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VESICULAR-ARBUSCULAR MYCORRHIZAE OF SOUTHERN ONTARIO FERNS AND FERN-ALLIES

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ABSTRACT

Thirty-nine species and varieties of common Pteridophytes of southern Ontario were surveyed for the presence of VAM. The sporophytes of the three *Equisetum* spp. and the two *Lycopodium* spp. examined were usually non-VA mycorrhizal. The two species of *Botrychium* were extensively VA mycorrhizal, while the Filicales were in general moderately colonized, though plants from isolated rock outcroppings and crevices were often non-VA mycorrhizal. These observations are discussed in light of Baylis' theory of root morphology and mycotrophism. In addition, various root-inhabiting fungi which could be confused with VAM are illustrated.

Key Words: vesicular-arbuscular mycorrhizae, ferns, fern-allies.

Since Mosse (**1959**) demonstrated the reassociation of vesicular-arbuscular (VA) fungi and their higher plant partners under artificial conditions, economic and academic interests have focussed on the potential of vesicular-arbuscular mycorrhizae (VAM) in agriculture and horticulture. But even before the causative fungi were identified, VAM had been recognized as a morphological category of plant root-fungus symbiosis. Gallaud (**1905**) described the basic structural components, most notably the arbuscules, and their variations in different host plants. From subsequent studies, three features of VAM have emerged to spark speculation about the evolutionary significance of this symbiosis. These are: 1) the mycorrhiza-dependence of certain agriculturally valuable plants under conditions of poor nutrition; 2) the apparent lack of host-specificity of the fungal partners, and 3) the ubiquity of the symbiosis in nature.

Baylis (**1972**, **1976**), discussing the relationship between root system morphology of angiosperms and the incidence and extent of VAM, concluded that "primitive" fleshy roots are highly colonized, "advanced" fine roots less so. Boullard (**1957**, **1979**) showed that this also applied to Pteridophytes. He suggested that the dependence of fern gametophytes on mycotrophism decreased with increasing photosynthetic ability. The sporophytes of the Ophioglossales, with "primitive" roots, were consistently and extensively VA mycorrhizal, while those of certain Filicales with "advanced" roots were, at least occasionally, non-mycorrhizal. Proceeding from this generalization, and from the presence in fossilized rhizomes of the early (Devonian) land plants *Rhynia* and *Asteroxylon* of structures reminiscent of the "ubiquitous endophyte," Pirozynski and Malloch (**1975**) proposed the mycotrophic origin of land plants.

The validity of these generalizations depends on accurate differentiation of VAM fungi from other root-colonizing fungi. Lewis (**1973**) restricted application of the term "vesicular-arbuscular" to those mycorrhizae induced by certain of the Endogonaceae. The arbuscule is the morphological and functional link between all fungi known to form VAM in the strict sense. Studies have indicated that this much-branched organ may be the primary site of nutrient transfer from

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the fungus to the plant cell (Gianinazzi *et al.*, **1979**; Gianinazzi-Pearson and Gianinazzi, **1978**; Hayman, **1974**).

Hepden's (**1960**) unverified claim of successful isolation of the causative fungus from endomycorrhizal roots of a leptosporangiate fern echoed other similar reports (Ham, **1962**; Hawker, **1962**). While the identification of these isolates as VAM fungi is questionable, to say the least, the isolation of a variety of true and protoctistan fungi (e.g., *Phoma* spp., *Pythium* spp.) from roots illustrates the complexity of root-fungus associations. Cooper (**1976**) distinguished twelve morphological groups of "endomycorrhizae" in New Zealand ferns, but only seven of these were arbuscular, others being described as orchidaceous, ericaceous, or ectendomycorrhizal. Some plants were typically ectomycorrhizal under certain ecological situations.

We assessed the mycorrhizal status of the sporophytes of some of the common Pteridophytes of mixed woodlands in southern Ontario, Canada. Because of the probable functional role of the arbuscule, the presence of a typical intracellular arbuscule was the criterion for classification of the root-inhabiting fungi as VAM.

MATERIALS AND METHODS

Sporophytes of 33 species and varieties representing 17 fern genera, plus 2 of *Lycopodium* and 4 of *Equisetum*, were collected, care being taken to retain adequate roots and adjacent soil. Part of the root mass of each plant was fixed in 50% formalin-acetic acid-alcohol (FAA); the rest, with the soil, was bagged and held a maximum of 3 months at approximately 4 C until examined.

The roots were cleared and stained by a method modified from Phillips and Hayman (**1970**). The roots were taken from the FAA, washed several times in tap water and placed in 10% (w/v) KOH in 100 ml Erlenmeyer flasks. They were heated to approximately 90 C for a minimum of 2 h, the time depending on the initial pigmentation of the roots. When the roots were cleared, the KOH was decanted and discarded. Several washes of cool water followed, the last being slightly acidified with a few drops of lactic acid. This treatment improved the intensity of staining. The roots, still in the final wash, were poured into a Petri plate with just enough solution to float them. The main roots were teased apart and the fine roots cut into 5-mm-long segments. One hundred fine root segments from each plant were stained in drops of 0.5% acid fuchsin in lactoglycerol heated to the point of fuming on glass microscope slides. Decoloration (differentiation) was carried out in lactoglycerol by the same procedure. Slides were sealed with Gurr's Glyceel (BDH Chemicals Ltd., Poole, England).

Root segments were recorded as VA mycorrhizal only if one or more of the cortical cells contained an arbuscule. The percentages calculated therefore express frequency rather than intensity of infection. Other features of the roots, such as the presence of other fungi and of root hairs, were noted but not quantified.

RESULTS

The incidence and frequency of VAM in fine roots of ferns and fern-allies collected in southern Ontario, expressed as the percentage of root segments containing one or more arbuscules, are presented in TABLE I. Each number represents one plant. Although the sample size for many species was relatively small, single plants for 16 of the species, certain trends are apparent.

The lycopods and species of *Equisetum* examined were essentially non-mycorrhizal, and their roots bore large numbers of root hairs. The roots of the

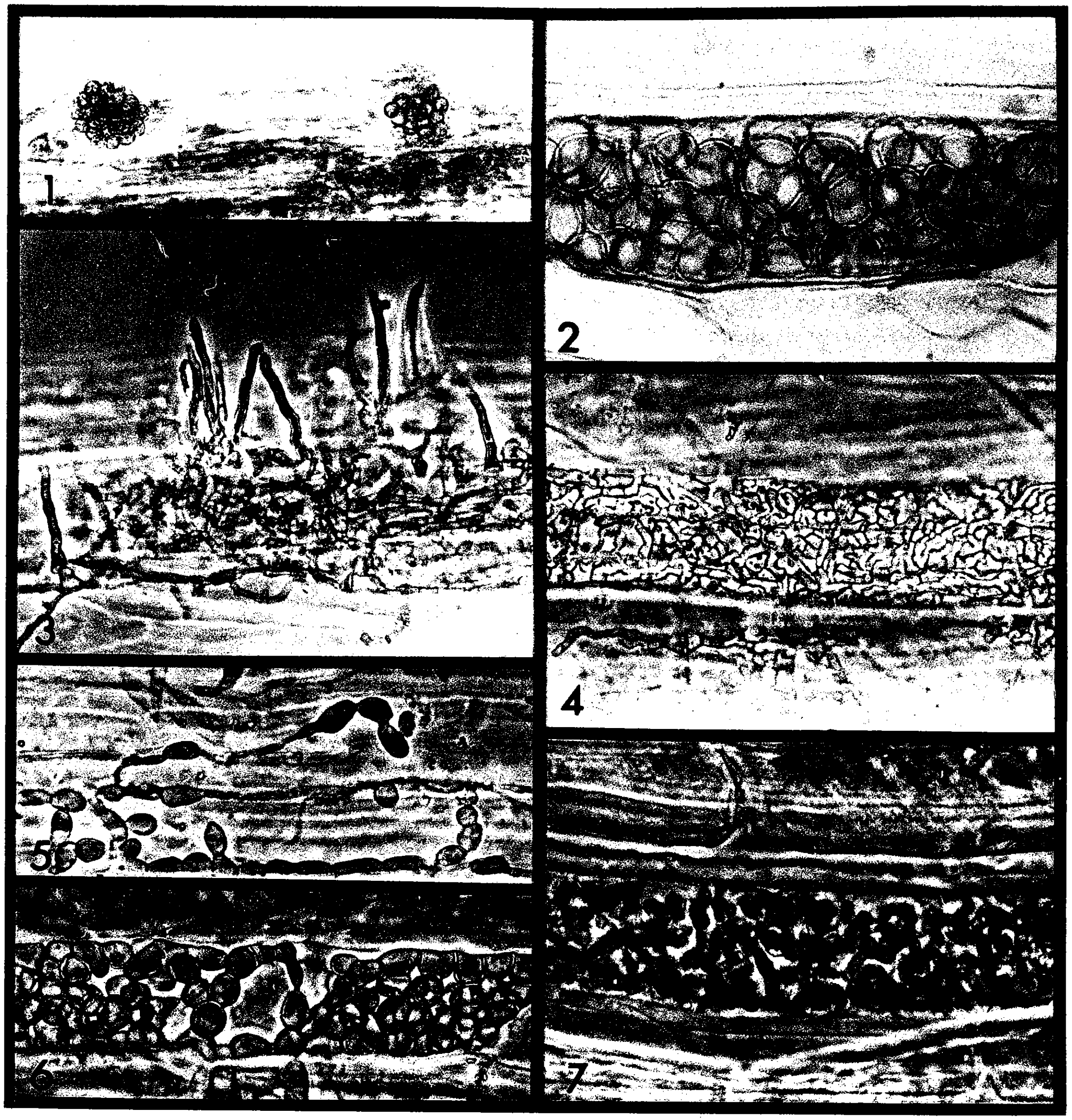
TABLE I

OCCURRENCE OF ARBUSCULAR MYCORRHIZAE IN FERNS AND FERN-ALLIES

LYCOPODIALES	
Lycopodium flabelliforme Blanchard	0[a]
L. tristachyum Pursh	0
EQUISETALES	
Equisetum arvense L.	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 20
E. hyemale v. *affine* Eaton	0, 0, 0
E. hyemale v. *elatum* Morton	0
E. scirpoides Michaux	0
OPHIOGLOSSALES	
Botrychium oneidense House	100
B. virginianum Swartz	100, 100, 80
FILICALES	
Adiantum pedatum L.	40, 80
Asplenium trichomanes L.	0, 0
Athyrium angustum Presl.	60, 100, 70, 70, 10, 80, 85
A. filix-femina Roth	60
A. pycnocarpon Tidestrom	1
A. thelypteroides Desvaux	40
Camptosaurus rhizophyllus Link	0
Cystopteris bulbifera Bernhardi	75, 50, 30, 80
C. fragilis Bernhardi	0, 0, 0, 0
C. fragilis v. *laurentiana* Weatherby	100
C. fragilis × *bulbifera*	90
Cryptogramma stelleri Prantl	0
Dryopteris cristata Gray	20, 80, 80, 60
D. dilatata Gray	85
D. intermedia Gray	25, 55, 40, 50, 65, 60
D. intermedia × *spinulosa* Wherry	50
D. marginalis (L.) Gray	60, 25, 25, 0, 80, 80, 65
D. spinulosa Watt	75, 75, 10, 95, 85
Gymnocarpium dryopteris (L.) Newm.	95, 85, 65, 55, 5
Matteuccia pennsylvanica Raym.	95, 95, 65
Onoclea sensibilis L.	25, 35, 0, 0, 40, 60, 10, 80, 5
Osmunda cinnamomea L.	35, 55, 90
O. regalis L.	0
Pellaea glabella Mettenius	50
Phegopteris hexagonoptera Fee	95, 75
Polypodium virginianum L.	0, 0, 0, 0, 0
Polystichum acrostichoides Schott	40, 75, 70, 90
Pteridium aquilinum Kuhn	70, 100, 90, 25
Thelypteris noveboracensis Nieuwland	75, 70, 80
T. palustris Schott	65, 90, 30, 30, 50
T. palustris v. *pubescens* Fernald	40, 45, 30

[a] Percentage of root segments containing arbuscules. Each percentage represents one plant.

primitive, eusporangiate *Botrychium* spp. (Ophioglossales) were almost completely converted to VA mycorrhizae. Their roots were thick, fleshy, infrequently branched and almost free of root hairs. Most of the Filicales were VA mycorrhizal, and the non-mycorrhizal exceptions were often from unusual habitats. Within a single species the extent of colonization varied; in *Onoclea sensibilis*, for example, it ranged from 0 to 80%. Root hairs were sometimes localized in dense clusters, sometimes spaced evenly along the fine roots, or sometimes non-existent. Occasionally either root hairs or VA mycorrhizae predominated, though both conditions occurred within the same root system as well as in different plants.



FIGS. 1–6. Non-VAM fungi and fungus-like structures in roots of various ferns. 1. Resting spores of Plasmodiophorales-like endophyte on *Cystopteris fragilis* × *bulbifera*, ×560. 2. Similar to 1 on *Matteuccia pennsylvanica*, ×560. 3. Regularly septate dikaryomycotan hyphae and conidiophores on *Onoclea sensibilis*, ×560. 4. Intracellular sclerotioid structure on *O. sensibilis*, ×560. 5. Monilioid hyphae on *O. sensibilis*, ×560. 6. Similar to 5 on *Matteuccia pennsylvanica*, ×560. 7. Tightly-coiled branched hyphae on *Gymnocarpium dryopteris*, ×560.

Since most plants were collected from the understory of mixed deciduous angiosperm and evergreen gymnosperm forests, the unusual habitats referred to above were those which lay outside this particular environment. Many of the species of smaller ferns found on bare rock (*Polypodium virginianum*) or in the crevices of cliffs (*Asplenium trichomanes*, *Camptosaurus rhizophyllus*, *Cystopteris fragilis* var. *fragilis*, *Cryptogramma stelleri*) were non-mycorrhizal. Because of the rarity of some of the species, single plants were examined, thus prohibiting assessment of variability of infection.

Many other fungi and fungus-like structures were present in the roots of various of the ferns: spherical or cell-filling clusters of resting spores of Plasmodiophorales-like endophytes (FIGS. 1, 2); regularly septate dikaryomycotan hyphae and conidiomata (FIG. 3); dark sclerotial structures (FIG. 4); monilioid, branched hyphae (FIGS. 5, 6), and tightly-coiled hyphae (FIG. 7).

The majority of the plants examined, however, were inhabited by typical VA mycorrhizal fungi. Because of the severity of the clearing and staining procedure, most of the hyphae extending into the soil (extramatrical) were detached. Appressoria which formed on the outer wall of root cortical cells (FIGS. 8, 9) or on the surface of root hairs (FIG. 11) were frequently observed. Below the appressoria, fine penetration pegs (FIG. 13) extended through the plant cell wall, the hyphae expanding again when inside the plant cell. In the outer cortical cells, hyphae looped or coiled (FIG. 12), or grew directly through to establish the deeper phase of the mycorrhiza in cortical cells surrounding the stele. Infection often appeared to have spread longitudinally in the root from a single point of entry. The stele and the apical meristems were free of hyphae.

Arbuscules (FIG. 14) are either ephemeral structures, or susceptible to digestion by the clearing and staining procedure, or both. In most infections they appeared as amorphous masses of stained material. The regular pattern of dichotomous branching of the slender arbuscular hypae (FIG. 15) was visible in a small number of the root segments observed. The hyphae associated with the arbuscular phase were often irregularly septate.

Intramatrical vesicles occurred frequently in living and moribund roots (FIG. 16) and were also found embedded in decaying root tissue (FIG. 17).

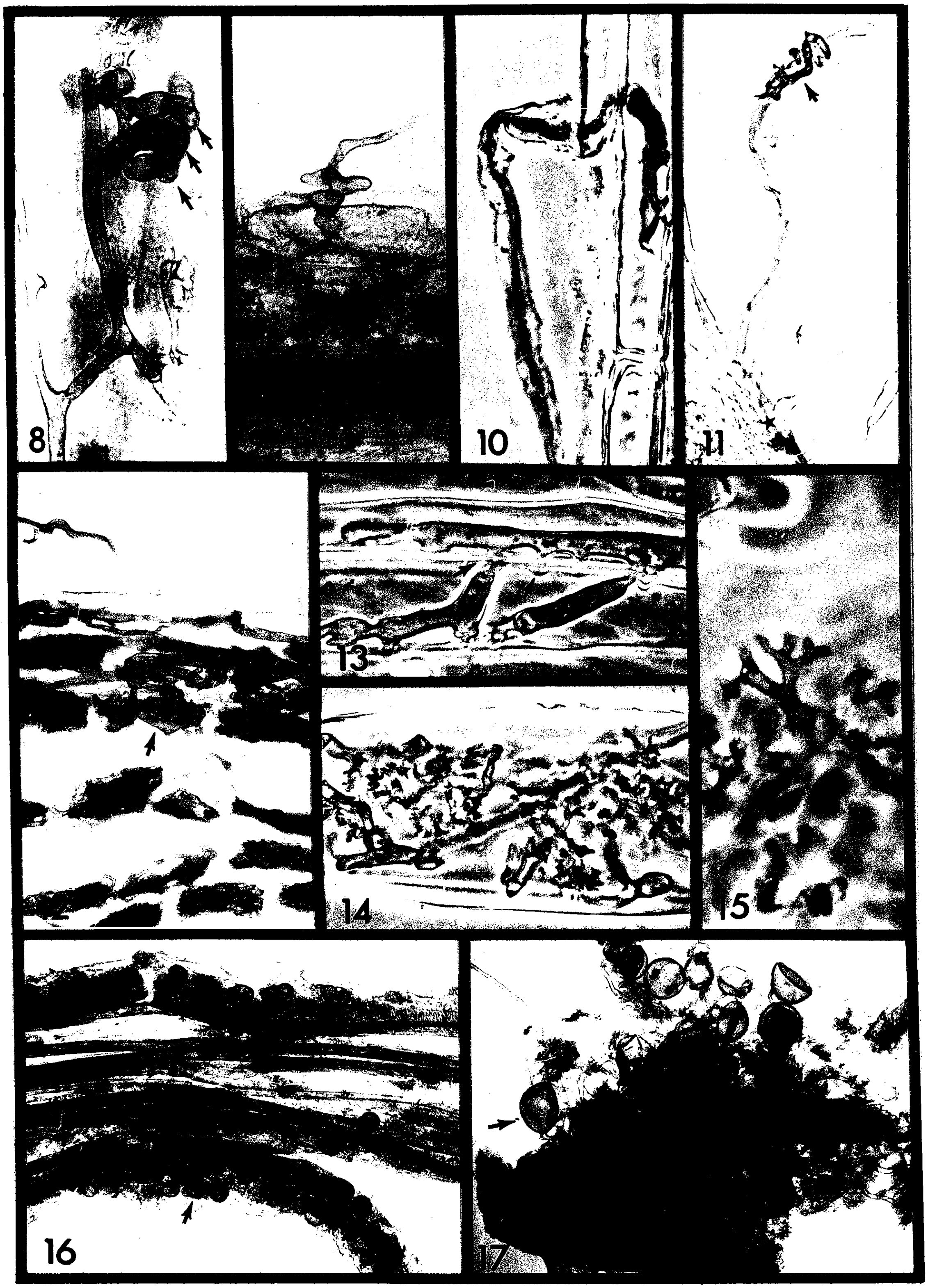
The features of VA mycorrhizae described here were common to all of the ferns observed. No species or individual formed mycorrhizae sufficiently distinct as to be considered separately from the rest.

Spores of potential VAM fungi were sieved from soil surrounding the roots of the plants collected. As it was not possible to determine which of the spores were formed by the actual mycorrhizal fungi of any given plant, no attempt was made to correlate spore numbers or species to the observed VAM. The fungal taxa collected will be described separately to emphasize the fact that there is no direct evidence linking spores to mycorrhizae. It is clear, however, that the absence of mycorrhiza, as in the case of *Equisetum* spp., was not necessarily due to a lack of potential inoculum, since all of the surrounding soils contained many kinds of spores.

DISCUSSION

The preponderance of VAM in the common woodland ferns of southern Ontario, Canada, largely agrees with reports from elsewhere. Ectomycorrhizae which Cooper (**1976**) found on ferns growing under pure stands of ectomycorrhizal forest trees in New Zealand, were not observed on the ferns in the present study. Ectomycorrhizae are, in any case, uncommon on herbaceous plants with annual absorptive roots, though they have been reported for certain tropical non-woody herbs (Warcup, **1980**). The roles of ecological factors in determining the type of mycorrhizal association formed by a plant are not well understood.

Ferns from bare rock surfaces and crevices were non-mycorrhizal. Run-off from overhead soils and circulation of dry soil and root particles in winds should carry VAM fungi to these otherwise remote sites, but the absence of soil from many of these niches will reduce the populations of microorganisms potentially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competing with the roots for available nutrients. The dynamics of nutrient movement and availability on bare rock must differ considerably from those in soils where certain nutrients may be preferentially adsorbed to soil particles. All of these factors may contribute to the nonmycorrhizal status of the ferns in these habitats.

The fleshy roots of the *Botrychium* species examined lacked root hairs and were extensively VA mycorrhizal, thus supporting one aspect of Baylis's theory concerning root system morphology and VAM dependence. The naked, smooth roots would conceivably be surrounded by a zone, depleted of available nutrients, which could be traversed by the conductive hyphae of the VAM fungi. Whether the absence of root hairs is characteristic of these species, or is a reaction to the presence of the mycorrhizal fungus, or is a result of other factors, is not known. The relationship between root hair formation and VAM warrants further study.

Certain reports in the literature support the observation made during the present study that the fern-allies *Equisetum* and *Lycopodium* are not VA mycorrhizal. Though relatively little information is available about symbiotic fungi in the Equisetales, Janse (in Kelley, **1950**) stated that forest-dwelling Equisetae in Java did not have root-inhabiting fungal endophytes. Lohman (**1927**) cited *E. arvense* as containing an endophytic "phycomycete." Boullard (**1957**) illustrated and described fungi in the root cortical cells of European *Equisetum* spp., but the fungi apparently did not form arbuscules, and resembled the "monilioid" hyphae observed in the present study.

If the extant Equisetales are, in fact, not mycorrhizal, the possibility must be considered that their ancestors, so successful during the Carboniferous, may have shared this peculiarity. Perhaps their geologically sudden demise was a result of competition from plants equipped with root-inhabiting fungi adept at gleaning scarce nutrients from the soil.

While Kelley (**1950**) indicated that 16 species of *Lycopodium* have mycorrhizal sporophytes, Boullard (**1957**) states that, in the gametophytes, ". . . l'infection y est très nette; par contre, les sporophytes ne semblent héberger que très irrégulièrement des rhizomycètes." The abundant literature dealing with the mycotrophic status of *Lycopodium* spp. (cf. Burgeff, **1938**, and Boullard, **1979**) indicates that gametophytes normally harbor endophytic fungi, some of them apparently VAM, but that sporophytes may exist independently.

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FIGS. 8–17. VAM of ferns. 8. Series of appressoria (arrows) on root surface of *Athyrium angustum,* ×560. 9. Single appressorium on root surface of *A. angustum,* ×200. 10. Hypha entering root through root hair of *Gymnocarpium dryopteris,* ×560. 11. Appressorium (arrow) on root hair (star) of *Dryopteris intermedia,* ×560. 12. Intracellular hyphal loops (arrow) in *Cystopteris fragilis,* ×200. 13. Hyphal pegs (arrows) at various stages of cortical cell wall penetration on *Dryopteris marginalis,* ×560. 14. Arbuscule in *Phegopteris hexagonoptera,* ×560. 15. Detail of dichotomous branching of arbuscule, same as in 14, ×1500. 16. Vesicles (arrow) within root of *Dryopteris dilatata,* ×75. 17. Intramatrically formed "vesicles" (arrow) liberated from root of *Dryopteris marginalis,* ×130.

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