Spiroplasmalike Organisms in a Vesicular-Arbuscular Mycorrhizal Fungus and Its Mycoparasite

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ABSTRACT

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Helical organisms, resembling spiroplasmas, were found in the cytoplasm of the vesicular-arbuscular mycorrhizal fungus, *Glomus* sp., that had already been infected by a fungal hyperparasite, *Phlyctochytrium kniepii*. Similar entities were also found in the infection tubes, apophyses, and rhizoids of the associated *P. kniepii*. The organisms were cell wall-free, and showed regular wave form and helicity. In thin section a common form of the organisms was a curved cell (0.14–0.16 μm wide and 0.4–0.6 μm long), which might represent a section through the helix. The longest filaments were 3.75 μm long and 0.14–0.16 μm wide. Attempts to cultivate the spiroplasmalike organisms in various cell-free media, however, have not been successful.

Davis et al (5) were the first to discover the helical, motile, cell wall-free microorganisms associated with corn stunt disease. The agent was classified as a spiroplasma because of its helicity (6). Chen and Liao (1) and Williamson and Whitcomb (22) cultured the corn stunt spiroplasma on a cell-free medium and demonstrated the pathogenicity of the cultivated spiroplasma. Similar organisms cause citrus stubborn (8,9,18), citrus little-leaf (4,12), and brittle root of horseradish (7,16). In addition to plants, spiroplasma also can infect insects (2) and mammals (19,20). Heath and Unestam (10) reported the presence of mycoplasmalike structures in the aquatic fungus *Aphanomyces astaci* Schikora. Ross et al (17) demonstrated a highly infectious mycoplasma capable of inhibiting meiosis of the fungus, *Coprinus*. Helical organisms, similar to spiroplasma or spirochaetes, have not been associated with fungi (15). During our study of the fungal hyperparasitic relationships between *Phlyctochytrium kniepii* Gaerthner and the vesicular-arbuscular mycorrhizal fungi (VAMF) of *Glomus* spp., spiroplasmalike organisms were observed in both of these fungi. The morphological characteristics of the spiroplasmalike organisms observed in *P. kniepii* and its host, *Glomus* sp., are reported in this paper.

MATERIALS AND METHODS

A *Glomus* sp., designated isolate BNa, was isolated from soil in a corn field and maintained in open-pot cultures of corn in a greenhouse. *Phlyctochytrium kniepii* was isolated from a naturally infected *Glomus clarum* Nicol. & Schenck spore and maintained in Miller's M-3 medium (14).

Spores of the *Glomus* sp. were retrieved from the rhizosphere by wet-sieving and decanting, or by dissecting the mycorrhizal roots. The spores were picked up with a glass micropipette. In order to induce the hyperparasitism the spores were surface sterilized with 2% sodium hypochlorite for 2 min. The spores were transferred to small petri dishes that contained sterile pond water and were inoculated with a piece of *P. kniepii* culture. After inoculation and incubation for 3–7 days, the spores were heavily infected by the hyperparasite. The infected spores were then double-fixed with buffered glutaraldehyde and osmic acid solution, dehydrated, and prepared for scanning and transmission electron microscopy (21).

To culture the helical organisms, spores of the *Glomus* sp., whether infected by *P. kniepii* or not, were transferred to dishes containing spiroplasma culture broth (11). Spores were examined and crushed under a dissecting microscope. The protoplasmic contents plus the culture broth were passed through a sterile Millipore (0.22 or 0.45 μm), and 0.5 ml of the filtrate was added to a test tube containing 5 ml of culture broth and incubated at 30 C. Culture broth in the inoculated test tubes was examined with a light microscope fitted with dark-field or differential interference optics, or observed by scanning and transmission electron microscopes according to methods described by Cole et al (3).

RESULTS AND DISCUSSION

P. kniepii is a destructive hyperparasite of vesicular-arbuscular mycorrhizal fungi of *Glomus* sp. The process of infection of the host by the parasite involves encystment of the parasite's zoospore on the surface of the spores of *Glomus*, germination and penetration through the host cell wall by an infection tube, and formation endobiotic apophyses and rhizoids in the host cytoplasm. The rhizoids presumably assimilated the nutrients and conveyed them back to the epibiotic cyst. The nourished cyst eventually develops into zoosporangia (Fig. 1). A thin section through an infected chlamydospore of *Glomus* sp. (BNa) is depicted in Fig. 2, which revealed the epibiotic zoosporangium, infection tube, and the endobiotic apophysis of *P. kniepii*. The host cell wall was about 2–5 μm and extremely electron-dense (Fig. 2). A serial section of the spore shown in Fig. 2 is illustrated in Fig. 3. In this thin section, some spiroplasmalike organisms are present in the apophysis and in the infection tube. It is estimated that three of 10 of the *Glomus* spores infected by the *P. kniepii* harbored the spiroplasmalike organism. Since the *Glomus* spores infected by *P. kniepii* were eventually deprived of cytoplasmic contents and died, the physiological effects of the spiroplasmalike organism could not be determined. The spiroplasmalike organisms are also found scattered in the rhizoid of *P. kniepii* (Fig. 4) and in the cytoplasm of the *Glomus* spores (Figs. 5, 7, and 8). A cluster of membrane-bounded spiroplasmalike organisms was sometimes located inside the apophysis of *P. kniepii* (Fig. 6). A possible developing sequence of the spiroplasmalike organisms is shown in Figs. 4–8. This might also represent part of the helical filament being sectioned through different planes. The helical organisms are cell wall-free (Fig. 5) and shown a regular wave form and helicity (Figs. 3,7, and 8). A shape commonly seen in thin sections is a curved cell of 0.14 μm wide and 0.4–0.5 μm long (Fig. 4). The longest filament is 3.75 μm long and

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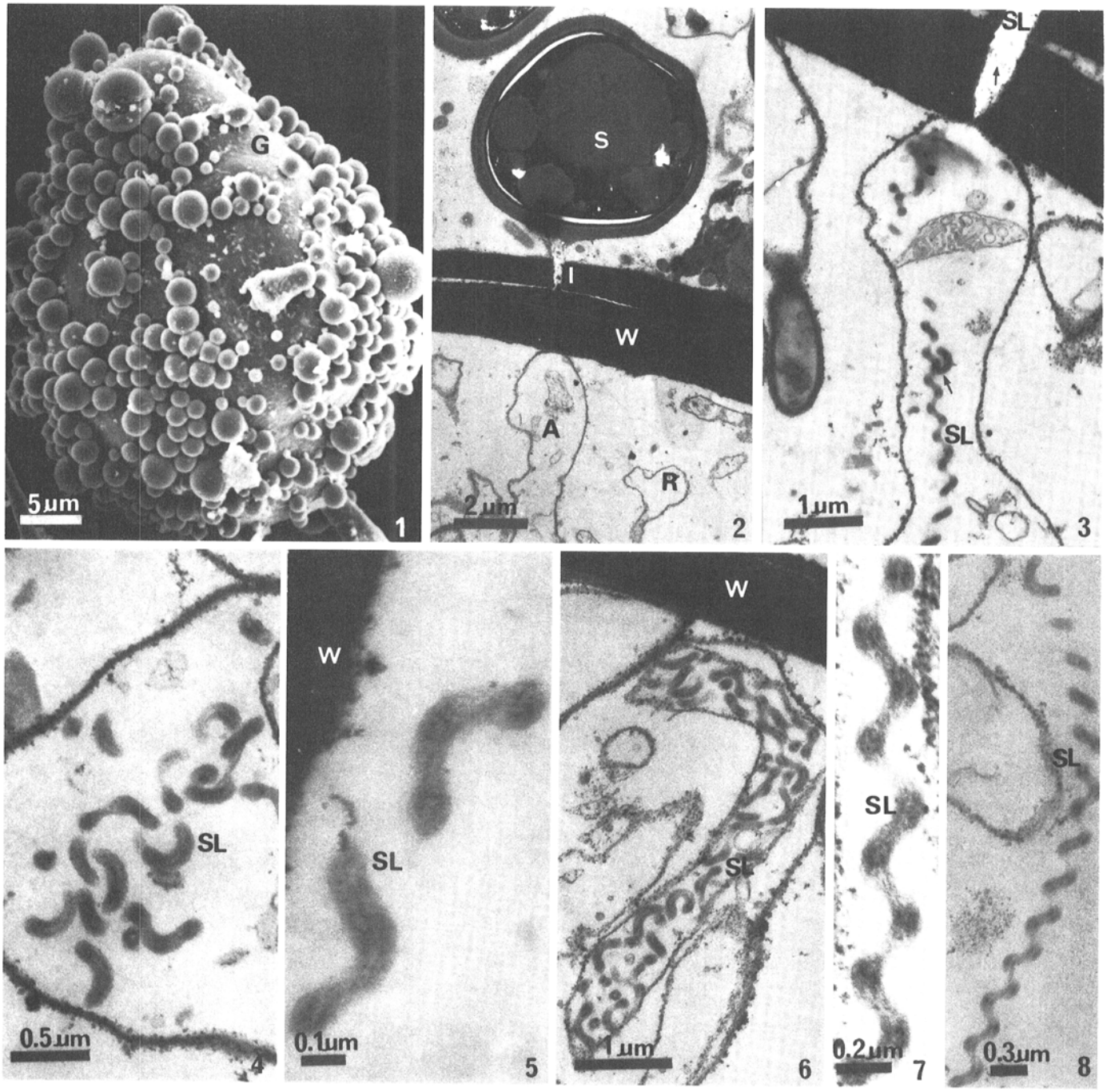
0.14–0.16 μ wide (Fig. 8). Helical filaments with a lateral branch were sometimes visible (Fig. 3, arrow), but the possibility that it is a superimposed second filament cannot be ruled out. Structures like cytoplasmic cylinders and axial fibrils as reported in spirochaetes have not been observed. Generally, the spiroplasmalike organisms in *Glomus* sp. and *P. kniepii* seem to be shorter and narrower than the spiroplasmas that cause the corn stunt or citrus stubborn diseases (1,6,8,18).

Large numbers of motile, extremely minute, somewhat coiled, filamentous microorganisms were found in the culture broth after inoculation and incubation for 4–7 days. Nevertheless, the incidence was low, and the microorganisms were in only 2–5% of the inoculated tubes. Unfortunately, some of these cultures were contaminated with coccoid bacteria. To eliminate the contaminating bacteria, the culture broth was further passed through a sterile Millipore filter (0.22 μm). A few drops of the filtrates were added to the culture media developed by Chen and Liao (1), Fudl-Allah and Calvan (8), and Saglio et al (18), in either the solid or liquid state, but none of the subcultures yielded the filamentous microorganisms. Further attempts to cultivate the spiroplasmalike organisms found in *Glomus* sp. and in *P. kniepii* were unsuccessful. This is the first report of the occurrence of the



Figs. 1–8. Scanning electron micrograph showing the attachment of various sizes of zoosporangia of *Phlyctochytrium kniepii* on the chlamydospore surface of a *Glomus* sp. **2.** Thin-section through the spore of *Glomus* sp. infected by *P. kniepii*. **3.** Serial thin-section of the spore shown in Fig. 2 revealing an infection tube of *P. kniepii* and the presence of spiroplasmalike organisms in the apophysis. **4.** Spiroplasmalike organisms in the rhizoid of the associated hyperparasite, *P. kniepii*. **5.** Spiroplasmlike organism distributed in the cytoplasm of *Glomus* sp. **6.** A cluster of spiroplasmalike organisms enveloped by a membranous structure and located inside the apophysis of *P. kniepii*. **7-8.** Spiroplasmalike organisms with a typical helical structure in the cytoplasm of *Glomus* sp. A = endobiotic apophysis of *P. kniepii*; G = spore of *Glomus* sp.; I = infection tube of *P. kniepii*; R = rhizoid; S = epibiotic zoosporangium of *P. kniepii*; SL = spiroplasmalike organisms; and W = cell wall of *Glomus* spore.

spiroplasmalike organisms in VAMF, *P. kniepii*, or any fungi. It is possible that fungi may act as vectors for transmitting spiroplasma to other fungi or to higher plants. Fungi acting as alternate hosts and vectors of plant viruses in nature have been reported (13).

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