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Short Communication

Scanning electron microscopic observation of resting spore clusters of *Polymyxa* spp.

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Resting spore clusters of *Polymyxa graminis* and *P. betae*, fungal vectors of virus diseases, were observed using the scanning electron microscope. The spore clusters in host plant cells were uncovered using the styrene resin cracking method. Resting spores of *P. graminis* were found to be spherical, while *P. betae* spores were more irregular in shape.

Key Words——*Polymyxa betae*; *Polymyxa graminis*; resting spore cluster; scanning electron microscopy.

Two members of the Plasmodiophoromycetes, *Polymyxa graminis* Ledingham and *P. betae* Keskin, are obligate parasites associated with soil-borne virus diseases of cereal crops and rhizomania of sugar beet, respectively. Several researchers have carried out morphological observations of these fungi with light microscopes or transmission electron microscopes (TEM) (Barr, 1979; Barr and Asher, 1996; D'Ambra and Mutto, 1977; Keskin, 1964; Keskin and Fuchs, 1969; Langenberg and Giunchedi, 1982; Ledingham, 1939). However, scanning electron microscopy (SEM) was employed in only a few studies (Ciafardini and Marotta, 1988, 1989) due to the difficulty in preparing suitable specimens. In this study, I used the styrene resin cracking method (Tanaka et al., 1974) to uncover the spore clusters in host cells. A brief report of this work was presented previously (Okabe et al., 1995).

Barley plants infected by *P. graminis* were grown in a breeding field infested with barley yellow mosaic virus at the National Agricultural Research Center. The sugar beet plants infected by *P. betae* were grown in rhizomania-infested soil at the Hokkaido National Agricultural Experiment Station.

Small pieces of plant roots were prefixed in 1% glutaraldehyde and cut into pieces about 2–3 mm in length. Root pieces containing resting spore clusters were selected by light microscopy and postfixed in 1% osmium tetroxide for 1 h. After dehydration in an ethanol solution series, they were immersed in a mixture of ethanol and styrene monomer (1 : 1) for 1 h, then in pure styrene over night at 4°C. The samples were embedded in small gelatin capsules which were filled with styrene containing 2% benzoyl peroxide. Polymerization was completed within 24 h at 60°C, and polymerized specimens were cracked under the dissecting microscope using a knife and hammer. The cracked pieces were glued onto cover slips with egg albumin solution containing glycerol, then the resin was removed from the root tissues by dipping them in propylene oxide for 2 h (the solvent was renewed every 30 min). Specimens were dried using the critical point method, coated with platinum-palladium, and observed using a Hitachi S-900 electron microscope.

A cross section of the sugar beet root is shown in Fig. 1A. Several cortex cells contain the resting spore clusters of *P. betae*. The spore clusters consist of a single or double layer of resting spores. Observation at higher magnification (Fig. 1B) indicates that the spores are irregular in shape, and some have ridges. The spores are about 4–5 μm in diam and have thick cell walls. There is a space between the cell wall and cytoplasm. Ciafardini and Marotta (1988) stated that immature resting spores are polyhedral and separated from each other, becoming "inflated" as they mature. The spore clusters shown in Fig. 1 seem to be in the mature stage.

A resting spore cluster of *P. graminis* in a barley root cell is shown in Fig. 2. The host plant cell seems to be packed with dozens of resting spores. Every spore is spherical, about 5 μm in diam, and has a thick cell wall. The morphological character of *P. graminis* is similar to that of *Plasmodiophora brassicae* (Buczacki and Moxham, 1979), the causal agent of the clubroot disease of cruciferous plants, but spines on the spore surface are not conspicuous.

This is the first report on a SEM observation of resting spores of *P. graminis* at high magnification. Although it has been said that there is no apparent morphological distinction between *P. graminis* and *P. betae*, this SEM observation showed that the resting spores of *P. graminis* are spherical, while *P. betae* are more irregular, as Barr (1979) and Ciafardini and Marotta (1988) reported. Ciafardini and Marotta (1989) also suggested that the upper area of spores was favorable to germination in their in situ observation. Although I did not ob-

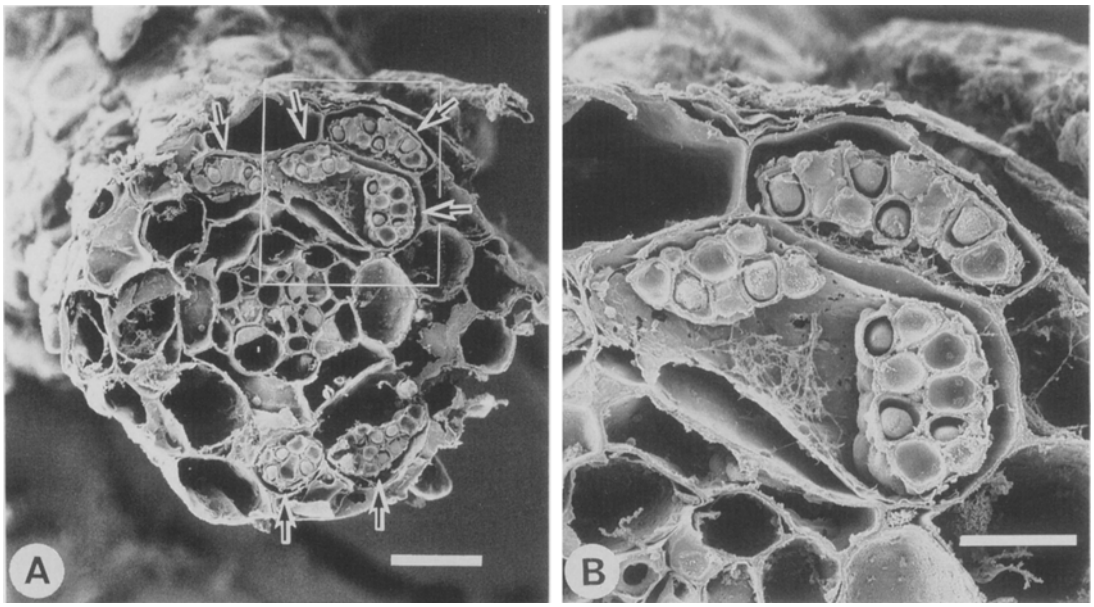

Fig. 1. Resting spore clusters of *P. betae* in the host plant root.
(A) A cross section of sugar beet root. Several cortex cells contain resting spore clusters (arrows). Scale = 20 μm. (B) Higher magnification view of framed area in (A). Scale = 10 μm.

serve germinating spores in this study, the styrene resin cracking method would make in situ observation easier, allowing resting spore clusters to remain in position in host cells. This technique also enabled observation of the host plant root at any position, because the cracking was done under the dissecting microscope at room temperature. The technique also showed the intracellular structure of some resting spores, although not very clearly. By integrating information about surface structures obtained by SEM with intracellular structures observed by TEM, we should be able to increase our morphological knowledge of resting spores of *P. graminis* and *P. betae*.

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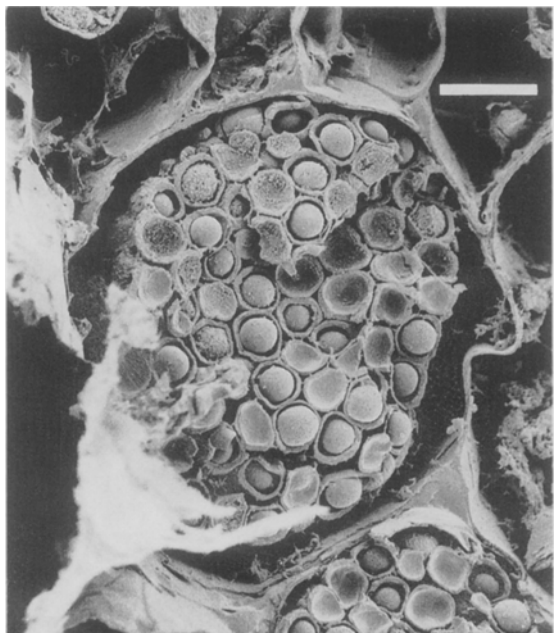

Fig. 2. A resting spore cluster of *P. graminis* in a barley root cell. Scale = 10 μm.

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