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Electron microscopy of infection of nematodes by *Dactylaria haptotyla*

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Infection of nematodes by *Dactylaria haptotyla*, a nematode-trapping hyphomycete, was studied by electron microscopy. The cytoplasm of the adhesive knob in the fungus contained a number of electron-dense, membrane-bound vesicles, 0.2–0.5 μm in diam. The vesicles were rarely seen in the stalk cell or vegetative cell cytoplasm. When the adhesive knob came into contact with the nematode's cuticle, it secreted an adhesive which was seen in ultrathin sections between the knob and the cuticle as an amorphous mass. At the same time, electron-dense vesicles in the cytoplasm were reduced in number and many small vacuoles developed. Soon after capture of a nematode, the cell wall of the adhesive knob became obscure at the prospective site of penetration, where a vesicle, 0.7 μm in diam, was found in serial thin sections of the knob's cytoplasm. At the site facing the vesicle, the peripheral part of the nematode's cell exhibited a high electron density. The vesicle, which appeared to be derived from smaller electron-dense vesicles coalesced with each other, released its enzymic contents toward the captured nematodes before penetration by the fungus.

Key Words——adhesive knob; *Dactylaria haptotyla*; electron microscopy; nematode; penetration vesicle.

Introduction

Nematode-trapping hyphomycetes produce trapping organs of various types, including constricting rings, non-constricting rings, adhesive nets and adhesive knobs. Although ultrastructural aspects of various of these traps in thin sections have been reported by several authors (Heintz and Pramer, 1972; Dowsett and Reid, 1977, 1979; Dowsett et al., 1977; Nordbring-Hertz and Stålhammar-Carlemalm, 1978; Wimble and Young, 1983a, b, 1984; Saikawa, 1985), the initial stages of fungal infection just before penetration into nematodes have not yet been fully examined. The ultrastructure of the initial stages of penetration by an adhesive knob of *Dactylaria haptotyla* Drechsler are shown in the present study.

Materials and Methods

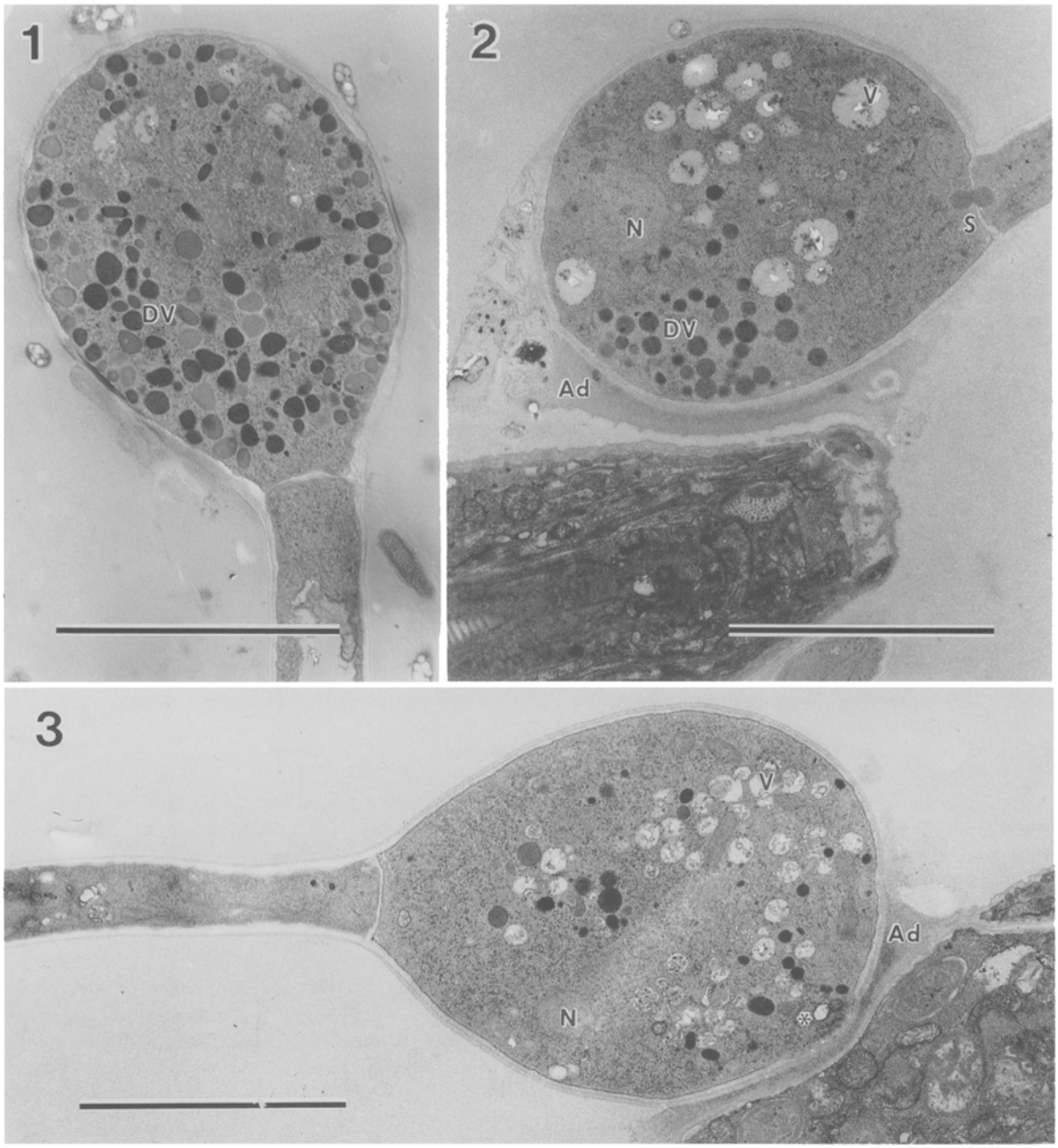
Dactylaria haptotyla parasitizing nematodes was found on a water agar plate in October 1992. General morphology of the fungus was identical to that reported by Drechsler (1950). The plate had been incubated for about 1 month with a pinch of moss collected in the campus of Tokyo Gakugei University, Koganei, Tokyo, Japan. The fungal materials capturing nematodes were prefixed in 2.5% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.2) by microwave irradiation three times for 4 sec at below 37°C (Mizuhira, 1988). After the prefixation the specimens were washed with the same buffer for 1.5 h, and postfixed in OsO_4 in the buffer at 4°C for 12 h. After dehydration through an acetone series, the fungal materials were embedded in Poly/Bed 812 (Polysciences, Inc., Warrington, PA, U.S.A.). Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a JEOL 100CXII electron microscope operating at 80 kV.

Results and Discussion

The trapping organ of *D. haptotyla*, a nematode-destroying hyphomycete, is a unicellular adhesive knob, subspherical or prolate ellipsoidal in shape, mostly 7.5–10 μm long and 6–9 μm wide, developed on a slender stalk of 1–3 hyphal cells. Electron micrographs showed that the cytoplasm of the adhesive knob contained a number of membrane-bound vesicles, 0.2–0.5 μm in diam and of various electron densities (Figs. 1–4), in addition to the usual cell constituents, such as nuclei, mitochondria and ribosomes. The electron-dense vesicles were rarely seen in the stalk-cell or vegetative cell cytoplasm (Figs. 1, 2). When the adhesive knob came into contact with the nematode's cuticle, it secreted an adhesive. This was seen in ultrathin sections between the knob and the cuticle as an amorphous mass with a moderate electron density (Figs. 2–7). At the same time, electron-dense vesicles in the cytoplasm were reduced in number and many small vacuoles developed (Figs. 2–7).

The hyphal cell wall of the fungus was composed of an outer, electron-dense layer and an inner, less dense layer. In thin sections, both layers exhibited similar thicknesses in the adhesive knob (Figs. 3, 4), while in other parts of the mycelium the former layer of cell wall was al-

ways thinner than the latter. When the adhesive knob came into contact with the nematode's cuticle, both layers of cell wall became obscure at the prospective site of penetration (Fig. 3), where a vesicle, 0.7 μm in diam, was found in the cytoplasm in serial ultrathin sections of the adhesive knob (Figs. 4-6). In addition, the periphery of the nematode's cell (Figs. 4-6), which was depressed by the adhesive knob, showed a high electron density at the site facing the vesicle. It is obvious that the vesicle had migrated there and discharged its enzymic contents



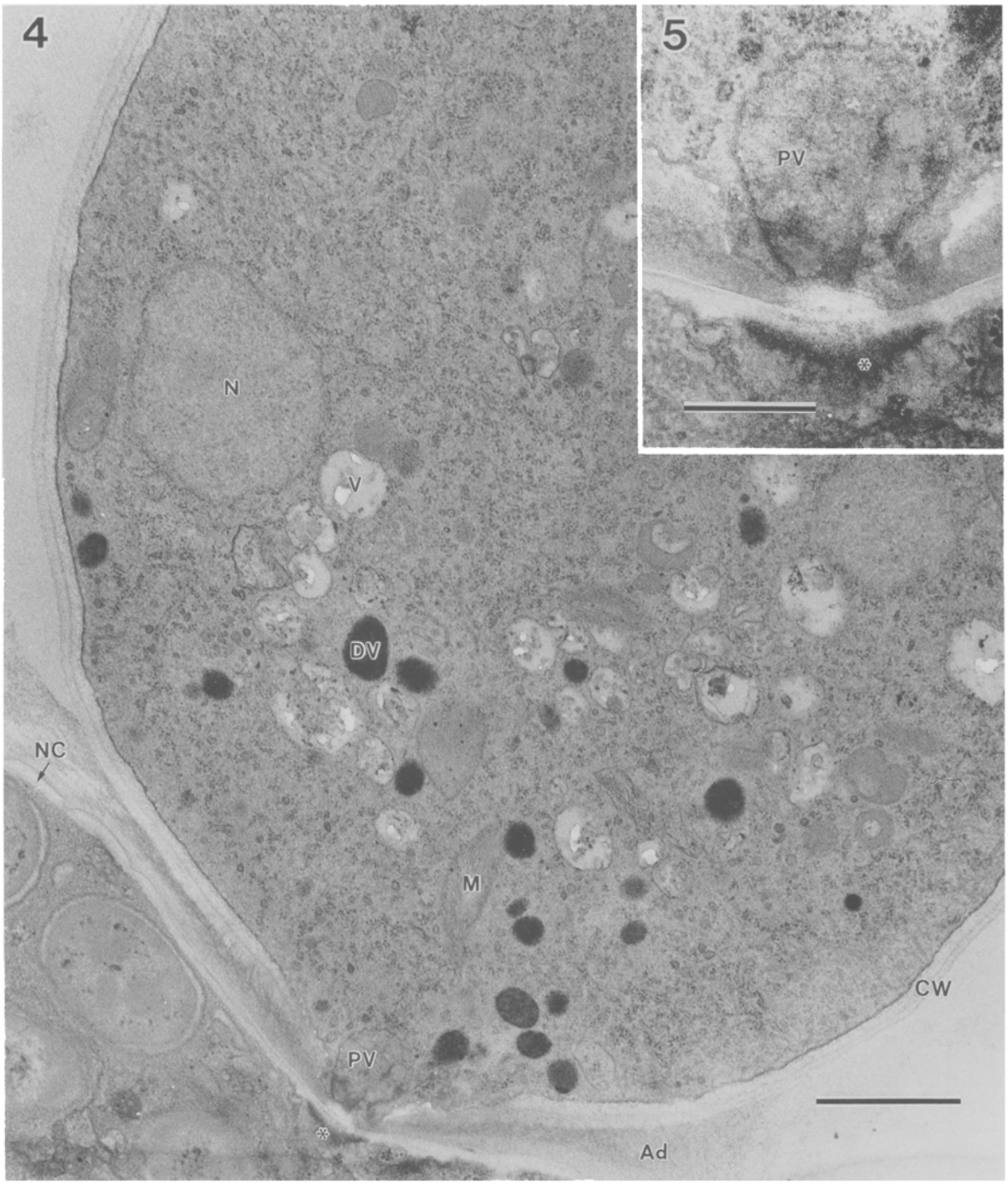
Figs. 1-3. Electron micrographs of adhesive knobs of *Dactylaria haptotyla* before penetration into nematodes.

1. Cytoplasm of an adhesive knob containing a number of electron-dense, membrane-bound vesicles of various electron densities (DV). Bar: 5 μm.
2. Adhesive (Ad) secreted from an adhesive knob seen between the knob and the nematode's cuticle. The knob's cytoplasm develops vacuoles (V), and electron-dense vesicles (DV) are seen in its peripheral region facing the adhesive. Bar: 5 μm.
3. Cell wall of an adhesive knob becomes obscure at the prospective site of penetration (*), where the knob's cytoplasm pushes against the nematode. Bar: 5 μm.

Abbreviations used in figures: Ad, adhesive; AK, adhesive knob; CW, cell wall; DV, electron-dense vesicle; IB, infection bulb; M, mitochondrion; N, nucleus; NC, nematode's cuticle; PV, penetration vesicle; S, septum associated with Woronin bodies; V, vacuole.

toward the captured nematode to degrade the cuticle, because the vesicle was not electron-dense at all and its membrane was wavy in appearance (Fig. 5). The vesicle could be derived from smaller electron-dense vesicles coalesced together.

Wimble and Young (1984) reported ultrastructures of *Dactylella lysipaga* Drechsler, a nematode-trapping fungus producing both adhesive knobs and nonconstricting rings. In thin sections of the fungus a vesicle undoubtedly identical to that found in *D. haptotyla* in the present study was seen at the site of penetration by an adhesive knob. In that case, the cytoplasm had already penetrat-



Figs. 4, 5. Electron micrographs of an adhesive knob of *Dactylaria hyptotyla* just before penetration into a nematode.
4. A vesicle (PV), 0.7 μm in diam and with moderate electron density, is seen at the prospective site of penetration, where the cell wall of the knob seems almost to be digested. Cytoplasm of the nematode shows a high electron density (*) at the site facing the penetration vesicle. Bar: 1 μm.
5. Enlargement of the vesicle at the site of penetration in Fig. 4, showing the wavy outline of the membrane. Bar: 0.5 μm.

ed slightly into the nematode to form a developing infection bulb, which was surrounded by an electron-dense material (Wimble and Young, 1984, fig. 30). Saikawa (1985) found another electron-dense, barrel-shaped, plug-like structure (0.5 × 0.7 μm in size) before penetration of nematodes by a nonconstricting-ring trap of *Dactylella leptospora* Drechsler. In the latter case, no sign of penetration was recognized in the nematode's cell. Thus, the electron micrographs in Figs. 4–6 in the present study show an intermediate stage in the infection process between that in *D. leptospora* reported by Saikawa and that in *D. lysipaga* by Wimble and Young. The vesicle for penetration, or "penetration vesicle," is an organelle that probably occurs commonly in this stage of infection by nematode-trapping hyphomycetes.

After breakdown of the nematode's cuticle, the

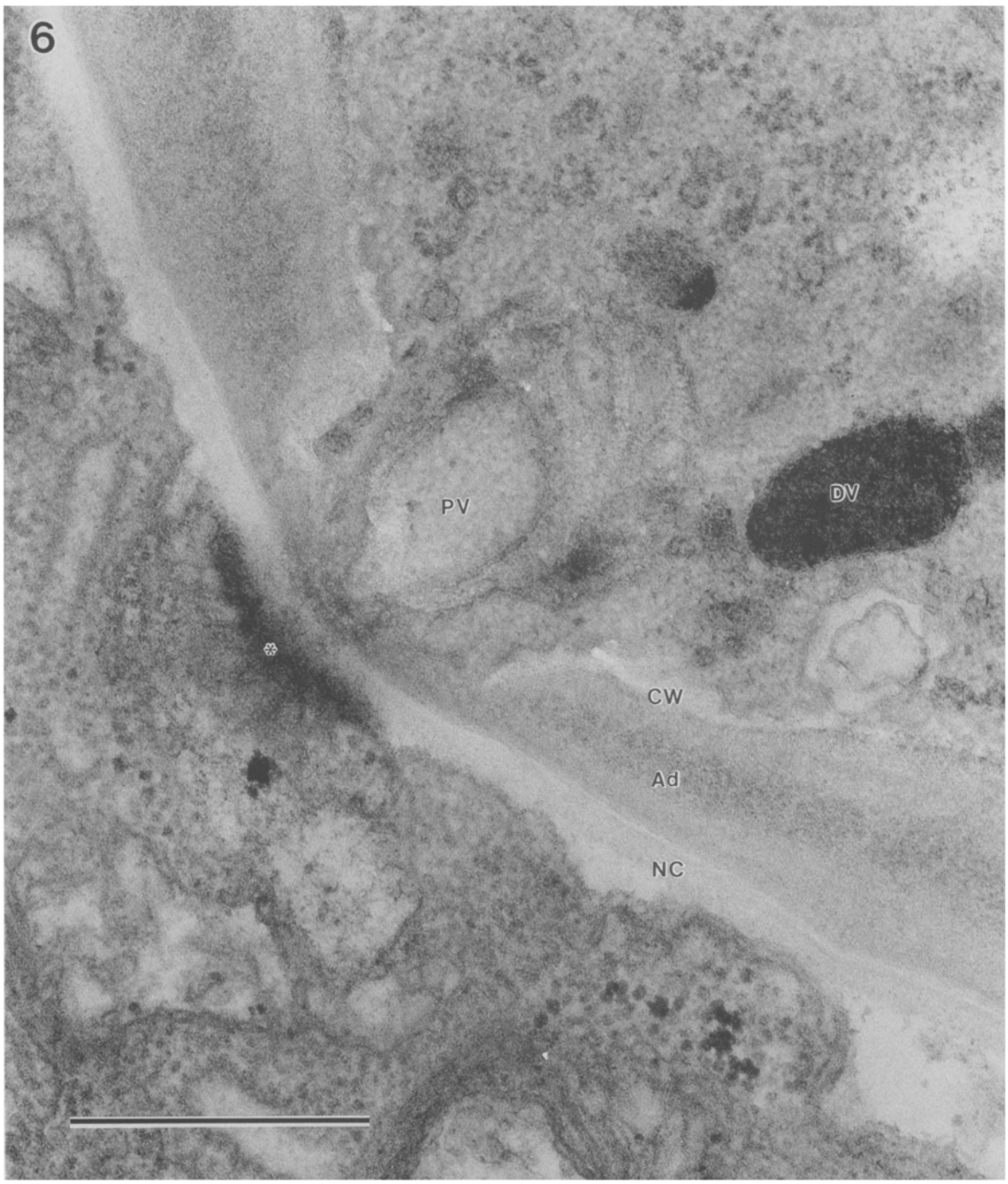
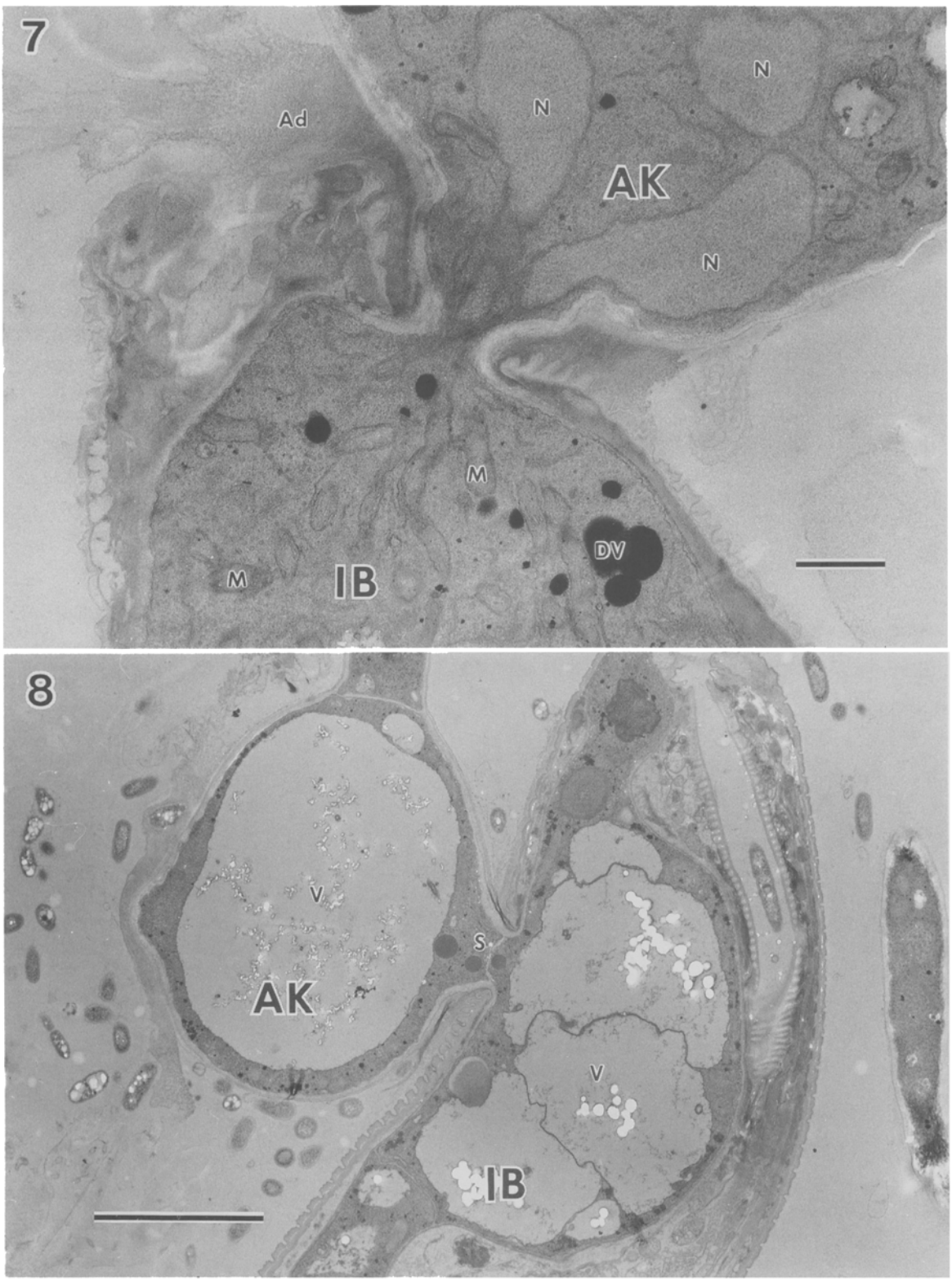


Fig. 6. Electron micrograph of another section of the same adhesive knob of *Dactylaria haptotyla* as in Fig. 4 at a higher magnification. The penetration vesicle (PV) with moderate electron density at the prospective site of penetration is cut slightly off center. Peripheral cytoplasm of the nematode shows a high electron density (*) at the site facing the vesicle. Bar: 0.5 μm.



Figs. 7, 8. Electron micrographs of adhesive knobs and infection bulbs of *Dactylaria haptotyla*.

7. Formation of the infection bulb (IB) by an adhesive knob (AK). Cell organelles are migrating into a newly formed infection bulb in the nematode body. Bar: 1 μm.
8. Adhesive knob (AK) and infection bulb (IB) at a later stage of infection. Most portions of fungal cells contain large vacuoles (V). Bar: 5 μm.

knob's cytoplasm in *Dactylaria haptotyla* migrated into a newly formed infection bulb in the nematode's body (Fig. 7), and a septum formed at the boundary between the knob and the infection bulb (Fig. 8). Similar features showing migration of cytoplasm into infection bulbs have already been reported by Wimble and Young (1984).

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