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Four hundred-million-year-old vesicular arbuscular mycorrhizae

(Endomycorrhizae/symbiosis/fossil fungi/mutualism)

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ABSTRACT **The discovery of arbuscules in *Aglaophyton major*, an Early Devonian land plant, provides unequivocal evidence that mycorrhizae were established >400 million years ago. Nonseptate hyphae and arbuscules occur in a specialized meristematic region of the cortex that continually provided new cells for fungal infection. Arbuscules are morphologically identical to those of living arbuscular mycorrhizae in consisting of a basal trunk and repeatedly branched bush-like tuft within the plant cell. Although interpretations of the evolution of mycorrhizal mutualisms continue to be speculative, the existence of arbuscules in the Early Devonian indicates that nutrient transfer mutualism may have been in existence when plants invaded the land.**

Perhaps the most widespread, and certainly significant, mutualism between plants and fungi is the root symbiosis termed vesicular arbuscular mycorrhiza (VAM) or arbuscular mycorrhiza. These fungal endosymbionts, which are nearly universal in their association with flowering plants, are represented by >100 species of Zygomycetes included in the Glomales (1). Not only are they an important component of the mycota, but, because of their interrelationships with angiosperms, they are an integral component of terrestrial ecosystems throughout the world (2). Members of the Glomaceae are believed to have been present as early as the Cambrian period (3). Arbuscular mycorrhizae have been reported in extant bryophytes, pteridophytes, and gymnosperms; however, the physiological interrelationships are not well understood in these instances. While it is well known that VAMs considerably increase the uptake of specific nutrients by the host, other functions attributed to the fungi include production of plant growth hormones, protection of host roots from pathogens, and increased solubility of soil minerals (4).

The widespread geographic and biologic distribution of modern arbuscular mycorrhizae suggested to some the antiquity of this symbiosis (5), while others hypothesized that this kind of mutualism was pivotal in the origin of the terrestrial flora (6). This hypothesis is based on the assumption that the various fungal structures found in the anatomically preserved Early Devonian plants represent components of an endosymbiont. These include terminal thick-walled spores that morphologically resemble VAM chlamydospores, nonseptate mycelia, coiled hyphae, and irregularly shaped, thin-walled spheres that resemble vesicles (7). Although it is difficult to document a physiological function based solely on morphology, the presence of arbuscules currently provides a reliable clue to the mutualistic nature of the association. To date no arbuscules have been identified in any Paleozoic plant, although convincing examples are known from the roots of a Triassic cycad (8).

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In this paper we report evidence of arbuscules of VAM fungi from plants preserved in the Rhynie chert. The fossils are preserved as permineralizations at this Lower Devonian (Siegenian) locality. Since the fossil plants containing the fungi are preserved in silica, it is necessary to prepare thin sections by cementing pieces of the rock matrix to microscope slides and then grinding the rock to a thickness of 50–150 μm with silicon carbide powder. Fungi were photographed under oil immersion objectives directly on the polished surface of the rock. Slides have been deposited in the collection of W.R.

The fungi occur in all regions of the axes of *Aglaophyton major*, an enigmatic plant that possesses features found in both vascular plants and bryophytes (9). *Aglaophyton* axes have a uniformly thick epidermis of rectangular cells overlying a two- to five-cell-thick hypodermis. Inside of this layer is a one- to four-cell-thick zone of thin-walled cells that appear similar to palisade parenchyma. To date this tissue has been observed only in *Rhynia* and *Aglaophyton*. It is in this region of the axis that the arbuscules occur (Fig. 1), even though the intraradical mycelium is extensively developed within the intercellular spaces in the remaining cortical tissues. The nonseptate hyphae range up to 4.0 μm in diameter and often show both Y- and H-shaped anastomoses. In extant VAM fungi arbuscules are formed when intercellular hyphal branches penetrate the cell wall of the host but do not rupture the plasmalemma (10). The hyphal trunk of the arbuscule is ≈1.3 μm wide (Fig. 2) and branches repeatedly to form a "bush-like" structure within the cell (Fig. 3). Secondary branches range from 0.7–2.0 μm wide, and the ultimate ones are beyond the resolving power of light microscopy (in the size range 0.2–0.5 μm). Fig. 4 shows the top of an arbuscule indicating the configuration of the more distal branches. In the arbuscules of extant VAMs, the walls are osmiophilic and decrease in thickness to <20 nm near the tips. In some arbuscules the tips may be slightly swollen, and this condition also occurs in the fossils.

In extant VAMs, the arbuscules are ephemeral and begin to break down at the tips of the smallest branches in 4–6 days, ultimately forming an amorphous mass within the cell (11). We have no way of gauging the longevity of the fossil arbuscules except to note that various stages of arbuscule morphology are present in a single section, including those that have collapsed and deteriorated. Boullard (12) suggested that in some living ferns the frequent presence of clumps indicates a short functional span of an arbuscule. Several authors working with modern VAM fungi reported the formation of a subapical septum that separates the functional and nonfunctional portion of an arbuscule during deterioration. We have not observed this structure in any of the fossil arbuscules to date. The fossil arbuscules appear morphologically identical to those of many extant VAMs (13). There is far less information available about the structural and phys-

Abbreviation: VAM, vesicular arbuscular mycorrhiza.

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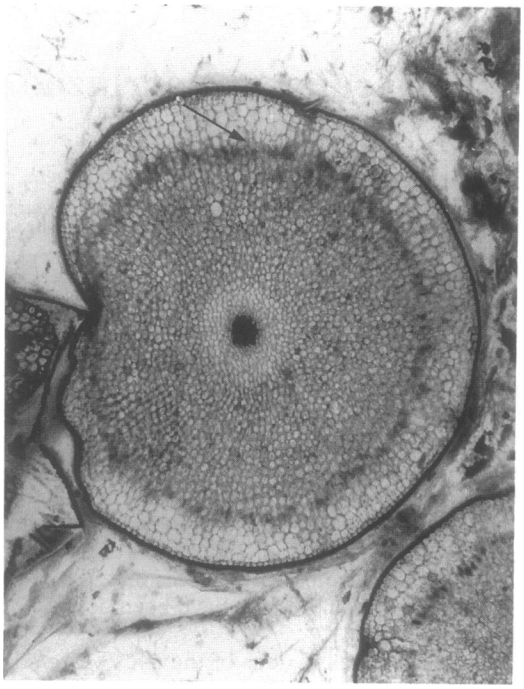

FIG. 1. Transverse section of *A. major* axis showing tissue preservation. Arrow indicates zone of arbuscule-containing cells. (×15.)

iological interrelationships between mycorrhizal fungi and their fern and bryophyte hosts, although both groups are characterized as having VAMs (10). In both, a major arbuscular trunk initially dichotomizes and each of the branches divides repeatedly to form smaller and smaller units. These are quite different morphologically from the arbuscules in a Triassic cycad, which are less branched and more robust (8). Many of the Rhynie chert arbuscules are also accompanied by a slight thickening in the cell wall. In modern VAMs this thickening represents an apposition that forms at the point of penetration of the host cell wall by the hypha. Thus, not only

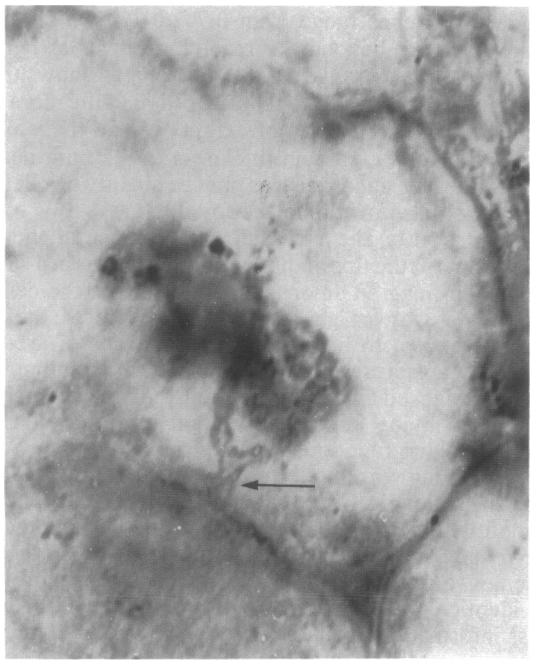

FIG. 2. Cortical cell showing arbuscule trunk (arrow) extending into the cell lumen. (×1500.)

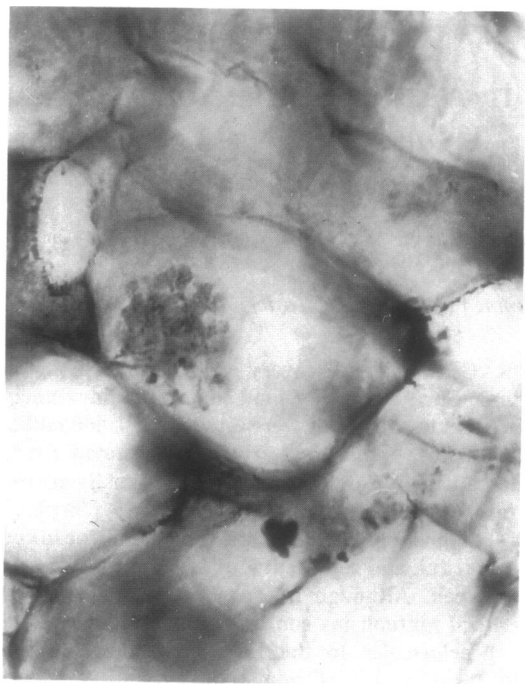

FIG. 3. Lateral view showing arbuscule. (×600.)

is there a morphological correspondence between the fossil and extant arbuscules, but the presence of appositions in the cells of *Aglaophyton* also indicates that these host cells were alive and responded by the formation of thickenings, similar to those of their modern counterparts.

As more anatomical and morphological information is assembled about the Rhynie chert plants, it is becoming increasingly clear that these organisms are structurally complex. For example, *Aglaophyton* and *Rhynia* are the only plants in the Rhynie chert that possess a zone of palisade-like cells beneath the hypodermis in which arbuscules develop. Many of the cells in this layer contain remnants of arbuscules (Fig. 1). These host cells also appear collapsed and disassociated, while arbuscule-free cells look normal. Since many of the cells in this zone are organized in files, we speculate that the arbuscule-containing zone may be meristematic, perhaps throughout the life of the plant, to produce new cells that provide sites for the infection of VAM fungi. This appears to be unlike the situation in modern VAM infections (14), where

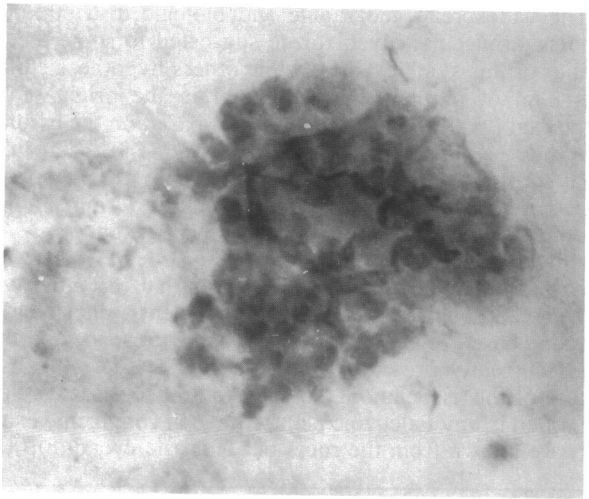

FIG. 4. View from the top of an arbuscule showing extensive branching. (×1500.)

nonfunctioning arbuscules are absorbed by the host cells. Although it is not understood how this takes place metabolically, once resorption is completed the host cells continue to function. If our assumption is accurate, it means that the meristematic zone in *Aglaophyton* may then represent a tissue-directed response that predates the capability of individual cells to resorb arbuscules.

Although it is generally assumed that members of the true fungi diverged ≈1 billion years ago (15), there continues to be no fossil record of these early forms. The oldest fossils believed to represent true fungi are nonseptate hyphae from Cambrian sediments (16) and hyphae and septate spores from the Silurian (17). In both instances nothing is known about the organization of these fungi or the type of interactions they had with other organisms. The first plants with conducting cells that resemble tracheids in vascular plants appeared during the Upper Silurian period. However, there is an increasing body of evidence that plants adapted to a terrestrial existence much earlier. At present, all known Silurian plants are preserved as either impressions or compressions, making it impossible to determine whether endophytic fungi were present or not. Therefore, the earliest land plants in which it is possible to identify mycorrhizae come from the Rhynie chert.

Some regard mycorrhizal symbioses to be the outcome of a parasitic interaction from which a mutualistic interrelationship subsequently developed (18). An alternative hypothesis views such endosymbionts as originating from saprotrophs that were capable of entering a living host. Both saprotrophs and biotrophs occur in the Rhynie chert mycota. Saprotrophs are represented by various tissue-degrading fungi (7) and biotrophs are represented by mycoparasites (19). Neither interaction provides any direct evidence of a relationship that preceded the endomycorrhizal condition. However, the fossil record does indicate that in some instances host cells invaded by some mycoparasites were not immediately killed (necrotrophy) because there is an observed response in the host cells. At least theoretically, this would suggest the ease with which an equilibrium might be established between parasite and host. Moreover, the shift from biotrophy to saprophytism and vice versa or from a mutualistic equilibrium to biotrophy can result from a change in environmental conditions (19). The discovery of arbuscules in *Aglaophyton* establishes VAMs by Early Devonian time but throws no additional light on the history and affinities of these fungi. However, the fossils support the time of origin of VAMs as recently deduced from the sequence data from small subunit rRNA obtained from spores of 12 living species of representative VAM fungi (20). According to these molecular data, VAM fungi originated between 462 and 353 million years ago, well within the approximate 400-million-year time frame of the Lower Devonian fossils.

The Early Devonian endophytes provide no additional support for the hypothesis that mutualistic symbioses between fungi and algae were the necessary catalyst in the early colonization of the land (21). However, one of the intriguing aspects of the Early Devonian mycota is the potential selective pressures that they had on their hosts. In the presence of modern endophytes, plants are known to increase the amount of vascular tissue and the lignification of the xylem (22). The presence of clusters of transfusion tracheids in *Aglaophyton* may represent such a response. In addition, some have argued the presence of endophytes was responsible for the initial lignification of cells during the evolution of vascular plants (23). Finally, Wilson (24) has recently suggested that certain types of chemical plant defenses may be the result of endophyte infection and as a result have contributed to the coevolutionary relationships between fungi and plants. While it is obvious that these chemical interrelationships cannot be documented from the fossil record, the realization that saprophytic, parasitic, and mutualistic fungi were well established by Early Devonian time underscores the fact that such interactions accompanied the rise of the terrestrial flora.

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