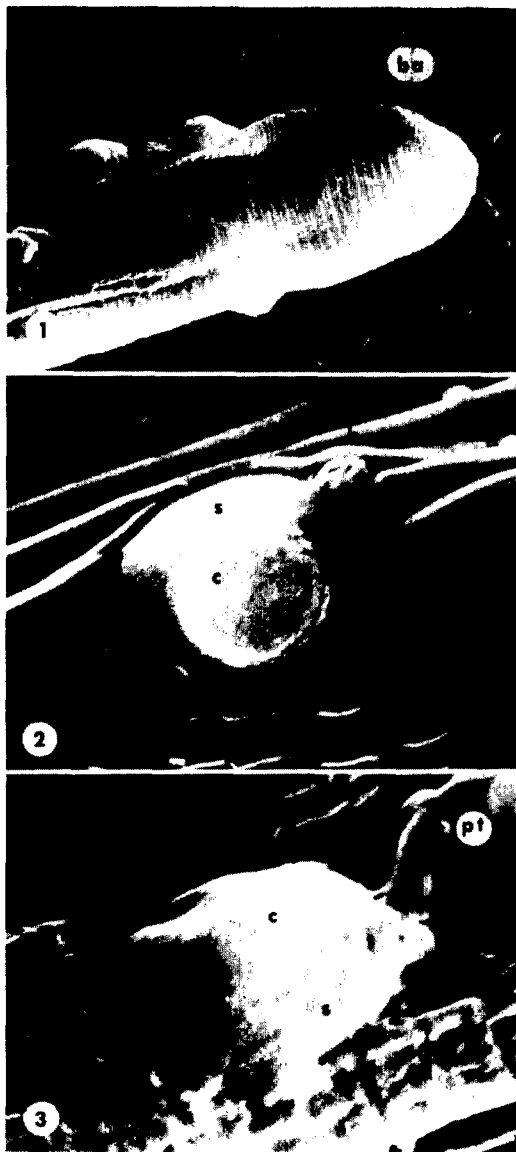
Larvae of *M. incognita* from a greenhouse culture infested with spores of *D. penetrans* were killed and fixed in lactophenol at room temperature and stained for 24 h in acid fuchsin. Then the larvae were washed in tap water for 10 min and

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FIG. 1-3. Scanning electron micrographs of *Meloidogyne incognita* parasitized by *Duboscqia penetrans*. 1) A side view of spores attached to a larva (X 3,000), 2) An overview of a *D. penetrans* spore showing the shoulder and cap parts of a penetration tube (X 15,000). 3) Another overview of *D. penetrans* spore showing a well-developed penetration tube (X 15,000). (h=bud, ba=bacteria, c=cap, pt=penetration tube, s=shoulder).



moved to a 22 mm², 0-size coverslip. The coverslip was mounted off-center on a 12-mm diam brass stub coated with clear fingernail polish, and the specimens were dried for scanning.

A Denton DV-502 high vacuum evaporator operating at 3×10^{-5} Torr was used to spread a standard layer of gold on the specimens. A Jeol JSM-2 SEM with an accelerated voltage of 35 kv scanned the parasitized larvae. A light compound microscope was also used to look at the specimens. Kodak Tri-X Ortho film No. 4163 was used to make the photographs.

SEM showed root-knot larvae parasitized by 7-12 *D. penetrans* spores attached to the nematode cuticles (Fig. 1). The spores were rounded above and flattened below. A round cap was part of the mature spore (Fig. 2, 3). The spores averaged 4.3 μm diam by 1.6 μm in depth, and the spore cap 2.8 μm diam by 0.4 μm in depth. Except for the wrinkled lines at their juncture, the spore and its cap were smooth. Pressure on the cuticle probably caused the flattened spore bottom.

Buds and tubes were seen on the spore bases (Fig. 3). The buds apparently form tubes that penetrate the host cuticle. SEM photos of buds and tubes on mature spores confirm the observations of Allen (1).

SEM photos of mature spore forms and caps generally confirm descriptions of *D. penetrans* provided by Thorne (5). Spore morphology is like that of a protozoan; spore size was greater than *Bacillus* spp. cells. Spores did not have the crescent shape of *Bacillus popilliae* sporangia nor of any known bacterium. Endospores or rod-shaped spores were not seen, although bacteria were noted near parasitized larvae (Fig. 1). Hyphae, thalli, and other fungal structures were not found. For these reasons, we prefer to keep this parasite in the protozoa.

LITERATURE CITED

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