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FULL PAPER

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Time-lapse photomicrography and electron microscopy on initiation of infection of nematodes by *Dactylella ellipsospora*

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Abstract Infection of nematodes by two strains of *Datylella ellipsospora* was observed using video photomicrography and electron microscopy. By light microscopy, each cell with adhesive knobs contained a number of particles that were distributed evenly before capture of a nematode. The cytoplasmic particles moved to and fro at random. At the moment when the knob cell came into contact with a nematode, the particles accumulated at the place where the cell wall of the knob stuck firmly to the nematode cuticle and exuded adhesive at the same time. The adhesive can be seen near the point of contact between the cell wall of the knob and the cuticle of the nematode. At that point, the knob cell produced an infection peg in most cases, and the cell showed a preference to invade the body of the nematode rather than the tail and head. During capture, accumulation of cytoplasmic particles was seen until infection-bulb formation began. In electron micrographs of ultrathin sections, most of the particles could be seen as electron-dense vesicles, 0.2–0.6μm in diameter. After attachment of the knob cell to the nematode cuticle, the vesicles were found to fuse with plasmalemma one after another to exude adhesive seen as an amorphous electron-dense substance.

Key words Adhesive knob · *Dactylella ellipsospora* · Electron microscopy · Infection of nematode · Video photomicrography

Introduction

Time-lapse photography was employed by Higgins and Pramer (1967) to show the formation and closure of a constricting-ring trap by *Arthrobotrys dactyloides* Drechsler in eight figures from films taken with a Bolex 16mm movie camera. It was clear that a tiny bud was formed in opposition to the advancing tip of a curved hook of hypha just before completion of a closed ring. However, Drechsler (1950) had already found such morphological changes, including bud formation, in a constricting ring of *Dactylella aphrobrocha* Drechsler and in a developing branch of hyphal networks of *Dactylaria eudermata* Drechsler. The reason Higgins and Pramer were not aware of the illustrations made by Drechsler may be the inconspicuousness of his line drawings, which were inserted into other many illustrations of conidia and other structures. It is obvious, however, that time-lapse photography would be a superior technique to illustration by line drawing for most purposes to show rapid real changes in fungus morphology. Using the time-lapse technique, infection of nematodes by adhesive knobs of *Dactylella ellipsospora* Grove is shown in the present study.

As seen in the light microscope, the cytoplasm of young hyphal traps is occupied by a number of particles before capture of nematodes. Most of the particles are thought to be identical with electron-dense vesicles in ultrathin sections. The vesicles, like particles in the light microscope, are found only in cells of adhesive traps and are thought to be sites where the adhesive is localized before secretion (Heintz and Pramer 1972). Among various types of adhesive traps, adhesive knobs had already been studied ultrastructurally in *Dactylella drechslerii* (Tarjan) R.C. Cooke & Dickinson (Heintz and Pramer 1972), *Dactylaria candida* Drechsler (Dowsett and Reid 1977b), *Dactylella lysipaga* Drechsler (Wimble and Young 1983a,b, 1984), and *Dactylaria haptotyla* Drechsler (Saikawa and Kaneko 1994). In the present study, electron micrographs of adhesive knobs at the initiation of infection of nematodes by *Dactylella ellipsospora* are also shown.

Materials and methods

The G and Y strains of *Dactylella ellipsospora* were recovered from soil collected from the campus of Tokyo Gakugei University, Koganei-shi, Tokyo in April 1998 and from an

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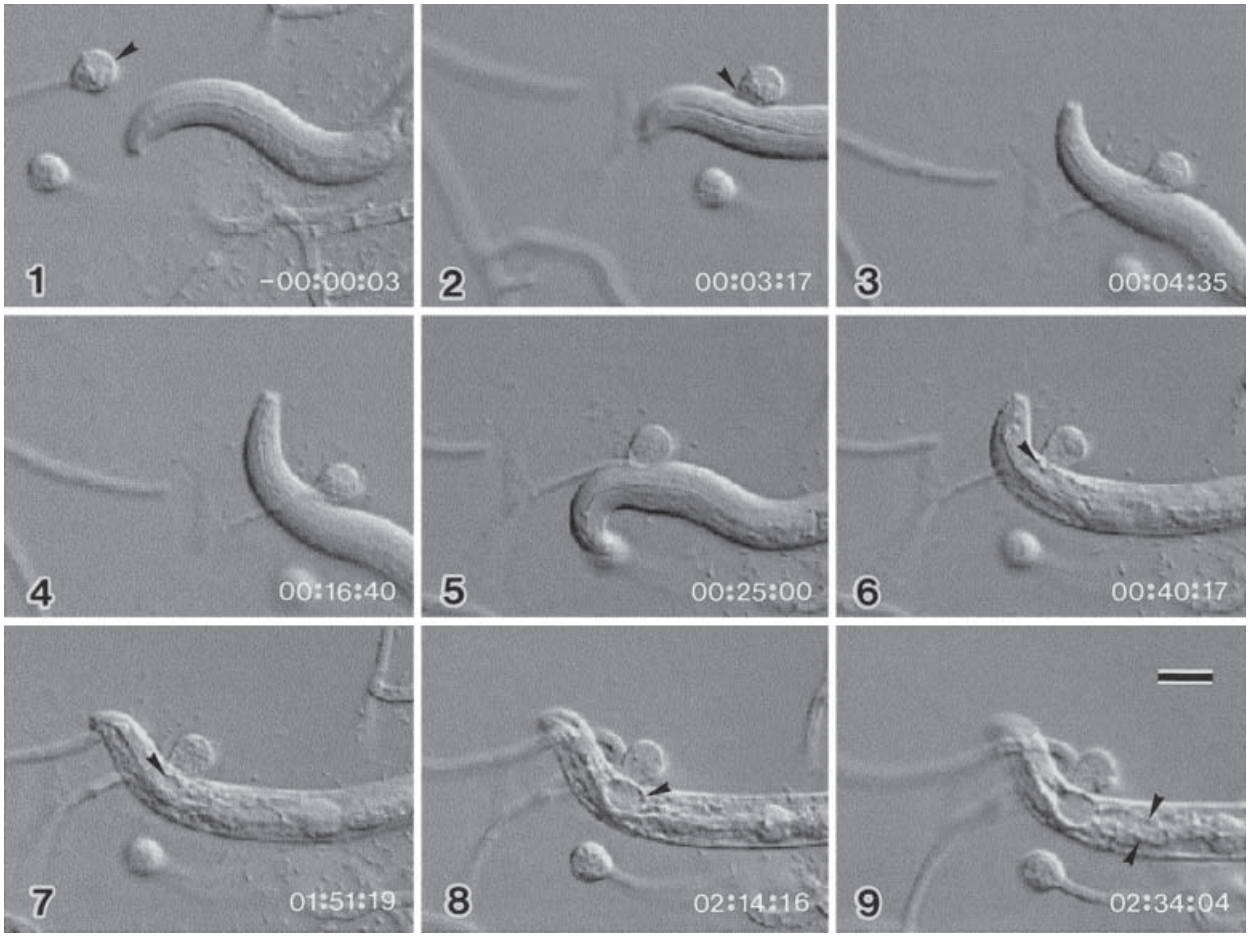
urban park, "Yakushi-ike," Machida-shi, Tokyo, in November 1998, respectively. The two fungal strains were cultured on water agar plates with nematodes (*Rhabditis* sp.), from which a small portion was transplanted onto fresh agar plates at an interval of 2 weeks. The nematode was multiplied on 1/10 diluted potato dextrose agar (PDA) in the same way. For light microscopy, 5 × 5 mm of agar plate including a fungus that captures nematodes was cut into pieces and placed onto a glass slide. After adding a droplet of water, the agar piece was covered with a cover glass for observation under a microscope (Zeiss Axioscope) equipped with interference contrast. Video photomicrographs of the fungus were taken with a digital video camera (Sony DXC-D30, Sony, Tokyo, Japan) that was connected to a videocassette recorder (Sony WV-D9000, Sony, Tokyo, Japan).

For electron microscopy, specimens were fixed in 3% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.2) for 1.5 h at room temperature, 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 epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a JEOL 100CXII electron microscope (JEOL, Tokyo, Japan) operating at 80 kV.

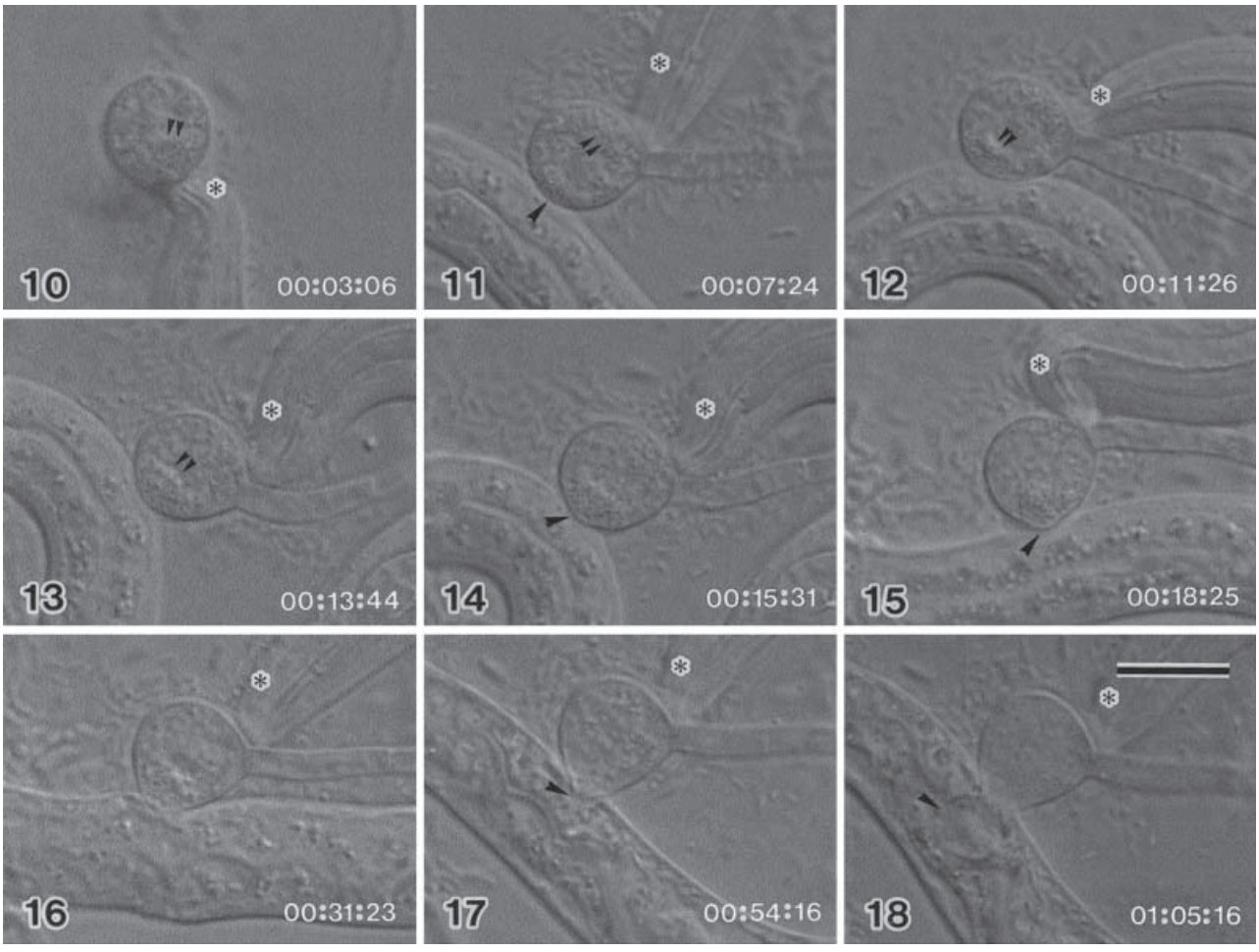
Results

Morphology of the two strains of *Dactylella* used in the present study resembled that of *Dactylella ellipsospora sensu* Drechsler (1937), except that the sizes of adhesive knobs, 9–19 × 8–11 μm in the G strain and 9–16.7 × 8–11.3 μm in the Y strain, were slightly larger than those in Drechsler's strain, 7–11.5 × 6.5–10 μm. The knob cell contained a number of particles evenly distributed throughout the cell before capture, although they always moved to and fro at random.

When a nematode came into contact with a knob cell (Fig. 1), the worm struggled violently for a while to escape from the knob (Figs. 2–5), and the knob cell eventually



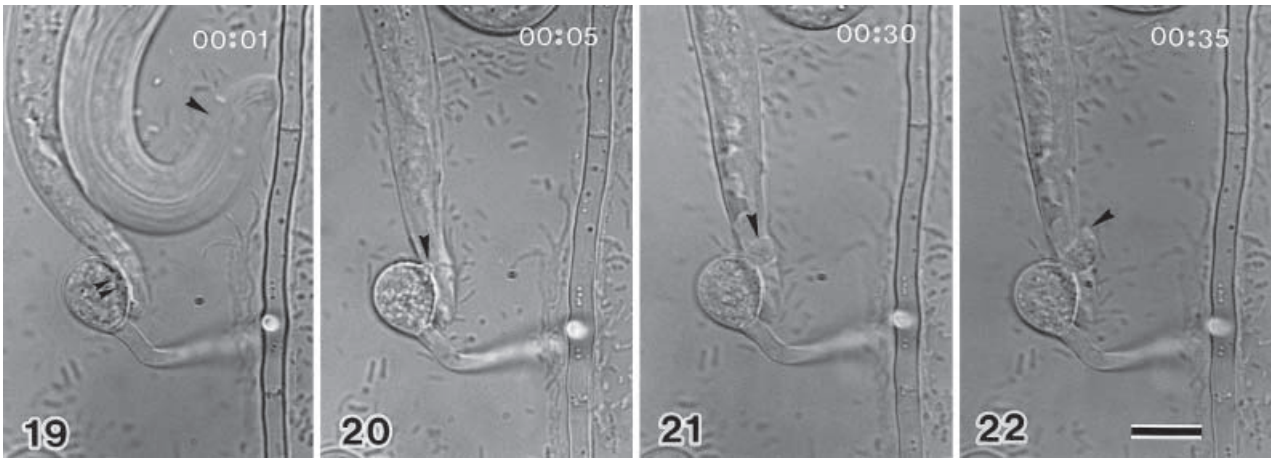
Figs. 1–9. *Dactylella ellipsospora*, G strain. Capture and invasion of nematode in 150 min. The times are shown as 00 (h): 00 (min): 00 (s), starting at initiation of infection of nematode. **1** A nematode right before being captured by one (*arrowhead*) of the two adhesive knobs. **2** The nematode just after being captured by one of the two knobs seen in **1**. *Arrowhead* shows the adhesive, which is not clear here but is recognized clearly when watched in video. **3–5** The worm, struggling violently to escape from the knob. **6** An infection peg (*arrowhead*) appeared from the knob cell. **7, 8** Infection bulb (*arrowhead*) developed from the infection peg in **6**. **9** A vegetative hypha (*arrowheads*) that developed from the infection bulb. *Bar* **9** 10 μm (for **1–9**)



Figs. 10–18. *Dactylella ellipsospora*, Y strain. The times are shown as 00 (h): 00 (min): 00 (s), starting at initiation of infection of nematode. **10** A knob cell at 00:03:06 after capture at the head region (*asterisk*) of a nematode. *Double arrowhead* shows an accumulation of cytoplasmic particles. **11** The knob cell, making contact with the body portion (*arrowhead*) of the same nematode at 00:07:24 after its original attachment to the head (shown in **10**). *Double arrowhead* shows an accumulation of cytoplasmic particles. **12, 13** Rapid accumulation of cytoplasmic particles (*double arrowhead*) to the portion where the knob cell attached secondarily to the nematode's body. **14** The knob shows the initiation of development of an infection peg at the position of contact with the nematode body (*arrowhead*) at 00:15:31 after capture of the nematode. **15, 16** Development of the peg (*arrowhead* in **15**) into which incorporation of cytoplasmic particles is seen. The portion of the peg is out of focus in **16** because of movement of the nematode. **17** Beginning of the formation of an infection bulb (*arrowhead*). **18** Infection bulb (*arrowhead*) seen at 01:05:16 after capture of the nematode. *Asterisk*, head region of nematode. *Bar* **18** 10 μm (for **10–18**)

developed an infection peg (Fig. 6) 5–40 min after its attachment to the nematode cuticle (Figs. 6, 17, 28–31). During these events, particles in the knob cytoplasm always accumulated near the point of attachment, still continuing random movement (Figs. 10–17, 19–22). In only one occasion in this study did an adhesive knob not produce the infection peg after capture of a nematode at its head (asterisk in Figs. 10–18) when the knob adhered to a body portion of the same nematode right after the first attachment. In this case, the accumulation of the cytoplasmic particles was originally seen near the head region of a nematode (Figs. 10, 11), and then most of the particles soon moved to the opposite side of the knob cell where the knob attached again to the body portion of the nematode (Figs. 12, 13). The infection peg appeared only from the latter portion of the knob cell. Soon after the formation of the infection peg, the distal end of the peg inflated to produce an infection bulb, 8.0–10.0 μm in diameter, in the nematode body 40–150 min after capture (Figs. 7–9, 18).

The capture of nematodes was performed by adhesive that was seen in video photomicrography between the knob cell and nematode, although it was obscure, unfortunately, in every still video image. It was clearly seen, however, as an electron-dense, amorphous substance in micrographs of ultrathin sections between the knob cell and nematode (Figs. 24–31). The substance was undoubtedly derived from electron-dense vesicles, 0.2–0.6 μm in diameter, in the cytoplasm of the knob; the cell wall of the knob was covered with a layer (Figs. 23–30) that was absent on any other parts of the cell wall of the vegetative hyphae. The electron-dense vesicles were evenly distributed before capture of a nematode (Fig. 23) or before adhesion among knob cells (Fig. 24). In these cases, the dense vesicles moved toward the point of contact with a nematode or neighboring knob cells



Figs. 19–22. *Dactylella ellipsospora*, G strain. The times are shown as 00 (h): 00 (min), starting at initiation of infection of nematode. **19** An adhesive knob just after its attachment to the tail portion of a nematode. In the knob cell, the cytoplasmic particles accumulate toward the tail portion (*arrowhead*). **20** Region of the nematode still under its struggling. An infection peg (*arrowhead*) developed on the knob cell. **21** The infection peg in **20** inflated to form an infection bulb (*arrowhead*) not inside but outside of the nematode body. **22** The bulb has begun to develop an infection hypha (*arrowhead*) that grows outside the worm. During these events in **19–22**, the nematode was infected by another knob that attached to the body portion of the nematode. *Bar* **22** 10 μm (for **19–22**)

(Figs. 24–27). Thus, the dense vesicles in ultrathin sections were almost identical with the cytoplasmic particles seen in the light micrographs, although the particles also include other cell organelles such as nuclei and mitochondria. The electron micrograph of Fig. 27 shows the beginning of the accumulation of electron-dense vesicles and exudation of adhesive, in which some of the vesicles are fusing with plasmalemma of the cell making a undulating, electron-dense layer. About 5–10 min after capture, an infection peg developed (Fig. 28). An accumulation of electron-dense substance remained at the distal portion of the developing infection peg (Fig. 29). The dense substance disappeared from the infection peg (Fig. 31) when the peg began to produce an infection bulb (Fig. 30), leaving a undulating, electron-dense layer that was thought to be the boundary between the cytoplasm and an accumulation of electron-dense material seen in Figs. 28 and 29.

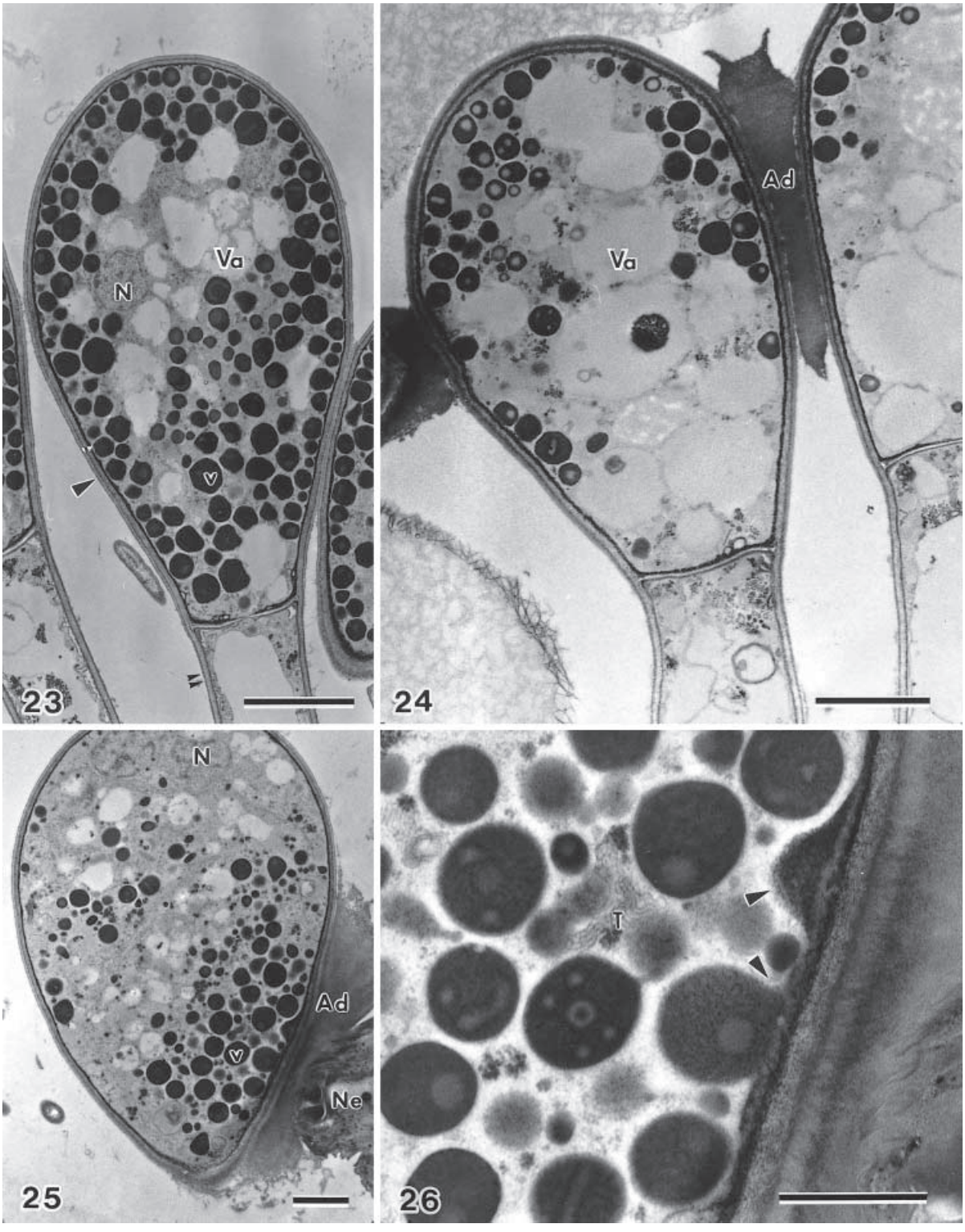
The adhesive did not perform perfectly in capturing a nematode, and escape from a knob cell was seen frequently. On a few occasions, the knob cell developed an infection bulb not inside but outside the nematode (Figs. 19–22). In such cases, the "external" infection bulb produced a hypha that grew outside the worm (Fig. 22). The nematode was invaded, in fact, by another adhesive knob of the same fungus that attached to the body portion of the nematode. This portion is out of the frame of these figures.

Discussion

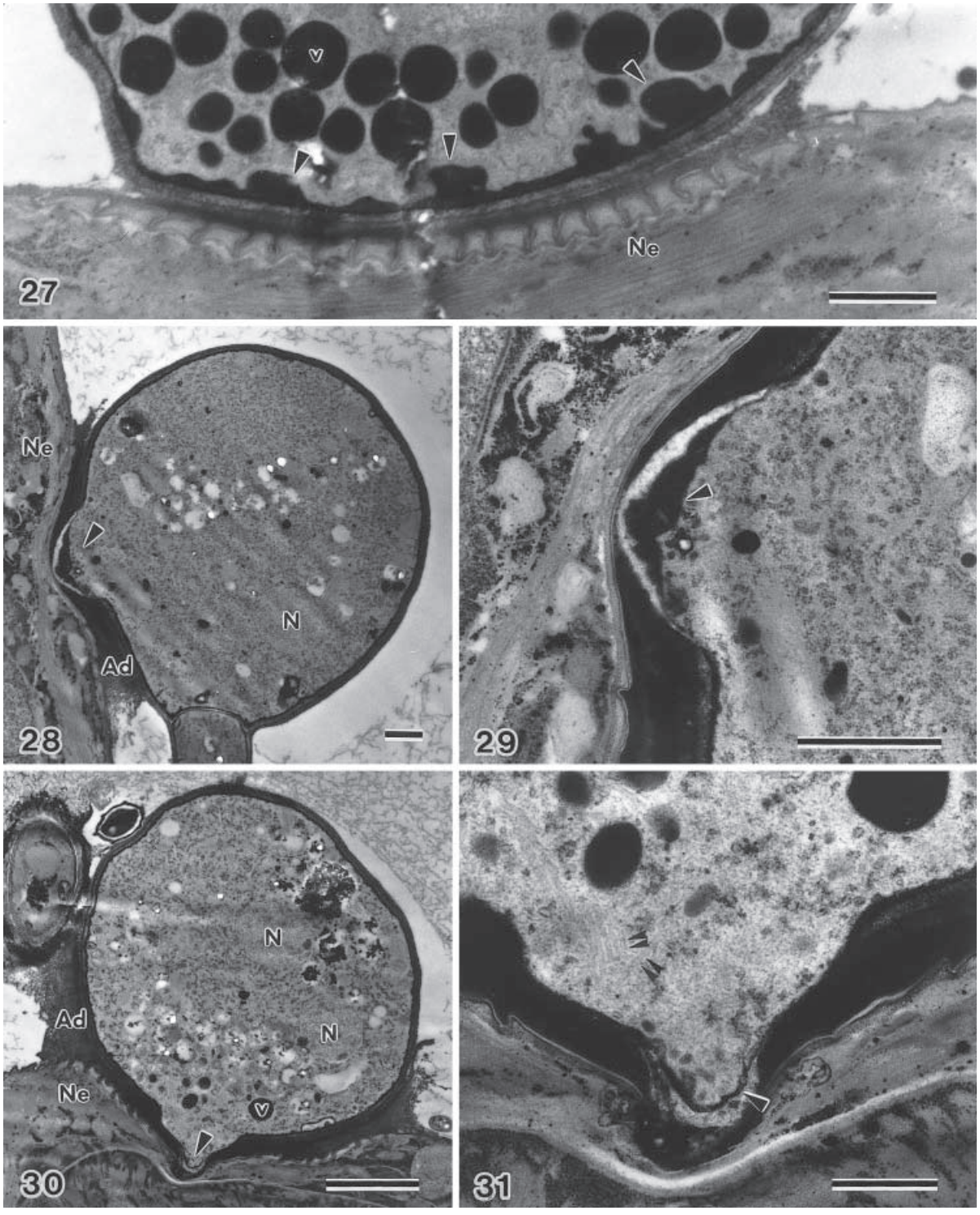
Although Shepherd (1955) illustrated the sequence of infection of a nematode by one of adhesive hyphal networks in *Arthrobotrys oligospora* Fres. by freehand, it is necessary to show such an event using photomicrography to obtain more precise records of it. The process of infection by adhesive knobs, therefore, is shown in the present study using the technique of time-lapse photomicrography, as well as electron microscopy, in two strains of *Dactylella ellipsospora*.

In the adhesive knob cell in both strains, G and Y, a number of particles were distributed evenly in the optical microscope to move actively at random throughout the cell before capture of a nematode. Most of these particles have been known as being equivalent to electron-dense vesicles in electron micrographs of ultrathin sections of various trapping organs (Heintz and Pramer 1972), including adhesive knobs in *Dactylella drechsleri* (Heintz and Pramer 1972), *Dactylaria candida* (Dowsett and Reid 1977b), *Dactylella lysipaga* (Wimble and Young 1983a, b), and *Dactylaria haptotyla* (Saikawa and Kaneko 1994). When the knob cell of *Dactylella ellipsospora* came in contact with a nematode, the particles soon accumulated at the position of contact, keeping their random movement. The accumulation and random movement continued until the infection bulb finished inflating, although total numbers in vesicles decreased gradually from the knob cell. The electron-dense vesicles in thin sections also were reduced in number during infection peg formation.

The adhesive was seen in the present video photographs between a knob cell and nematode, although it was obscure in every still image of the video film. It was clearly seen, however, as an electron-dense amorphous substance in electron micrographs of ultrathin sections (Figs. 24–31). As reported by Heintz and Pramer (1972), the substance was undoubtedly derived from the electron-dense vesicles, ~0.3 μm in diameter, in the cytoplasm of the knob cell, although the size was measured as 0.2–0.6 μm in the present study. In the video images the release of adhesive was in accord with the beginning of accumulation of the cytoplasmic particles in the knob cell facing the captured nematode. The electron micrograph of Fig. 27 shows the beginning of the accumulation of electron-dense vesicles



Figs. 23–26. *Dactylella ellipsospora*, G strain. Electron micrographs of ultrathin sections. **23** The knob cell, which is occupied by a number of electron-dense vesicles, is ~0.2–0.6 μm in diameter. *Arrowhead* shows outermost layer of knob cell wall. This layer is absent on the cell wall of the stalk (*double arrowhead*). *N*, nucleus; *v*, electron-dense vesicle; *Va*, vacuole. **24** The knob cell, in connection with other knobs with adhesive (*Ad*). The central portion of the knob cell is occupied by vacuoles (*Va*). **25** An accumulation of electron-dense vesicles (*v*) in the knob cell, right after capturing a nematode (*Ne*) with adhesive (*Ad*). **26** Enlargement of **25**; electron-dense vesicles are fusing with plasmalemma one after another (*arrowheads*). Small clusters of undulating tubules (*T*) are also seen. *Bars* **23–25** 2 μm; **26** 1 μm



Figs. 27–31. *Dactylella ellipsospora*, Y strain. Electron micrographs of ultrathin sections. **27** A part of a knob cell in contact with the nematode cuticle (*Ne*) at initiation of infection. Electron-dense vesicles (*v*) become fused with the cytoplasmic membrane one after another (*arrowheads*), making an electron-dense, undulating layer. **28** A knob cell fixed at almost 5 min after capturing a nematode (*Ne*), under the development of an infection peg (*arrowhead*). **29** Enlargement of **28**. Electron-dense substance (*arrowhead*) made by fusion between several electron-dense vesicles and plasmalemma still remains in the infection peg. **30** A knob cell fixed at 10 min after capturing nematode (*Ne*) shows initiation of development of the infection bulb (*arrowhead*). *Ad*, adhesive; *N*, nucleus; *Ne*, nematode. **31** Enlargement of **30** shows the developing infection bulb covered with an extracellular, electron-dense substance. An electron-dense layer (*arrowhead*) is seen in the cytoplasm ~2 μm behind the growing tip. *Double arrowheads*, microtubules. *Bars* 1 μm

and release of adhesive, because some of the vesicles are fusing with plasmalemma of the cell, making a undulating, electron-dense layer, and in addition the adhesive is not yet exuded as much. Thus, Fig. 27 shows an infection stage before making a penetration vesicle (~0.7–2.0 μm) that was shown in *Dactylella lysipaga* (Wimble and Young 1984), *Dactylaria haptotyla* (Saikawa and Kaneko 1994), and *Dactylella leptospora* (Saikawa 1985), although the vesicle in the latter fungus was found not in the adhesive knob but in the nonconstricting ring. The stage that is shown in Fig. 27 would be earlier than that in figures reported previously.

The ultrastructure of adhesive knobs in *Dactylella ellipsospora* is similar to that of adhesive hyphal branches in *Zoophagus insidians* Sommerstorff (Whisler and Travland 1974), although the latter fungus is classified into Zoopagales, Zygomycota. That is, (1) the traps of *Z. insidians* are packed with a number of vesicles filled with an electron-dense matrix before secretion of adhesive; (2) the adhesive is derived from secretion of the matrix of the electron-dense vesicles and takes the form of a homogeneous fibrous material; and (3) the outer cell wall of the trap separates from the inner wall and the intervening space is filled with adhesive. According to the authors, the adhesive in *Z. insidians* also contained a multivesiculate foamy mass in the homogeneous fibrous material (Whisler and Travland 1974). Although the adhesive in *Dactylella ellipsospora* did not contain such a foamy mass, Wimble and Young (1984) depicted clearly such a figure of another adhesive knob in *Dactylella lysipaga*. Thus, the mode of infection of microanimals, such as nematodes and rotifers, by adhesive hyphal traps would be common among fungi classified into both Hyphomycetes and Zoopagales, Zygomycota.

The knob cell in *Dactylella ellipsospora* was known in the present study to prefer to invade at a portion of the body of a nematode rather than at the tail or head. In the former case (see Figs. 19–22), the knob cell did not invade after attachment to the tail portion of a nematode, because another knob cell attached to the body of the same nematode just after the primary attachment. Another example of such a preference of the adhesive knob was seen in one occasion in which a knob cell chose a body portion of a nematode rather than the head, although the knob cell attached to the head at first (Figs. 10–18). The adhesive did not perfectly capture the nematode, and its escape from a knob cell was frequently seen. Unlike *Dactylaria candida* and *Dactylella leptospora* (Drechsler 1937; Barron 1977; Dowsett and Reid 1977a), however, there was no opportunity to see a nematode carrying adhesive knobs detached from stalks of hypha. The two species of fungi are known to produce nonconstricting rings in addition to the knobs (Drechsler 1937).

At the beginning of the formation of the infection bulb, however, most of both the cytoplasmic particles in the light micrographs and the electron-dense vesicles in the electron micrographs on the knob cell disappeared. Thus, although it is difficult to distinguish one from another, the particles or the vesicles that accumulated right after attachment of knobs to the nematode cuticle were those for exudation of adhesive, and the rest of the particles or the vesicles seen in the infection peg formation would supply an enzymatic substance to dissolve the nematode cuticle.

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