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Anatomy and Physiology of Vesicular-Arbuscular and Nonmycorrhizal Roots

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The roots of most species of vascular plants except the Coniferales are extensively colonized by the ubiquitous soil-inhabiting fungi classified in the Endogonaceae. The species of primary concern are in the genera *Glomus*, *Acaulospora*, and *Sclerocystis* since they form endomycorrhizae characterized by the production of both vesicles and arbuscules (VA mycorrhizae) (16,35). Although *Gigaspora* spp. only rarely produce vesicles in infected roots, other aspects of the mycorrhizae they form are generally comparable to infections caused by the three previously indicated genera of VA fungi. However, since most VA mycorrhizal research has involved various *Glomus* spp., those observations are the primary bases for our current understanding of these associations.

Macroscopic alterations of normal root morphology, which typically accompany ectomycorrhizal development, are absent in VA infections. In some hosts, a yellow pigmentation may be seen in freshly harvested mycorrhizal roots (4) but confirmation of VA mycorrhizal development, as well as quantitative evaluations of the extent of VA infections within the root system, requires microscopic examination of roots which have been chemically cleared and stained to reveal endophytic fungal structures (30,39). With carefully harvested and prepared roots grown under field conditions, VA infections involving half of the total root length of many host species are not uncommon and levels substantially exceeding 75% of the total root length may be obtained in pot cultures employing appropriate fungal symbionts and sterilized soil media. Although the fungi are obligate parasites and exhibit extremely broad host ranges, the magnitude of host response is not

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necessarily related to the extent of root invasion. Benefits to the host plant due to VA associations are influenced not only by activities of the VA endophytes *within* the root, but also by their relative abilities to compete with indigenous soil microflora and fauna, to effectively colonize the rhizosphere, and to fulfill certain nutritional requirements of the particular host species involved. In this paper, we have emphasized developmental characteristics that can be detected with a light microscope, since relatively little modern descriptive work has been conducted at that level of magnification. Since space is limited and excellent recent presentations of physiological aspects of VA mycorrhizal associations are available elsewhere (41,44), nutritional interactions in these systems are considered rather briefly here.

STRUCTURE OF VA MYCORRHIZAE

A large number of host-VA endophyte combinations have been studied since these associations were first described by Janse (25), Gallaud (14), and others at the turn of the century. Consequently, there is a rapidly expanding body of information relating to the occurrence and distribution of VA associations, effects of specific host-fungal combinations on host growth under diverse experimental conditions, influences of VA infections on other mutualistic or pathogenic microorganisms, physiological interactions between the symbionts, and many other aspects of VA endophyte-host plant interactions. Since the primary objectives in most of these studies have related fundamentally to an elucidation of increasingly diverse *effects* of VA associations, the majority of microscopic examinations have involved simply either confirmation of the presence of VA infections, or a quantitative evaluation of the magnitude of VA infection levels established. However, there are very few data for most host-endophyte combinations studied in the last half century that permit an evaluation of differences in morphological features or developmental characteristics of VA infections in relation to the variable effects they may exert on a given host. To fully understand how and why VA associations function as they do, it would seem desirable to determine, not only how functionally effective symbionts behave within specific hosts, but also how infection, colonization, and developmental patterns differ in associations exhibiting minimal growth responses or even suppression of growth in the host. Virtually no developmental data are available relating to the latter aspects of VA combinations. Developmental studies of "functionally effective" associations are also far from complete.

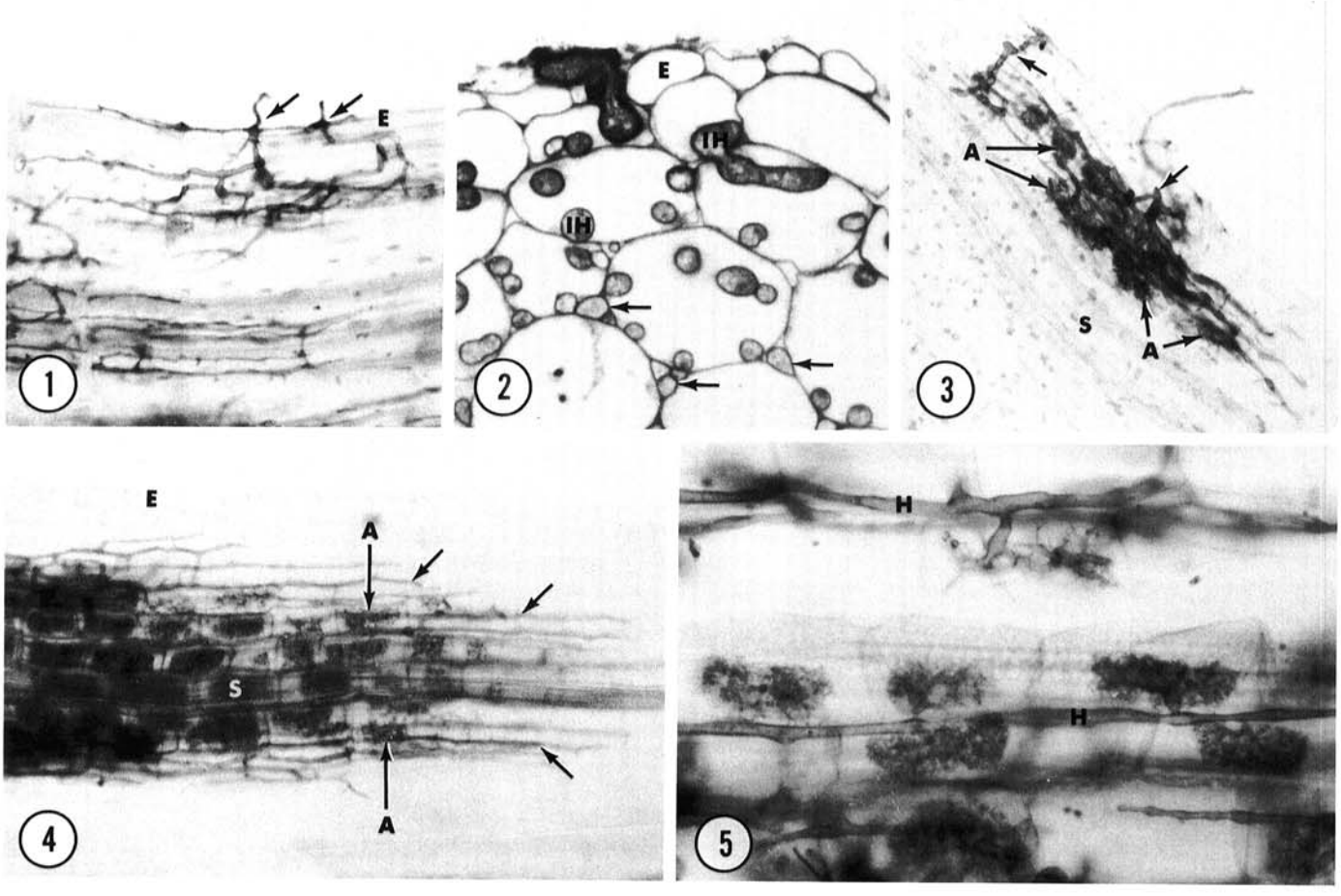
Structures produced by VA fungi within host roots include: a hyphal system contiguous, through initial penetration points, with a hyphal network extending into the soil; short-lived, intracellular arbuscules generally thought to function in nutrient transfer between the symbionts; and enlarged intercalary or terminal vesicles that appear to function as endophytic storage organs. Vesicles are not typically produced by *Gigaspora* spp. Although vesicles are normally produced in older infections by representatives of the other genera of VA fungi (Figs. 9 and 10), occasionally they may form abundantly in some infections with very little prior development of arbuscules. In some hosts, individual infections are sufficiently dispersed at maturity to permit identification of initially discrete "infection units." In onion, for example, *G. mosseae* infection units may reach a maximum length of 5 mm (9). In many VA associations, however, individual infection units become obscured at an early stage of development as a consequence of numerous, closely spaced penetrations along the root axis (41) often arising from external stolonlike, "runner" hyphae (37). Even though each individual infection unit may not be conveniently distinguished in mature VA mycorrhizae, it is important to emphasize that endogenous development from any single penetration point is limited and, as indicated by Cox and Sanders (9) that each "infection unit therefore presents an age-sequence, the oldest hyphae being those nearest the entry point, the youngest those nearest the advancing tips of the mycelium." A well-established VA association on a given host, therefore, does not represent a single, or even a few, extensive infection unit(s) of a particular fungal symbiont but rather, a very large number of discrete, often intermingled, infections of different ages which are established progressively in concert with new root development. Consequently, random selection of VA mycorrhizal root segments essentially guarantees: that the structures observed will represent developmental stages of endophytic structures comprising more than one infection unit and that most of the fungal structures observed will be either fully developed or senescent, since a relatively small percentage of the total axial length of the root system supports young infections. The situation is further complicated by the absence of external morphological characteristics that permit the investigator to preferentially select root segments containing early stages of fungal development. It is not surprising, therefore, that few developmental studies of VA infections have been performed with either the light or electron microscope. The following discussion of VA mycorrhizal development illustrates characteristics of infections by *Glomus mosseae*, *Glomus caledonius*, and *Glomus fasciculatus* on soybean. Each of the fungal strains illustrated produces consistently high infection levels as well as highly significant growth responses in the host.

Development and anatomy of infections. Infective propagules of VA fungi include soilborne chlamydospores (or in *Gigaspora* spp., azygospores), vesicles in residues of previously infected roots, or (possibly) viable remnants of previously established hyphal systems in the soil. VA infections are initiated following contact of a hypha issuing from any one of these structures with a suitable host root and formation of an appressorium followed by penetration through or between epidermal cells, or by direct penetration without appressorium formation. While we have observed all three modes of penetration in soybean, penetration between epidermal cells following appressorium development is the most frequent means of ingress. Penetration and subsequent colonization (Figs. 1–3) generally occurs in the areas of differentiation and elongation of active "feeder" roots. Meristematic tissues and older, often heavily pigmented, roots exhibiting pronounced secondary growth are free of infection. Once penetration of the epidermis has been achieved, fungal development is restricted to the root cortex. Inter- and/or intracellular hyphae develop from the point of penetration, normally at a greater rate longitudinally than in a radial or circumferential direction (Figs. 1 and 3). In soybean, longitudinal intracellular hyphae predominate in the outer cortex and intercellular hyphae form with increasing frequency in the inner cortex where arbuscules are later formed (Figs. 2, 8, and 10). This hyphal colonization of the host root takes place without damaging the integrity of cortical cells and without eliciting conspicuous restrictive responses by the host, even though intracellular development may be quite extensive (Figs. 2 and 6). There is usually no significant increase in the size of host nuclei or in host cytoplasmic volume in cells invaded by intracellular hyphae. At the ultrastructural level, the cytoplasmic content and composition of cells containing intracellular hyphae, in general, are quite comparable to those of uninfected cortical cells (Fig. 11). The intracellular hyphae are enclosed by a thin sheath of host cytoplasm but are separated from it by, presumably, newly synthesized host plasmalemma and a compact zone of material similar in appearance and, perhaps, in composition to the host cell wall (9,27). Observations and analyses of the material exterior to intracellular hyphal walls are too few at this time to permit meaningful evaluations but it is probable, on the basis of cytochemical analyses of the interfacial matrix surrounding arbuscules (13,47), particularly arbuscular trunks, that the material is deposited by the host cell rather than the endophyte.

Morphological patterns and distribution of the hyphal system within VA mycorrhizal roots vary considerably but are obviously influenced by the host. In one of the most intensively studied VA associations (ie, *G. mosseae* on onion), the hyphal system is intercellular, and intracellular arbuscules are produced 0.5 mm or more behind the advancing hyphal tips (9). Our observations of *G. mosseae* infections in soybean, in common with other *Glomus* spp. illustrated here, indicate the development of numerous longitudinally oriented intracellular hyphae, often appressed to the

inner surfaces of the host cell walls, as well as similarly oriented intercellular hyphae closer to the stele (Figs. 2, 7, 8, and 10). Arbuscules are produced from both intercellular and intracellular hyphae (Figs. 7 and 10) a short distance behind the hyphal apices (Figs. 4 and 5). In yet another host species, yellow poplar (tulip tree), *G. mosseae* produces virtually no intercellular hyphae but intracellular loops and coils, usually oriented tangentially to the root axis, are formed abundantly in the cells of the outer cortex (Fig. 6). Similar differences in the morphology of infections of *G. fasciculatus* in maize and tulip tree have been reported previously (15). Abbott and Robson (1) have succeeded recently in developing a key based upon morphological characteristics of VA infections, staining characteristics, and external features of the symbionts, etc. which may provide some insight relative to certain host-endophyte combinations. At the present time, however, only infections caused by *Glomus tenuis* may be identified with confidence in the absence of sporulating structures; this particular symbiont produces minute hyphae (2 μm or less in diameter) and a distinctive hyphal branching pattern within host roots (22).

The arbuscular cycle. Of the structures that characterize VA infections, arbuscules have attracted the greatest attention. Arbuscules are highly branched, haustorialike structures that develop within the innermost cortical cells surrounding the vascular cylinder. Primarily, because of their logistically significant position within the root, large surface area contact with host cytoplasm, and alterations in host cell cytology associated with rather drastic changes in arbuscular morphology, arbuscules have been considered to be the most probable sites of nutrient exchange between the symbionts. Arbuscular development is initiated by penetration of the host cell wall by a lateral branch produced from an adjoining intercellular or intracellular hypha. This penetration hypha, which becomes the arbuscular trunk, grows into the host cell in a repeatedly dichotomous pattern and, ultimately, terminates in a series of short bifurcate branches that may be less than 1 mm in diameter. At maturity, arbuscules typically occupy a large portion of the host cortical cell volume. As arbuscular development begins, there is a dramatic increase in the volume of cytoplasm in those host cells containing them and, concurrently, there is an enlargement of the host nuclei which is easily seen with a light microscope (Fig. 7). With increasing maturity, the cytoplasm within the arbuscules becomes highly vacuolated and individual branches or portions of the branching system collapse. Deteriorating portions of the arbuscular system, often involving progressive degradation toward the trunk, aggregate into dense clumps (Fig. 8). Ultimately, the entire arbuscule deteriorates and aggregates into an irregular dense mass near the original point of entry into the host cell. The volume of host cell cytoplasm decreases concurrently with arbuscular deterioration (Fig. 8) and the

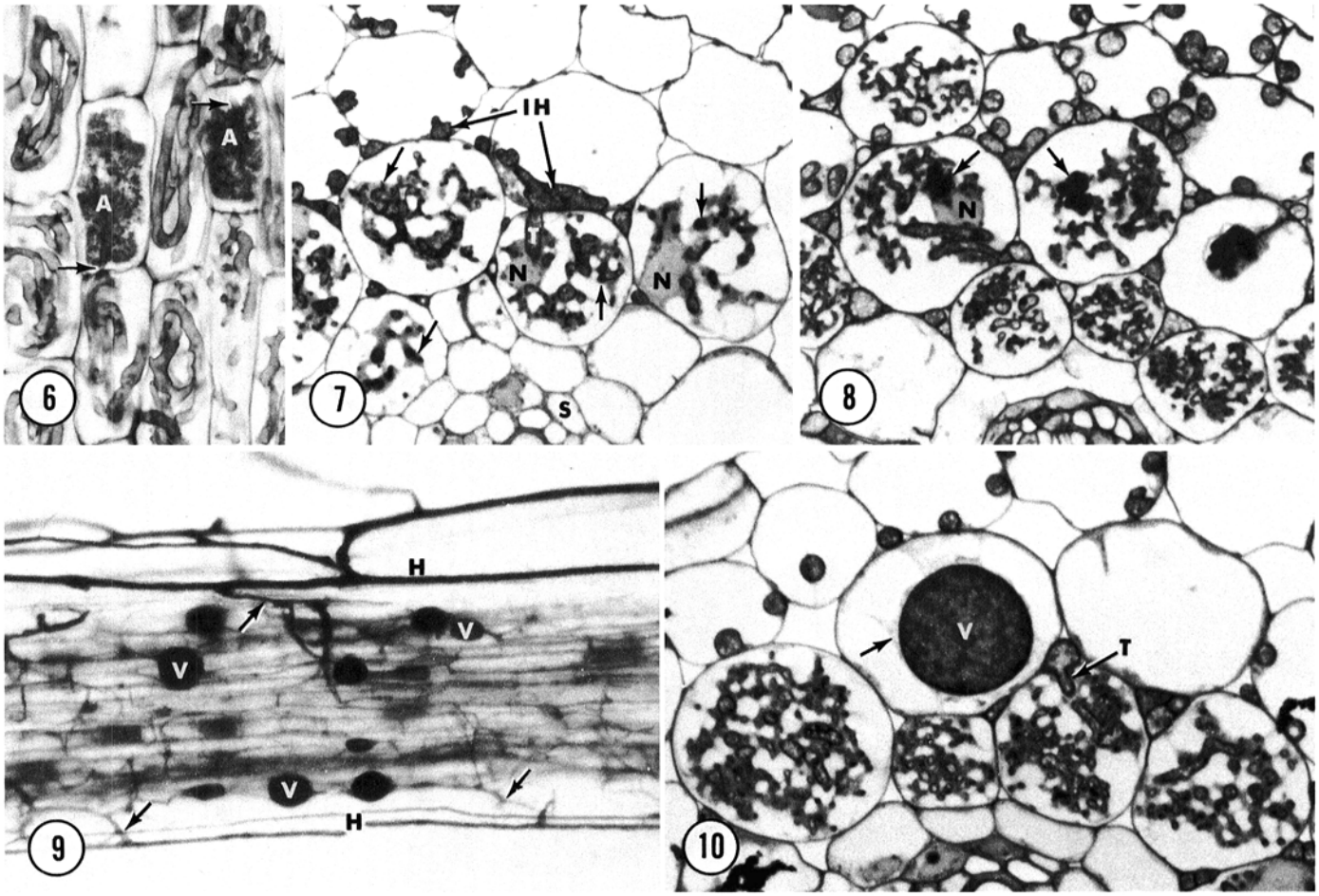


Figs. 1–5. Anatomy of vesicular-arbuscular (VA) mycorrhizae of soybean produced by *Glomus fasciculatus* (Figs. 1, 3, 4, and 5) and *Glomus caledonius* (Fig. 2). **1,** A cleared and stained whole mount showing an early stage of VA hyphal development arising from two closely-spaced penetration points (arrows) through the root epidermis (E). Hyphae extending from another infection are present in the lower left (×125). **2,** A 0.5 μm epoxy cross section of an older infection showing fungal penetration through an epidermal cell and development of intracellular hyphae (IH) in the outer cortex. Cell-to-cell penetration by intracellular hyphae is common. Epidermis (E) with intercellular hyphae (arrows) (×550). **3,** Early infections produced from two points of penetration (arrows). The more advanced (lower) infection has produced a hyphal system and several arbuscules (A) within the root. Stele (S) (×75). **4,** Leading edge of a well developed VA infection unit illustrating the progression of development in soybean. Enlargement of the infection occurs through growth of intercellular and intracellular hyphae (arrows) along the root axis (see also Fig. 2) followed by initiation of arbuscule (A) formation from these hyphae a short distance behind their apices. Older, more densely stained arbuscules occupy most of the inner cortical cells toward the central portion of the infection unit (left). Epidermis (E), stele (S) (×140). **5,** Production of arbuscules from the longitudinal hyphae (H) in soybean. The sparsely branched arbuscule (top center) is not yet fully developed. All five of the fully developed arbuscules (center) were produced in adjoining cells from a single hypha (×375).

cytological characteristics of uninfected cortical cells are ultimately reestablished (Fig. 11). The period over which the arbuscules remain structurally and, presumably, functionally intact is quite short, ranging from 4 to 15 days (5,11).

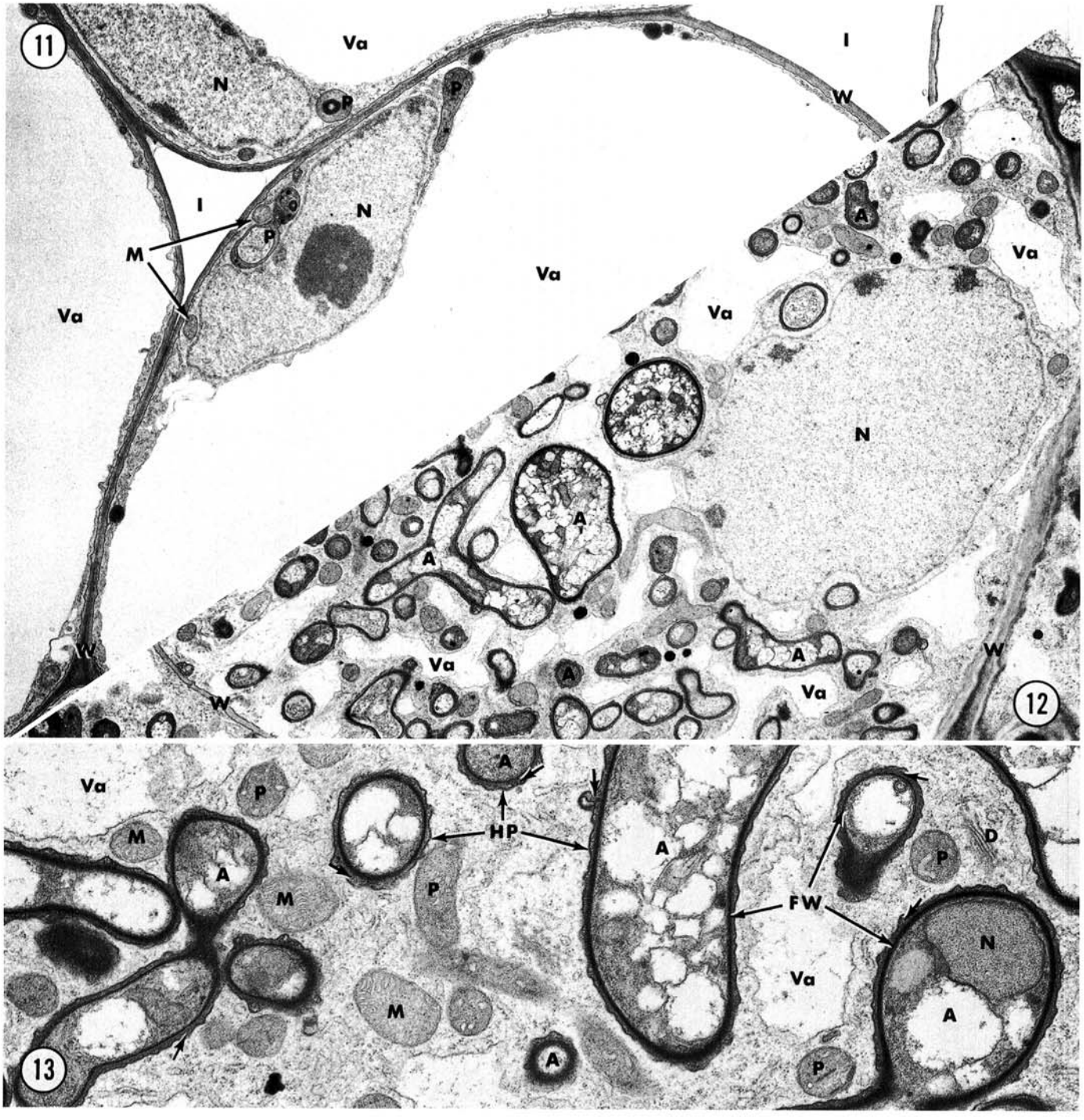
While VA mycorrhizae on several hosts have been examined ultrastructurally (9,23,26,28,29,46,47), most appear to have involved infections with either fully developed, vacuolate arbuscules similar to those in Figs. 12 and 13, or with arbuscules in varying states of deterioration. Since most of the arbuscules in any given infection unit are not in early developmental stages (9,29), random selection of VA mycorrhizal root segments for electron microscopy favors examination of mature and deteriorating stages of the endophyte. There is currently no ultrastructural documentation of the earliest stages of arbuscular development; ie, from host wall penetration to vacuolization of arbuscular cytoplasm. Nevertheless, the previously cited studies clearly show that throughout their life cycle, arbuscules are enclosed by host cytoplasm and the host plasmalemma, both of which dramatically increase in quantity. A 23-fold increase in host cytoplasm and a threefold increase in host plasmalemma has been calculated for onion cortical cells containing arbuscules of *G. mosseae* (11). Elevated numbers of host organelles (Figs. 11–13), clearly involving de novo synthesis, has been reported in cells containing arbuscules with each host-endophyte interaction examined. Starch grains in amyloplasts, often seen in uninfected cortical cells, are characteristically lacking in cells with arbuscules.

The interfacial zone between the host plasmalemma and arbuscular cell wall has attracted considerable attention since the arbuscules are generally assumed to be the structures involved in nutrient transfer from the fungus to the host. The appearance and distribution of materials found in the interfacial zone may differ somewhat depending upon the particular association studied, the location along the arbuscule (ie, from the trunk to the smallest branching tips), and the morphological condition of the arbuscule itself (9,28,29,46). With the demonstration, by cytochemical analyses, of pectic materials within this zone (13,47), there is now little doubt that much of the interfacial material is produced by the host. In general, interfacial material deposited around penetration points and extending inward along the arbuscular trunk has been reported by most workers to be comparable in structure and density to the host primary wall. Further within the host cell, outward along viable arbuscular branches, this material reportedly



Figs. 6–10. Anatomy of vesicular-arbuscular (VA) mycorrhizae produced by *Glomus mosseae* in yellow poplar (Fig. 6) and by *Glomus caledonius* (Figs. 7, 8, and 10) and *Glomus fasciculatus* (Fig. 9) in soybean. **6,** Longitudinal paraffin section of yellow poplar showing distinctive intracellular hyphal coils, oriented tangentially to the root axis in this host, arbuscules (A) and arbuscular trunks (arrows) produced from hyphae in adjoining cortical cells (×225). **7,** Epoxy cross section of soybean illustrating developing arbuscules within several cortical cells (center) surrounding the stele (S). At this stage of development the cytoplasm within intracellular hyphae (IH), arbuscular trunk (T) and arbuscular branches (arrows) is dense and nonvacuolated. The volume of host cytoplasm greatly increases in cells containing arbuscules and encloses these fungal structures. The host nucleus (N) also enlarges and is usually located among the branches of the arbuscule (×490). **8,** Epoxy cross section of soybean illustrating fully developed vacuolate arbuscules in different states of decline. Portions of two arbuscules have deteriorated and aggregated into dense irregular clumps (arrows). Deterioration and aggregation generally progress toward the trunk and ultimately destroy the structural integrity of the arbuscule (right center). The volume of host cytoplasm surrounding the arbuscule decreases during this process. Host nucleus (N) (×490). **9,** Several vesicles (V) and the hyphal network in a cleared and stained soybean root. Stolonlike hyphae (H) are produced in abundance along the surface of the root and yield numerous closely spaced penetration points (arrows) along the root axis (×100). **10,** Epoxy cross section of soybean showing four fully developed vacuolate arbuscules and an immature vesicle (V). The vesicle, which contains carbohydrate and lipid reserves at maturity, is enclosed by a thin layer of host cytoplasm (arrow) of lesser density than that surrounding arbuscules. Arbuscular trunk (T) produced from an intercellular hypha (×550).

decreases in quantity and, around the finest branches, may be present only as a sparse, flocculent to loosely fibrillar substance adjacent to the arbuscular wall. An electron-lucent zone of varying width, bounded at its outer limit by the host plasmalemma, has been considered by some to be an integral component of the interfacial zone (9,13,47, and others). However, with a fixation technique unlike those normally employed with VA infections, Carling et al (8) show that in soybean this electron-lucent "periplasmic space" represents a fixation artifact. In their illustrations of *G. caledonius* on soybean, similar to Figs. 12 and 13, periplasm is absent and the host plasmalemma conforms closely to the outline of all arbuscular branches. In infections of soybean by some *Glomus* spp., a narrow electron-lucent area may be present (E. J. King and M. F. Brown, *unpublished*). The interfacial material surrounding the arbuscular branches of all *Glomus* spp. we have examined in soybean is moderately electron dense and



Figs. 11–13. Ultrastructural comparison of cortical cells of nonmycorrhizal soybean roots and cortical cells containing arbuscules of *Glomus caledonius*. **11,** Portions of several control cortical cells in which the vacuole (Va) comprises nearly all of the cell volume. The nucleus (N) and cytoplasm containing relatively few plastids (P), mitochondria (M) and other organelles is appressed to the cell wall (W). Intercellular space (I) (×5,600). **12,** Portion of a cortical cell containing a fully developed vacuolate arbuscule comparable to those shown in Figs. 8 and 10. The host nucleus (N) is enlarged and all arbuscular branches (A), which can be identified by their dense walls, are enclosed within a greatly increased volume of host cytoplasm. Host organelles are far more abundant than in uninfected cells and numerous small vacuoles (Va) are interspersed among the arbuscular branches. Host cell wall (W) (×5,600). **13,** The arbuscular branches (A) are surrounded by host cytoplasm containing numerous plastids (P) and mitochondria (M) but are separated from it by the host plasmalemma (HP) and an interfacial zone containing moderately dense material (arrows) exterior to the dense fungal wall (FW). Host vacuole (Va), dictyosome (D), fungal nucleus (N) (×16,800).

finely granular in texture around larger branches grading into a granular-to-weftlike texture around the smallest branches. There is a need to employ critical preparative procedures in additional critical ultrastructural studies of the interfacial zone of other host-endophyte combinations to determine the full range of differences that exist in a broader range of host-arbuscular interactions.

Arbuscules begin to deteriorate as soon as morphological development is complete. It is not known whether this process is autolytic or results from physiological activities of the host cell. Deterioration starts with the breakdown of fungal membrane systems, usually within smaller branches in one or more portions of the arbuscule, and is followed by collapse, then aggregation of the residual fungal walls into the dense clumps shown in Fig. 8. Deterioration generally progresses toward the trunk and may be accompanied by the formation of cross walls within the arbuscule which appear to isolate degrading from still viable components of the structure (26,29). The integrity of the host plasmalemma surrounding the arbuscule is maintained throughout this sequence, but it is apparent that some host cytoplasm and plasmalemma adjoining portions of the original branching system are entrapped and degraded as the collapsed branches aggregate (9,13,29). The condensed arbuscular clumps, consequently, include: compressed but apparently undegraded fungal walls; condensed and degraded remnants of entrapped host cytoplasm; and (usually) a substantial amount of interfacial material. Coincident with the degradative sequence, there is a corresponding increase in host vacuolar volume and a reduction in cytoplasmic volume as the host cell reestablishes cytological characteristics typical of uninfected cortical cells.

STRUCTURE vs FUNCTION

Many of the physiological differences between VA-mycorrhizal and nonmycorrhizal plants reflect an altered nutritional status within the host conferred by activities of the mycorrhizal root system. When available phosphorus levels in the soil are low, VA infections stimulate significant increases in P uptake, resulting in dramatic increases in host growth (16,35). In addition, increased P uptake in mycorrhizal legumes stimulates nitrogen fixation by *Rhizobium*, thus indirectly causing an increase of N concentrations in the host (7,48). Changes in concentrations of other elements (ie, S, Zn, Cu, Sn, and others) in host tissues are also known to be influenced by VA mycorrhizae (19,21,24,32), but these changes are less dramatic than those in P. Several hypotheses relating to potential mechanisms by which increased P uptake by VA mycorrhizal roots might occur has been presented by Tinker (49). Currently, the most tenable of these hypotheses is that hyphae extending into the rhizosphere from infected roots increase the effective P absorbing surface of the root by exploration of a larger volume of soil than is accessable to nonmycorrhizal roots. It has been thought that this additional surface area, and the distribution of P-absorbing sites on the hyphae in the soil might wholly account for the superior absorbing capabilities of VA mycorrhizal roots. However, recent studies of the kinetics of P absorption by mycorrhizal and nonmycorrhizal tomatoes suggest that mycorrhizal roots not only have more P-absorbing sites, but that these sites on mycorrhizal roots have a greater affinity for P than those on control roots (12). Additional studies of this sort might significantly clarify the mechanisms involved in nutrient absorption by VA mycorrhizae.

Translocation of absorbed P within hyphae to the host is quite rapid as indicated by the recovery of significant concentrations of ^{32}P in both root and leaf tissues of onion only 2 days after injection of the isotope into soil 3–5 cm from mycorrhizal roots (40). Currently, evidence gained by several different analytical techniques (6,10,50) seems to support the scheme proposed by Callow et al (6): that absorbed phosphate is converted by the fungus to polyphosphate which is then translocated, as vacuolar granules, to components of the fungus within root tissues where it is subsequently degraded and made available for transfer to the host. Very little is known about the mechanisms involved in the transfer of P from the endophyte to the host plant, but the exchange process seems to require energy (38) and evidence now indicates that the exchange takes place, primarily, across living membranes of fungus and host (11) rather than by deterioration of fungal components of VA infections. Gianinazzi-Pearson and Gianinazzi (17) recently implicated a mycorrhiza-specific alkaline phosphatase in onion infected by *G. mosseae* which exhibited maximum activity when the VA infections were "100% arbuscular." Maximal enzyme activity coincided with the observation of initial growth responses in the host. In a subsequent study (18) alkaline phosphatase was reported to be active in vacuoles of intercellullar and intracellular hyphae, and in arbuscular branches as they become vacuolated. Activity disappeared with senescence and collapse of arbuscular branches. The authors suggested that the vacuolar alkaline phosphatase may be involved in the active mechanism of phosphate transport within hyphae of VA fungi. Should these observations be confirmed in other VA associations, another link in the chain of events involved in the transfer of P from fungal symbiont to host may be established.

Another aspect of VA mycorrhizal interactions that may shed some light on processes involved in the development and degeneration of endophytic structures relates to the initial observation by Mosse (36) that the ability of VA fungi to colonize host roots is generally suppressed or eliminated if high levels of P are present in the soil. Later studies (34,43) show that it is not soil P levels that cause the inhibition but, rather, concentrations of P within the host. Very recently, alterations in root cell membranes and corresponding changes in root exudation, regulated by P levels in the host (sudangrass), have been shown to be responsible for the P inhibition phenomenon (20). These workers have proposed that increased root membrane permeability, under conditions of low P availability, yields host exudates at levels sufficient to sustain the fungal symbiont during preinfection stages of development and colonization of the host. As the VA infection produces elevated P levels within the host, however, there is a reduction in host membrane permeability, a corresponding reduction in the release of root exudates, and (presumably) a decrease in the quantity of host metabolites accessible to the endophyte for further development. While this proposal is based upon analyses of entire VA mycorrhizal roots, it is not inconceivable that similar processes may also be involved in the sequence of events surrounding arbuscular development and deterioration within individual host cortical cells in VA infections. It is also possible that, during arbuscular development, the fungus may *induce* localized alterations in host plasmalemma structure and permeability as a consequence of its own metabolic activities.

It is now generally accepted that VA mycorrhizal infections do not function as low-resistance channels for transport of water from the soil into host roots (42,45) as was earlier suggested. However, VA mycorrhizae have been shown to increase stomatal conductance and photosynthesis after water stress of rough lemon (33), and to increase both transpirational and photosynthetic rates, as well as chlorophyll concentrations, in the grass *Bouteloua gracilis* (3). Allen et al (2) have reported elevated cytokinin levels in mycorrhizal *B. gracilis*, and noted that increased transpirational and photosynthetic rates may result from cytokinin increases. Further, Krishna et al (31) observed that bundle sheath chloroplasts were larger and more numerous, and that the veins and mesophyll cells of mycorrhizal finger millet were larger than those of nonmycorrhizal plants. These workers also suggest that the observed differences may have been caused by increased cytokinin levels stimulated by the VA mycorrhizal infections. These very recent studies certainly suggest that host responses to VA infections may involve far more complex physiological activities, perhaps ultimately expressed in host cytological and anatomical alterations, than can be presently explained solely on the basis of increased P and/or trace element uptake.

Although knowledge relating to VA associations has expanded rapidly over the past two decades, there is clearly a need for additional anatomical, cytochemical, and physiological investigations in order to achieve a more thorough understanding of those mechanisms and processes which ultimately result in improved growth and vigor of mycorrhizal plants.

LITERATURE CITED

1. Abbott, L. K., and Robson, A. D. 1978. Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil. New Phytol. 81:575-585.
2. Allen, M. F., Moore, T. S., Jr., and Christensen, M. 1980. Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhizae: I. Cytokinin increases in the host plant. Can. J. Bot. 58:371-374.
3. Allen, M. F., Smith, W. K., Moore, T. S., Jr., and Christensen, M. 1981. Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis* H. B. K. Lag ex Steud. New Phytol. 88:683-693.
4. Becker, W. N., and Gerdemann, J. W. 1977. Colorimetric quantification of vesicular-arbuscular mycorrhizal infection in onion. New Phytol. 78:289-295.
5. Bevege, D. T., and Bowen, G. D. 1975. Endogone strain and host plant differences in development of vesicular-arbuscular mycorrhizas. Pages 77–86 in: F. E. Sanders, B. Mosse, and P. B. Tinker, eds. Endomycorrhizas. Academic Press, London. 626 pp.
6. Callow, J. A., Capaccio, L. C. M., Parish, G., and Tinker, P. B. 1978. Detection and estimation of polyphosphate in vesicular-arbuscular mycorrhizas. New Phytol. 80:125-134.
7. Carling, D. E., Riehle, W. G., Brown, M. F., and Johnson, D. R. 1978. Effects of a vesicular-arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nodulating soybeans. Phytopathology 68:1590-1596.
8. Carling, D. E., White, J. A., and Brown, M. F. 1977. The influence of fixation procedure on the ultrastructure of the host-endophyte interface of vesicular-arbuscular mycorrhizae. Can. J. Bot. 55:48-51.
9. Cox, G., and Sanders, F. E. 1974. Ultrastructure of the host fungus interface in a vesicular-arbuscular mycorrhiza. New Phytol. 73:901-912.
10. Cox, G., Sanders, F. E., Tinker, P. B., and Wild, J. A. 1975. Ultrastructural evidence relating to host-endophyte transfer in a vesicular-arbuscular mycorrhiza. Pages 297–312 in: F. A. Sanders, B. Mosse, and P. B. Tinker, eds. Endomycorrhizas. Academic Press, London. 626 pp.
11. Cox, G., and Tinker, P. B. 1976. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. I. The arbuscule and phosphorus transfer: A quantitative ultrastructural study. New Phytol. 77:371-378.
12. Cress, W. A., Throneberry, G. O., and Lindsey, D. L. 1979. Kinetics of phosphorus absorption by mycorrhizal and nonmycorrhizal tomato roots. Plant Physiol. 64:484-487.
13. Dexheimer, J., Gianinazzi, S., and Gianinazzi-Pearson, V. 1979. Ultrastructural cytochemistry of the host fungus interfaces in the endomycorrhizal association *Glomus mosseae*/*Allium cepa*. Z. Pflanzenphysiol. 92:191-206.
14. Gallaud, I. 1905. Études sur les mycorrhizes endotrophes. Rev. Gen. Bot. 17:5-48, 123-136, 223-239, 313-325, 423-433, and 479-500.
15. Gerdemann, J. W. 1965. Vesicular-arbuscular mycorrhizae formed on maize and tuliptree by *Endogone fasciculata*. Mycologia 57:562-575.
16. Gerdemann, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annu. Rev. Phytopathol. 6:397-418.
17. Gianinazzi-Pearson, V., and Gianinazzi, S. 1978. Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. II. Soluble alkaline phosphatase specific to mycorrhizal infection in onion roots. Physiol. Plant Pathol. 12:45-53.
18. Gianinazzi, S., Gianinazzi-Pearson, V., and Dexheimer, J. 1979. Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. III. Ultrastructural localization of acid and alkaline phosphatase in onion roots infected by *Glomus mosseae* (Nicol. & Gerd.). New Phytol. 82:127-132.
19. Gilmore, A. E. 1971. The influence of endotrophic mycorrhizae on the growth of peach seedlings. J. Am. Soc. Hortic. Sci. 96:35-38.
20. Graham, J. H., Leonard, R. T., and Menge, J. A. 1981. Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. Plant Physiol. 68:548-552.
21. Gray, L. E., and Gerdemann, J. W. 1973. Uptake of sulfur-35 by vesicular-arbuscular mycorrhiza. Plant Soil 39:687-689.
22. Hall, I. R., and Fish, B. J. 1979. A key to the Endogonaceae. Trans. Br. Mycol. Soc. 73:261-270.
23. Holley, J. D., and Peterson, R. L. 1979. Development of a vesicular-arbuscular mycorrhiza in bean roots. Can. J. Bot. 57:1960-1978.
24. Jackson, N. E., Miller, R. H., and Franklin, R. E. 1973. The influence of vesicular-arbuscular mycorrhizae on uptake of ^{90}Sr from soil by soybeans. Soil Biol. Biochem. 5:205-212.
25. Janse, J. M. 1897. Les endophytes radicaux de quelques plantes javanaises. Ann. Jard. Botan. Buitenez. 14:53-212.
26. Kaspari, J. 1973. Elektronenmikroskopische Untersuchung zur Feistruktur der endotrophen Tabakmykorrhiza. Arch. Mikrobiol. 92:201-207.
27. Kinden, D. A., and Brown, M. F. 1975. Electron microscopy of vesicular-arbuscular mycorrhizae of yellow poplar. II. Intracellular hyphae and vesicles. Can. J. Microbiol. 21:1768-1780.
28. Kinden, D. A., and Brown, M. F. 1975. Electron microscopy of vesicular-arbuscular mycorrhizae of yellow poplar. III. Host-endophyte interactions during arbuscular development. Can. J. Microbiol. 21:1930-1939.
29. Kinden, D. A., and Brown, M. F. 1976. Electron microscopy of vesicular-arbuscular mycorrhizae of yellow poplar. IV. Host-endophyte interactions during arbuscular deterioration. Can. J. Microbiol. 22:64-75.
30. King, E. J., Schubert, T. S., and Brown, M. F. 1981. Techniques for developmental studies of VA mycorrhizae. Page 46 in: Proc. Fifth North American Conference on Mycorrhizae. (Abstr.) Univ. Laval, Quebec, P.Q., Canada.
31. Krishna, K. R., Suresh, H. M., Syamsunder, J., and Bagyaraj, D. J. 1981. Changes in the leaves of finger millet due to VA mycorrhizal infection. New Phytol. 87:717-722.
32. Lambert, D. H., Baker, D. F., and Cole, H. 1979. The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. Soil Sci. Soc. Am. J. 43:976-980.
33. Levy, J., and Krikun, J. 1980. Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. New Phytol. 85:25-31.
34. Menge, J. A., Steirle, D., Bagyaraj, D. J., Johnson, E. L. V., and Leonard, R. T. 1978. Phosphorus concentration in plant responsible for inhibition of mycorrhizal infection. New Phytol. 80:575-578.
35. Mosse, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Annu. Rev. Phytopathol. 11:171-196.
36. Mosse, B. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. IV. In soil given additional phosphate. New Phytol. 72:127-136.
37. Nicolson, T. H. 1959. Mycorrhiza in the Gramineae. I. Vesicular-arbuscular endophytes, with special reference to the external phase. Trans. Br. Mycol. Soc. 42:421-438.
38. Pearson, V., and Tinker, P. B. H. 1975. Measurement of phosphorus fluxes in the external hyphae of endomycorrhizas. Pages 277–287 in: F. E. Sanders, B. Mosse, and P. B. Tinker, eds. Endomycorrhizas. Academic Press, London. 626 pp.
39. Phillips, J. M., and Hayman, D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.
40. Rhodes, L. H., and Gerdemann, J. W. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. 75:555-561.
41. Rhodes, L. H., and Gerdemann, J. W. 1980. Nutrient translocation in vesicular-arbuscular mycorrhizae. Pages 173–195 in: C. B. Cook, P. W. Pappas, and E. D. Rudolph, eds. Cellular interactions in symbiosis and parasitism. Ohio State Univ. Press, Columbus. 305 pp.
42. Safir, G. R., Boyer, J. S., and Gerdemann, J. W. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant Physiol. 43:700-703.
43. Sanders, F. E. 1975. The effect of foliar-applied phosphate on mycorrhizal infection of onion roots. Pages 261-276 in: F. E. Sanders, B. Mosse, and P. B. Tinker, eds. Endomycorrhizas. Academic Press, London. 626 pp.
44. Sanders, F. E., Mosse, B., and Tinker, P. B., eds. 1975. Endomycorrhizas. Academic Press, London. 626 pp.
45. Sanders, F. E., and Tinker, P. B. 1973. Phosphate flow into mycorrhizal roots. Pestic. Sci. 4:385-395.
46. Scannerini, S., and Bellando, M. 1968. Sull' ultrastruttura della micorrize endotrofiche di *Ornithogalum umbellatum* L. in attivita vegetativa. Atti Accad. Sci. Torino, C. Sci. Fis., Mat. Nat. 102:795-809.
47. Scannerini, S., and Bonfante-Fasolo, P. 1979. Ultrastructural cytochemical demonstration of polysaccharides and proteins within the host-arbuscule interfacial matrix in an endomycorrhiza. New Phytol. 83:87-94.
48. Schenck, N. C., and Hinson, K. 1973. Response of nodulating and nonnodulating soybeans to a species of *Endogone* mycorrhiza. Agron. J. 65:849-850.
49. Tinker, P. B. H. 1975. Effects of vesicular-arbuscular mycorrhizas on higher plants. Symp. Soc. Exp. Biol. 29:325-349.
50. White, J. A., and Brown, M. F. 1979. Ultrastructure and X-ray analysis of phosphorus granules in a vesicular-arbuscular mycorrhizal fungus. Can. J. Bot. 57:2812-2818.