An Introduction to Mycorrhizae

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Biotechnology can be defined as the controlled use of living organisms or their components for the benefit of mankind. One aspect of biotechnology involves the special relationships formed between certain fungi and plants, called mycorrhizae, which have been shown to dramatically enhance the establishment, growth, development and survival of plants. The term mycorrhiza (pl. mycorrhizae or mycorrhizas), means "fungus root" and can be defined as a beneficial symbiotic association between plant roots and specific soil fungi. In the mycorrhizal relationship, the fungus penetrates secondary roots during periods of active root growth. The anatomy of the root is changed, and a new organ, "the mycorrhiza," is formed (Hacskaylo 1972). Mycorrhizal organs can exist in many forms, their morphology is determined by the characteristics of each partner involved and by the specific plant-fungus combination (Harley & Smith 1983). During the infection process, the fungus invades the root epidermis and cortex (Figure 1), but does not enter the vascular cylinder (specialized tissues that transport food and water) or the meristem (region of cell division) that is covered by the root cap.

Classes of Mycorrhizae

Mycorrhizae are classified according to the relationship of the fungus to the root cells. The two main types are *ectomycorrhizae* in which fungal hyphae penetrate the spaces between cells, and *endomycorrhizae* where projections of the fungus enter the interior of the cell (Figure 1).

Ectomycorrhizae

Ectomycorrhizae are formed by "higher fungi" found in many families of Basidiomycetes and Ascomycetes (Alexopoulus & Mims 1979). Ectomycorrhizae form on many important woody species including plants in the following families: pine, spruce, hemlock, oak, chestnut, walnut, beech, birch, eucalyptus and willow.

The pattern of ectomycorrhizae formation is somewhat specific. The fungus gains contact with a sus-

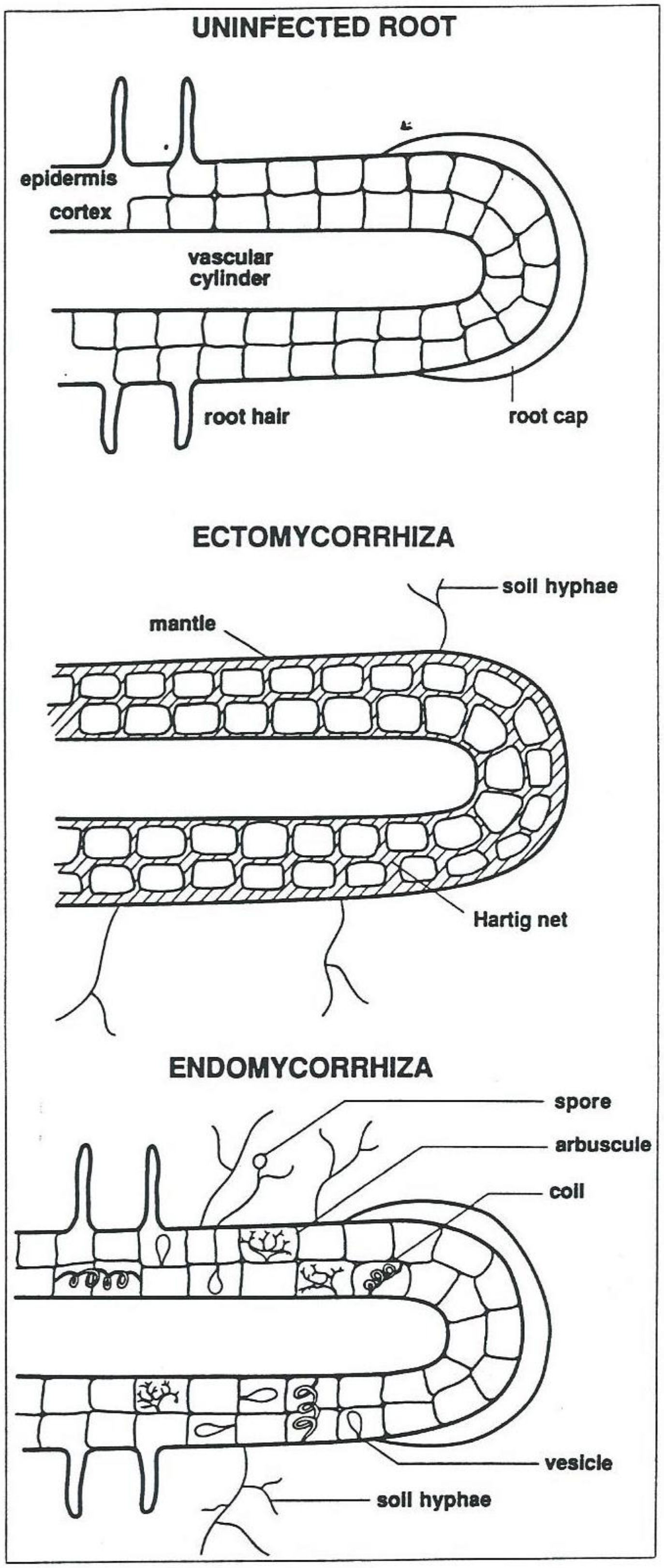

Figure 1. Diagramatic representation of the relationship of the symbionts in mycorrhizal associations.

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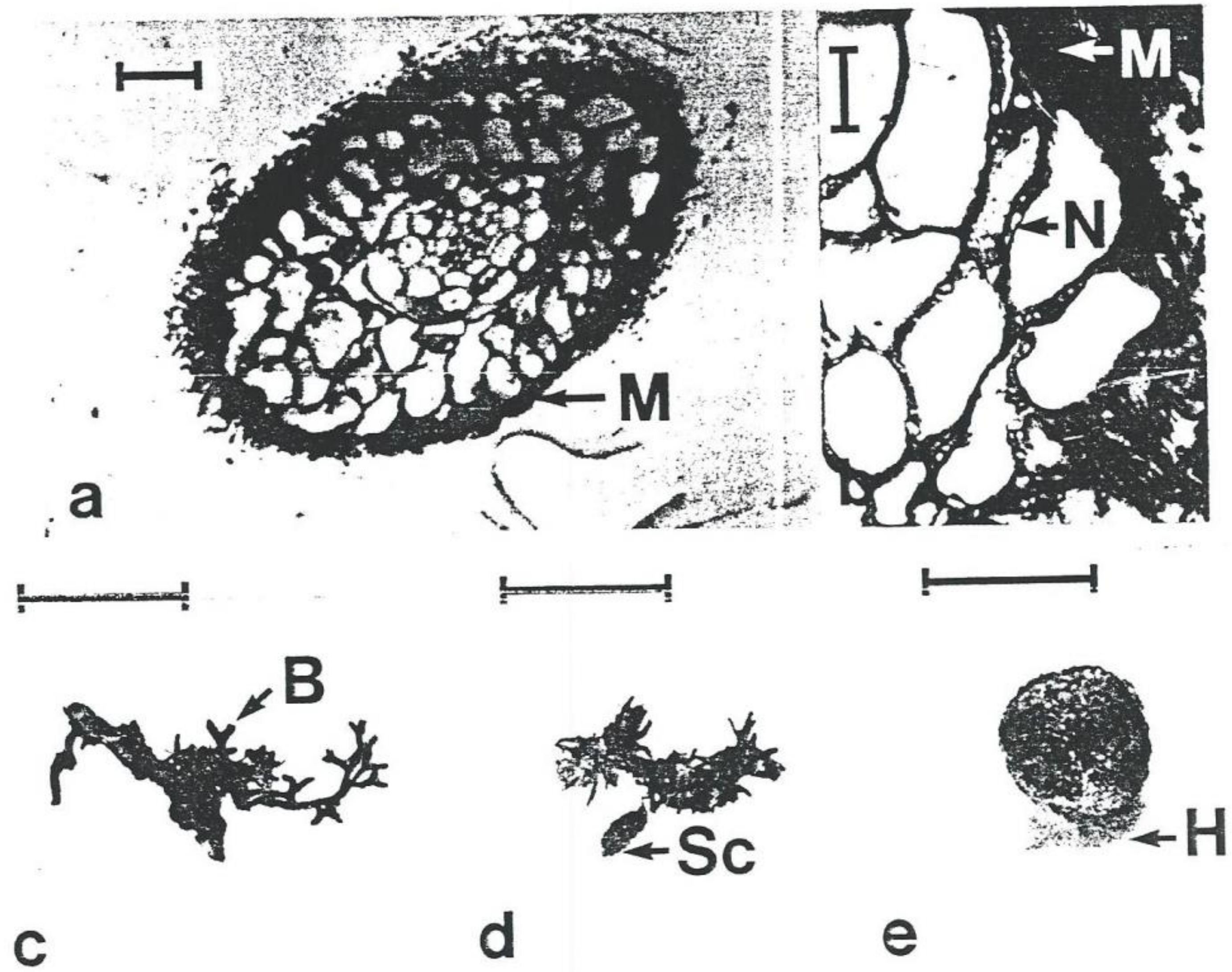

Figure 2. Ectomycorrhizae: a. Cross section of ectomycorrhiza formed by *Pisolithus tinctorius* and loblolly pine clearly showing the mantle (M) surrounding the root (bar = 116 μm); b. Enlarged portion of ectomycorrhiza in (a) showing the relationship of the hartig net, mantle and root cells (bar = 46 μm); c. Ectomycorrhizal pine root with branching (B) of short roots (bar = 1 cm); d. Ectomycorrhizal pine root with sclerotium (Sc) attached (bar = 1 cm); e. *Pisolithus tinctorius* sclerotium producing hyphae (H) at germination (bar = 2 mm).

ceptible root, and when stimulated by root exudates hair-like projections of the fungus called "hyphae" envelop the root and form a dense sheath called a *Mantle* that physically separates the root from its surroundings. The fungus secretes enzymes which enable hyphae from the mantle to penetrate the root and extend into the cortex. These hyphae do not enter cells, but are restricted to the spaces between root cells where they form an interconnected network called a *Hartig net* which plays a role in the exchange of materials between the plant and the fungus (the symbionts). As the mycorrhiza develops, the fungus secretes growth regulating compounds which cause changes in root development. When compared with an uninfected root, the mycorrhizal root is shorter, appears swollen, is often branched and does not form root hairs (Figure 2). Root hairs normally play a primary role in plant nutrition by absorbing nutrients and water from the soil. Since root hairs are absent in ectomycorrhizae, this function is assumed by an extensive network of hyphae from the mantle that extend into the soil.

Some ectomycorrhizal fungi require specific mycorrhizal associations in order to form sexual reproductive structures. Many of the mushrooms, puffballs, earth stars and truffles found beneath trees are sexual reproductive structures (called sporocarps) of ectomycorrhizal fungi which develop from hyphae attached to the mycorrhizal root (Hacskaylo 1972).

Endomycorrhizae

Endomycorrhizae are much more common than ectomycorrhizae and occur in more than 90 percent of all land plants (Harley & Smith 1983). Endomycorrhizae are usually associated with herbaceous (nonwoody) plants such as wheat, corn, tomatoes, onions, strawberries, legumes, grasses and many others. Endomycorrhizae also form in some trees such as apple and orange trees.

The most common types of endomycorrhizae are formed when roots are penetrated by hyphae from germinating spores. Spores (Figure 3) are fungal reproductive structures that can consist of one or many cells (Hawksworth et al. 1983). In fungi, reproduction can be sexual (producing sexual spores) or asexual (producing asexual spores or other structures). When spores germinate, both sexual and asexual, hyphae of the parent fungus are produced. Since neither the identity of the fungi that produce the spores that form endomycorrhizae nor the method of formation are known, their classification is based upon the morphology of the spores or spore-bearing structures. At present it is thought that the fungi that form these spores are "lower fungi" in the class Zygomycetes and all of those that are known have been placed in one family, the Endogonaceae (Alexopoulus & Mims 1979). These nearly microscopic spores are common soil inhabitants that infect susceptible roots when environmental conditions are favorable. During the infection process, spores or the hyphae attached to them come in contact with a receptive root and secrete enzymes that dissolve a small portion of the cell wall which permits hyphae to penetrate root hairs and other epidermal cells (Hacskaylo 1972). Penetration is sometimes limited to the epidermis, but frequently hyphae grow into cells of the cortex.

From the outside, the infected roots look normal, and the only way to be sure that the root is infected is by microscopic examination. Then one can see that the threadlike hyphal projections of the fungus have invaded the interior of the cells where they may remain as hyphal strands or form terminal hyphal swellings (vesicles), highly branched hyphae (arbuscles) or coils (Figure 4). These endomycorrhizae are referred to as vesicular-arbuscular mycorrhizae or "VAM," because of the presence of vesicles and arbuscles in the cells.

Arbuscles are believed to participate in nutrient transfer between symbionts while vesicles are thought to have a storage function; both arbuscles and coils increase the contact area between the fungus and the cell (Harley & Smith 1983; Harley 1969). Hyphae from the endomycorrhiza extend into the soil and aid root hairs in absorbing nutrients. The combination of hyphae and root hairs can absorb more nutrients than root hairs alone.

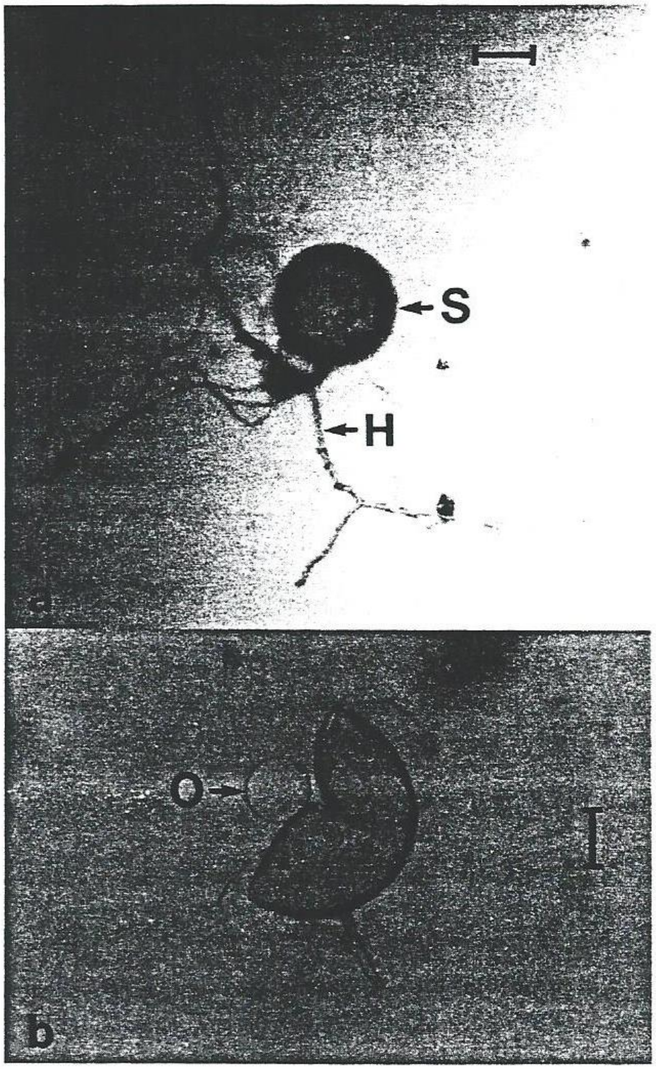

Figure 3. Spores that form endomycorrhizae: a. *Glomus deserticola* spore (S) with hyphae (H) attached (bar = 104 μm); b. Broken *Glomus intraradices* spore with escaping oil (O) droplet (bar = 19 μm).

Ectendomycorrhizae

While most plants form endomycorrhizae or ectomycorrhizae, a third type, *ectendomycorrhizae*, is also recognized. Ectendomycorrhizae are usually found on trees that normally form ectomycorrhizae and have been detected more often in nurseries than in other tree habitats. Ectendomycorrhizae exhibit anatomical characteristics of both ectomycorrhizae and endomycorrhizae. A mantle may be present or absent; if present, it can be thin and often difficult to detect. A hartig net is also present along with hyphae inside root cells. Some scientists believe that ectendomycorrhizae represent a transitional stage between ectomycorrhizae and endomycorrhizae (Melin 1948). However, definite proof is lacking.

Why Are Mycorrhizae Important?

A search of the existing mycorrhizae literature yields hundreds of articles of which 90 percent report

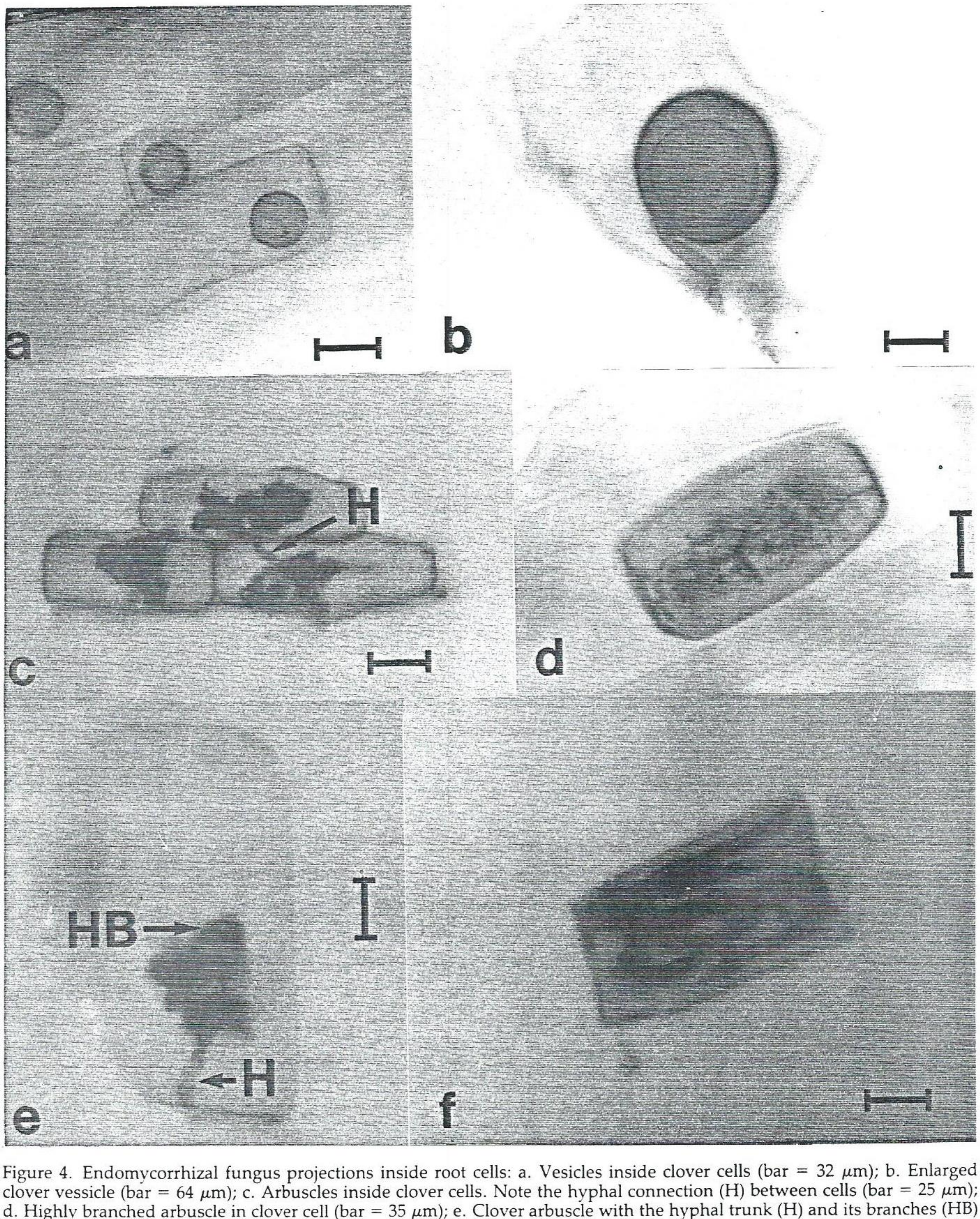

Figure 4. Endomycorrhizal fungus projections inside root cells: a. Vesicles inside clover cells (bar = 32 μm); b. Enlarged clover vessicle (bar = 64 μm); c. Arbuscles inside clover cells. Note the hyphal connection (H) between cells (bar = 25 μm); d. Highly branched arbuscle in clover cell (bar = 35 μm); e. Clover arbuscle with the hyphal trunk (H) and its branches (HB) demonstrating the tree-like appearance characteristic of many arbuscles (bar = 28 μm); f. Fungal coils inside wheat cell (bar = 30 μm).

the association of a specific fungus with a specific plant such as *Pisolithus tinctorius* with loblolly pine (*Pinus taeda*) or *Glomus mosse* and raspberries (*Rubus idaeus*). There are also descriptions of newly found species and of new habitats for existing species. The remaining articles deal with more fundamental aspects of mycorrhizae physiology, and among them there are conflicting data and gaps in knowledge (Morton 1988). However, it is apparent that in nature the mycorrhizal condition is the rule and not the exception.

Under suitable environmental conditions, most higher plants can form mycorrhizae. In fact, there are some plants that cannot become established or maintain proper growth without them (Marx 1975; Hacskaylo 1972). The conditions necessary for mycorrhizae formation exist when compatible symbionts (plant and fungus) are in close proximity; light, water and temperature necessary for normal plant growth are present; and soil nutrients, especially phosphorus (P), are in low concentrations. Many studies have demonstrated that mycorrhizal plants absorb P from the soil more efficiently than uninfected plants (Stribley 1987; Maronek et al. 1981). This effect on P uptake is especially important, because it can reduce the need for P fertilizer that has to be obtained from mining phosphate which is a nonrenewable natural resource.

Studies of fossils of some of the earliest land plants have revealed that mycorrhizae were as frequent hundreds of years ago as they are today (Pirozynski & Malloch 1975). Based upon this evidence and the knowledge that prior to plant colonization of land, early soils were nutrient-poor, some scientists have suggested that the evolution of mycorrhizae played an important role in the establishment of plants on land. This hypothesis has been strengthened by many contemporary studies which show that mycorrhizal plants are some of the first to become established and survive in low fertility soils such as those that have been poorly managed, ruined by severe erosion or devastated by coal mining.

Another factor that has stimulated interest in mycorrhizae is the large number of published studies showing that the presence of mycorrhizae dramatically increases the rate of plant growth. One classic example occurred during the 1950s in Puerto Rico where foresters had been trying to establish pine trees for more than two decades. Seeds imported from around the world would germinate, start to grow and then die no matter how drastically the growing conditions were manipulated or how much fertilizer was added. United States Department of Agriculture researchers thought that the needed mycorrhizal fungi were absent from soil on the island and introduced soil from a flourishing southeastern United States pine forest into the Puerto Rican experiment. Within three years the trees inoculated with the forest soil were up to 2.7 m tall while controls were no more than 45 cm high, if they were alive at all (Hacskaylo 1967). Further studies proved that mycorrhizal fungi in the forest soil caused enhanced tree growth (Hacskaylo & Vozzo 1967).

Table 1. Examples of enhanced plant growth due to the presence of mycorrhizae (Powell 1984; Menge et al. 1980, 1978).

Plant	*% Increase in Growth*
Avocado	254
Barley	290
Cowpea	50
Sour orange	1089
Soybean	53
Yellow poplar	167

Growth rate increases similar to those observed in the Puerto Rican Experiment have been reported for many other plant species (Table 1), both woody and herbaceous (Hayman 1987; Nemec 1987).

Mycorrhizal associations have been shown to benefit plants by increasing:

1. nutrient and water absorption
2. root health and longevity
3. tolerance to drought, high soil temperatures, toxic heavy metals, extremes in pH and transplant shock.

In addition, the fungal symbiont in this relationship can transport metabolites from one plant to another, produce plant growth regulators and antibiotics and may protect roots from invasion by pathogens (Harley & Smith 1983; Schneck 1982; Maronek et al. 1981; Marx 1981; Harley 1969). In the mycorrhizal relationship, the fungus also benefits by receiving housing and simple sugars needed for its metabolism.

The application of mycorrhizae technology into everyday practice could have a major impact on world agriculture by:

1. aiding the establishment of plants in poor soils
2. enabling nurserymen to produce vigorous mycorrhizal plants prior to outplanting
3. increasing plant size in a shorter time period which would lead to an increase in biomass and plant products and
4. reducing fertilizer requirements and thus cutting production costs and reducing fertilizer contamination of the environment.

Problems Associated with the Application of Mycorrhizae Technology

The term "mycorrhiza" was coined by A. B. Frank in 1885. Since that time, a considerable amount of research on mycorrhizae has been done, and the dynamic growth increases and other benefits to the plant attributed to the mycorrhizal association have been accepted for years. Since this knowledge is commonplace, one might ask why mycorrhizae technology is not a part of everyday agricultural practice. The answer to that question is rather complex. Although mycorrhizal fungi are normal soil inhabitants, they may be absent in a particular locale, and if present, they may not be those that form mycorrhizae with the desired plant. Inoculating the soil or plants with selected fungi known to form mycorrhizae with the plant of interest could lead to the production of the most efficient associations. A number of researchers have done field inoculations with mycorrhizal fungi; some have reported success while others have not. Marx (1981) and Molina and Trappe (1982) have attributed most problems in unsuccessful inoculations to the lack of adequate mycorrhizae inoculum (the form of the fungus that is transferred to the plant or soil).

Soil surrounding mycorrhizal plants in the field has often been successfully used for plant inoculations in many parts of the world. This method requires the use of large amounts of infected soil which can also transmit plant pathogens (Molina & Trappe 1982; Marx 1981).

The production of endomycorrhizae inoculum from specific spore types requires considerable effort. The spores (Figure 3) that form endomycorrhizae cannot be obtained from their unknown fungal parents, and these nearly microscopic spores must be sifted out of the soil and separated from the multitude of spores produced by other soil fungi. Once identified and separated, the spores must be inoculated onto a receptive plant if they are to remain alive and proliferate; the soil in which the inoculated plants grow, along with pieces of infected roots make up the inoculum that is used to infect other plants (Hayman 1982; Schenk 1982). Since successful methods for sterilizing these spores in mass have not been developed, inoculum produced in this way may also transmit plant pathogens. Currently, there are several companies that market endomycorrhizae inoculum that have been produced in this manner.

Some of the fungi that form ectomycorrhizae can be grown axenically (in a pure culture consisting of one organism), and have been used in field inoculations. While this method has produced some successful results, it has had limited use, because most of these fungi grow very slowly and often die when introduced into the soil in this form (Hacskaylo 1972). Inoculum produced this way is also expensive and difficult to handle (Molina & Trappe 1982). A number of researchers use basidiospores (sexual spores) of ectomycorrhizal fungi in a carrier, like sand, or encrusted on seeds as inoculum. However, the sporocarps that contain these spores must be collected by hand from the field. The availability of sporocarps can vary greatly from year to year which makes it difficult to plan broad scale inoculations. Reliable methods of germinating basidiospores have not been developed, and their long term storage requirements are unknown (Molina & Trappe 1982; Marx 1981). Basidiospore collections are not sterile, and they may transmit microorganisms and insects that are harmful to plants.

Commercial marketing of an ectomycorrhizal inoculum was first attempted in the United States about 12 years ago (Marx & Kenney 1982). This inoculum was prepared from axenic cultures of *Pisolithus tinctorius*, and has been successfully used in a number of studies; however, this product has a shelf life of only five to six weeks (Marx et al. 1984). A product with a shelf-life of this duration would have to be used shortly after production, and careful coordination of inoculum purchase, planting and inoculation is mandatory. This would indicate that an inoculum with a shelf-life longer than six weeks should contain resting forms of the fungus, like sclerotia (Figure 2), which are asexual structures composed of a compact mass of hardened hyphae that remains viable for long periods of time permitting the fungus to survive adverse environmental conditions and germinate (Figure 2) when conditions are favorable for fungal growth (Butler 1966; Snell & Dick 1957).

Only a few ectomycorrhizal fungi are known to form sclerotia; however they are produced by two of the most successful, *Cenococcum geophilum* (Trappe 1969) and *Pisolithus tinctorius* (Piche & Fortin 1982; Janerette 1981; Dennis 1980). If methods could be developed that would consistently induce axenic cultures of these fungi to form sclerotia or other resting structures, this would be a major step toward the production of persistent ectomycorrhizal inoculum. Before mycorrhizae technology can be applied on a daily basis, persistent, sterile, easily-handled inoculum with a long shelf life must be commercially available. An ideal inoculum would contain resting forms of the fungus whose presence could increase its shelf life and make it easier to handle by reducing the strict environmental requirements needed for the maintenance of axenic cultures. However, there is still a lot of work to be done and knowledge to be gained before an inoculum such as this is a reality.

There is strong interest in the commercialization of mycorrhizae technology. When persistent, cost effective inoculum is commercially available, some researchers believe that this technology will have a greater impact on slow-growing plants (like trees), container-grown perennials and field-produced cultivars than on crops that are grown on a short term basis, for flower production or those that are naturally infected in the field (Nemec 1987). The importance of mycorrhizae technology will be more apparent as world phosphate reserves near depletion and there are greater attempts to establish plants on land devastated by stripmining or other disasters, to decrease fertilizer pollution in the environment and to reduce global warming by revegetating rainforests that are being destroyed by overzealous cutting.

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