SEM Observations on Nematode Cuticle Penetration by *Bacillus penetrans*

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Because of the small size of the endoparasite, *Bacillus penetrans* (Thorne, 1940) Mankau, 1975 (2), it has been difficult to study, and disagreement about the method of its penetrating nematode cuticle has appeared in the literature (3). Concepts previously presented (3) are herein substantiated by *en face* scanning electron micrographs (SEM) of several infected specimens of *Dolichodorus* from Mozambique.

Specimens routinely prepared for taxonomic studies (4) had *B. penetrans* sporangia (spores) attached to the lip region (Fig. 1, 3, 4). Sporangia are most frequently attached to the anterior or neck region of nematodes [Fig. 2 *(Merlinius tessellatus)*] but they may occur anywhere on the body. The large, disc-like lip region of *Dolichodorus* sp. apparently provides sufficient surface area for sporangial attachment in that location. The sporangium attached to the lip region of the nematode in Fig. 1 has blocked the buccal opening and surrounding pits within which labial papillae apparently occur. If such sporangia became attached with sufficient force that the nematode could not dislodge them with its substantial stylet, the nematode would probably starve because of the parasitism. The attached sporangium in Fig. 1 is about 3.2 μm diam and consists of a central, raised region (Arrow A) 1.6 μm in diam surrounded by a second raised area (Arrow B). The central region corresponds in size to the endospore, and the outer area corresponds to the remainder of the sporangium.

During processing of one *Dolichodorus* specimen for viewing by SEM, a sporangium became detached from the lip region and the cuticle beneath the spore could be clearly examined (Fig. 3, 4). A hole approximately the size of the nuclear region of the endospore (0.5 μm) penetrates the cuticle. The presence of this characteristic tends to support the concept of enzymatic penetration proposed by Mankau and Imbriani (3). It appears that penetration is accomplised by means of a germ tube approximately the same size as the nuclear region of the endospore protoplast. A circular area 0.7 μm thick surrounding the hole is raised and has the appearance of having been softened by chemical action. Another slightly raised, contiguous ring 0.8 μm thick with a fimbriated edge surrounds the previous area. The swollen inner ring conforms in size to the endospore of *B. penetrans* and the outer circle delineates the circumference of the detached sporangium. Similar raised areas of the cuticle have been observed beneath attached sporangia in sections of root-knot nematode larvae prepared for light microscopy, but the areas were not as clear as those in the present material (3). Optical sections through the cup-shaped sporangia attached to nematodes give the

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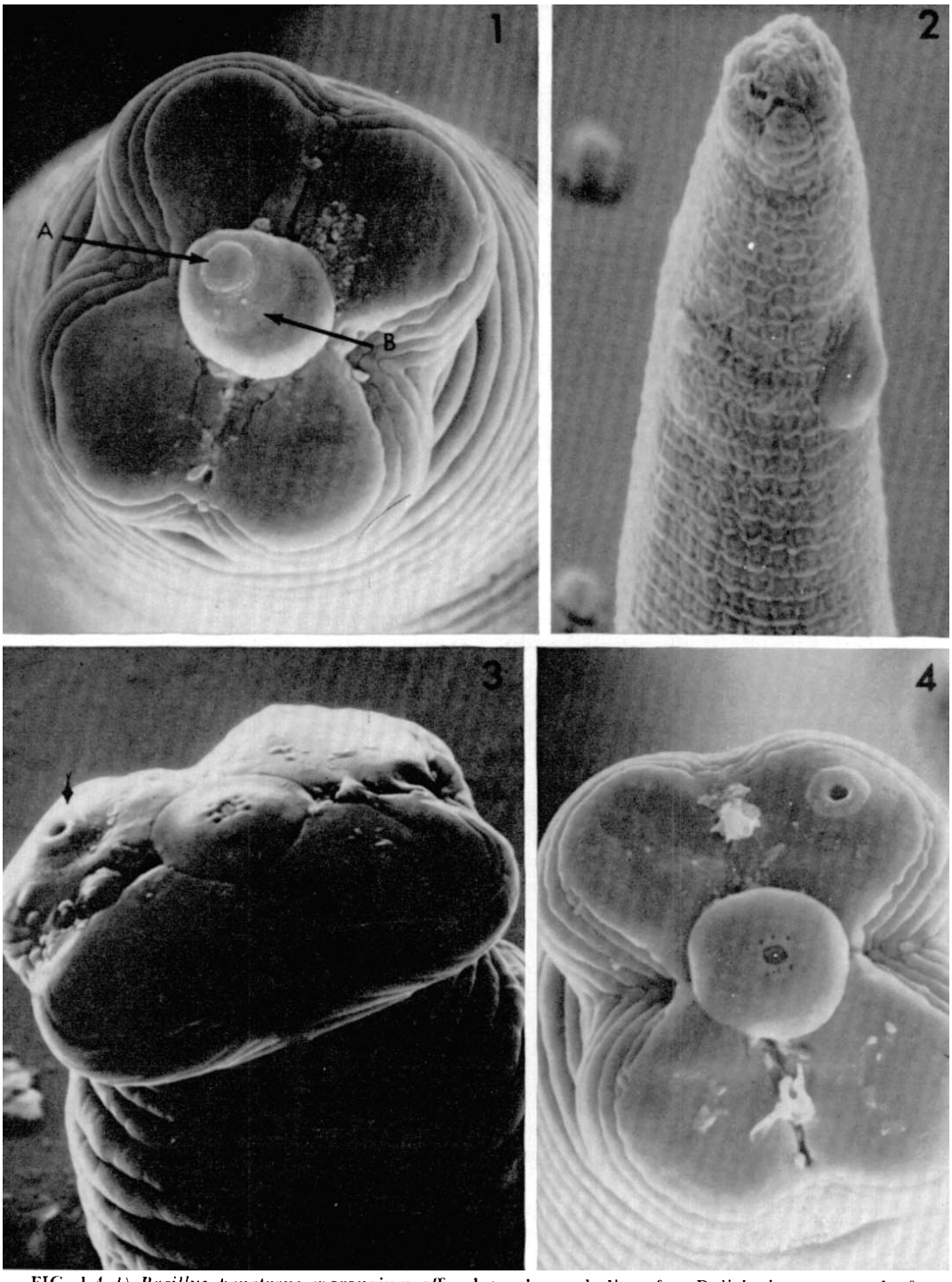

FIG. 1-4 *1) Bacillus penetrans* sporangium affixed to the oral disc of a *Dolichodorus* sp. male from Mozambique [buccal opening and oral pits are completely blocked by the "spore";—inner raised disc is endospore (A) and surrounding area (B) is sporangium; X5,000]. *2) B. penetrans* sporangium attached to the cuticle in the neck region of a *Merlinius tessellatus* (Goodey) Siddiqi male from the Netherlands (X4,000). *3-4)* Side and *en face* view, respectively, of penetration port of a detached *B. penetrans* sporangium on lip region of *Dolichodorus* sp. male from *Mozambique* (the hole is surrounded by two concentric bands of cuticle altered by the parasite; X5,000).

impression of a suction cup-like force applied to a chemically altered cuticle. This impression is borne out in the present observation.

LITERATURE CITED

1. MANKAU, R. 1975. Bacillus penetrans (n. comb.) causing a virulent disease of plant parasitic nematodes. J. Invert. Pathol. 26:333-339.
2. MANKAU, R., and J. L. IMBRIANI. 1975. The life cycle of an endoparasite in some tylenchid nematodes. *Nematologica* 21:89-94.
3. SHER, S. A., and A. H. BELL. 1975. Scanning electron micrographs of the anterior region of some species of Tylenchoidea (Tylenchida: Nematoda). J. Nematol. 7:69-83.