Chapter X

Mycorrhiza Agriculture Technologies

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Chapter X

Mycorrhiza Agriculture Technologies

INTRODUCTION-WHAT ARE MYCORRHIZAL FUNGI?

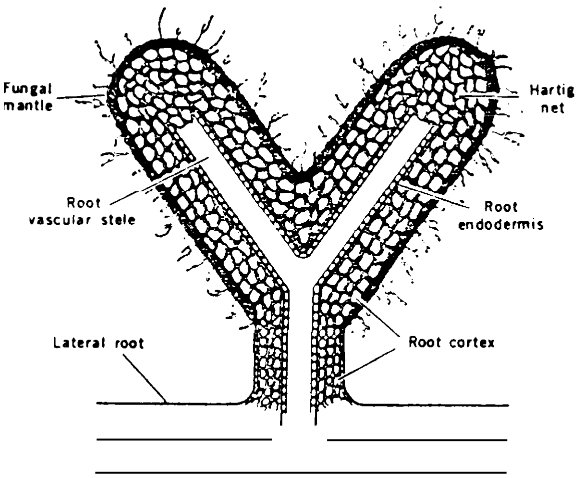
Mycorrhizal fungi are beneficial fungi that are associated with plant roots via a symbiotic association whereby both the host plant and the fungus benefit. Mycorrhizae are the structures formed by the symbiotic association between plant roots and mycorrhizal fungi. Mycorrhizae contain both plant roots and fungal tissues. In nature, mycorrhizae are far more common than non-mycorrhizal roots (24,92,94). Nearly all plant species are associated with mycorrhizal symbionts. Because of their importance to plants and their widespread distribution, mycorrhizae must be considered in all aspects of plant ecology, crop science, and agriculture.

Mycorrhizal fungi are divided into four very different types (66): ectomycorrhiza, vesicular-arbuscular mycorrhiza (abbreviated as VA mycorrhiza), ericaceous mycorrhiza, and orchidaceous mycorrhiza. As indicated by their names, ericaceous mycorrhiza and orchidaceous mycorrhiza are associated with ericaceous plants (blueberries, cranberries, azaleas, etc.) and orchidaceous plants (orchids), respectively. Because of the relatively low economic impact of these plants and the small amount of available data on these types of mycorrhiza, they will not be discussed further.

Ectomycorrhizae

Ectomycorrhizae are associated primarily with trees such as pine, hemlock, spruce, fir, oak, birch, beech, eucalyptus, willow, and poplar, Ectomycorrhizae are formed by hundreds of different fungal species belonging to the Basidiomycetes (mushrooms and puffballs) and Ascomycetes (cup fungi and truffles). These fungal symbionts are stimulated by root exudates and grow over the surface of host feeder roots to form a thick fungal layer known as a fungal mantle (figure 1). Hyphae of ectomycorrhizal fungi penetrate between the cells of the host root, develop around the root cortical cells, replace the host middle lamella, and form what is called the "Hartig net"—the distinguishing feature of ectomycorrhizae. In response to the fungal invasion, the host roots usually swell substantially and may branch dichotomously or in a coralloid manner. The root cells are not injured, however, and function of the roots is enhanced, as we shall discuss.

Figure 1.—Diagram of a Typical Ectomycorrhiza Including the Hartig Net, Fungal Mantle, and External Hyphae (courtesy D. H. Marx)



Vesicular-Arbuscular (VA) Mycorrhizae

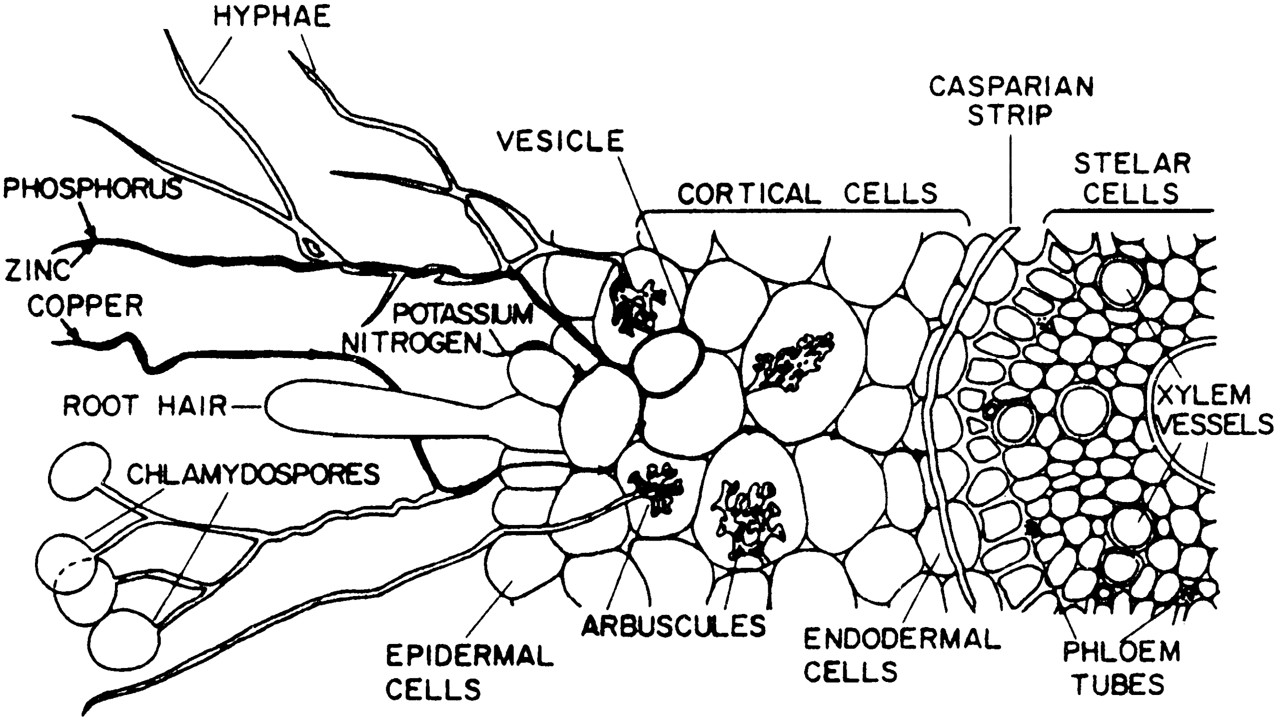
VA mycorrhizal fungi have the widest host range and form by far the most common type of mycorrhizae. VA mycorrhizae occur on liverworts, mosses, ferns, some conifers, and most broad-leaved plants. Only 14 families that are considered primarily non-mycorrhizal (28). The important crop families that are non-mycorrhizal are Cruciferae (cabbage, broccoli, mustard, etc.); Chenopodiaceae (spinach, beet, etc.); Cyperaceae (sedges); and Caryophyllaceae (carnation, pinks, etc.). wetland rice also is usually non-my corrhizal. Nearly all other important agronomic crops including wheat, potatoes, beans, corn, alfalfa, grapes, date palms, sugar cane, cassava, and dryland rice are associated with VA mycorrhizal fungi. Although many trees have ectomycorrhizae, most have VA mycorrhizae. Sixty-three of sixty-six tropical trees in Nigeria (77) are associated with VA mycorrhizae. So are most important tree crops such as cocoa, coffee, rubber, and citrus. Some trees such as juniper, apple, and poplar can have either ectomycorrhizae or VA mycorrhizae.

The fungi that form VA mycorrhizae, about 80 species, are in a few genera in the Zygomycetes class of fungi. They are so common in soils that literally any field soil sample from arctic to tropical regions will contain these fungi (66).

The hyphae of VA mycorrhizal fungi penetrate directly into the root cortical cells of host plants. Inside of the host plant cells, VA mycorrhizal fungi form minute coralloid structures known as arbuscules (figure 2). Arbuscules are thought to be the site of nutrient transfer between the symbiotic partners. The host plants obtain fertilizer nutrients from the mycorrhizal fungus while the fungus obtains sugars or other food materials from the plant. Although the arbuscule of VA mycorrhizal fungi occurs inside root cells, they remain covered by the host cell membrane and so are not in direct contact with the host cytoplasm. Vesicles are balloon-like mycorrhizal fungus structures that usually form inside the host root. These structures are thought to be storage organs that the fungus produces to store nutrient materials inside of the plant host.

VA fungi also produce abundant spores either inside or outside of host roots. These

Figure 2.—Diagram of a Typical Vesicular-Arbuscular Mycorrhiza Including Vesicles, Arbuscules, Spores, and External Hyphae



spores are the survival structures of VA mycorrhizal fungi. They are long-lived and extremely resistant to most unfavorable soil conditions. These spores are responsible for the widespread occurrence of VA mycorrhizal fungi in nearly all soils throughout the world. Despite the intracellular penetration by VA mycorrhizal fungi, they do not affect the roots' outward appearance except by inducing a yellow coloration in some hosts (4). Detection of VA mycorrhizal roots is best done by staining roots and examining them microscopically for the presence of hyphae, arbuscules, or vesicles (73).

Arbuscules of VA mycorrhizal fungi are short-lived and generally survive for less than 2 weeks before they are digested by the host plant (61,90). Plant roots normally release large quantities of chemical "exudates" into the root zone (8). Since the arbuscules are covered by the host membrane it is thought that the symbiotic association is regulated by the host plant via the cell membrane. The more nutrient materials released by the plant membrane to the arbuscule of the mycorrhizal fungus, the more abundant the mycorrhizal colonization (76). By restricting nutrients passing through the plant membrane the plant is capable of restricting mycorrhizal infection in roots. A similar mechanism can be postulated for the regulation of ectomycorrhizae by plant roots.

HOW DO MYCORRHIZAL FUNGI IMPROVE GROWTH OF AGRICULTURAL PLANTS?

The VA mychorrhizal symbiosis results in marked increase in crop growth and development. For example, inoculation of fumigated sand or soil with VA mycorrhizal fungi will increase the growth of citrus by as much as 1600 percent (figure 3); (42), grapes by 4,900 percent (74), soybeans by 122 percent (84), pine by 323 percent (100), and peaches by 80 percent (44). Growth responses due to VA mycorrhizal fungi have been observed in cotton (82), tomatoes (16), corn **(27),** wheat (41), clover (75), barley (5), potatoes (7), ornamental plants (99), and in many other crops.

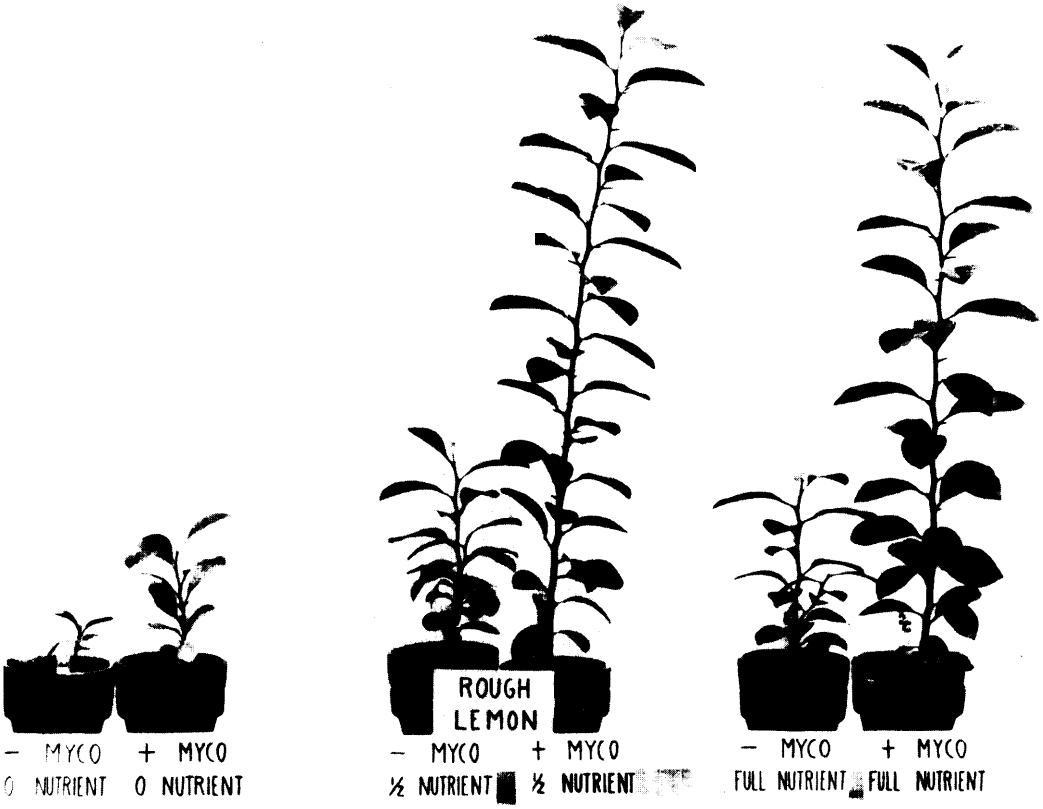
VA mycorrhizal fungi stimulate plant absorption of phosphorus (85,74,28,62), zinc (44,61), calcium (84), copper (84,85,60,42), iron (60), magnesium (36,61), and manganese (84,61). Increased uptake of phosphorus is perhaps the most important benefit provided by mycorrhizal fungi,

Most researchers agree that the increase in effective nutrient absorbing surface provided by mycorrhizal fungi is primarily responsible for the increase in uptake of soil nutrients by mycorrhizal plants. Hyphae from figure 3 mycorrhizal plant roots can extend up to 8 cm into the surrounding soil and transport nutrients this distance back to the roots (83).

VA mycorrhizal fungi may increase the effective absorbing surface of a host root by as much as 10 times (6). Nutrient ions such as phosphorus, zinc, and copper do not diffuse readily through soil. Because of this poor diffusion, roots deplete these immobile soil nutrients from a zone immediately surrounding the root. Mycorrhizal hyphae extend into the soil past the zone of nutrient depletion and can increase the effectiveness of absorption of immobile elements by as much as 60 times (6). Others have calculated that approximately 50 cm of mycorrhizal hyphae per cm root is necessary to account for the uptake of phosphorus by mycorrhizal plants (89), Experimental observations indicate that plant roots can have more than 80 cm of mycorrhizal hyphae, more than the amount necessary to account for the observed phosphorus uptake,

Plant uptake of mobile soil nutrients such as nitrogen and potassium is rarely improved by mycorrhizal fungi, Normal soil diffusion is adequate to supply roots of plants with these nutrients whether the roots have a large absorbing surface or not. Generally, plants that are most dependent on mycorrhizal fungi for nutrient uptake are those having roots with a low surface to volume ratio; that is, plants with coarse, fleshy roots with few root hairs (2).

Figure 3.—The Growth of Citrus (Rough lemon) Seedlings With (+Myco) and Without (−Myco) VA Mycorrhizal Fungi and at Different Nutrient Levels



Although some scientists speculate that mycorrhizal fungi can solubilize and absorb nutrients that are unavailable to plant roots, there is little evidence to support this claim. Sanders and Tinker (88) showed conclusively with ^{32}p-labelled phosphate that mycorrhizal fungi use the same phosphorus sources as do plant roots but they are able to absorb from a larger soil volume and so are responsible for the vast majority of phosphorus absorption by crop plants.

Mycorrhizal fungi can also enhance water transport in plants (87) and prevent water stress under some conditions (54). This probably is not a direct effect of mycorrhizal fungi, but instead is because of the improved nutrient status provided by the mycorrhizal fungi. Mycorrhizal fungi can endure much dryer soil conditions than can most plants and it is thought that plants may benefit from mycorrhizal infection under drought or water-stressed conditions (66,86). Ectomycorrhizae, in particular, with their mantle surrounding the roots, may provide a physical barrier against root dessication.

Considerable evidence exists to suggest that mycorrhizal plants may be better equipped to withstand the toxic effects of salt. Calcium, magnesium, and sodium concentrations in non-mycorrhizal citrus were 41 percent, 36 percent, and 150 percent greater than in mycorrhizal citrus (55). Hirrel and Gerdemann (35) found that mycorrhizal fungus increased bell peppers tolerance to salinity. Trappe, et al. (98), indicated that VA mycorrhizal fungi provided resistance to the toxic effects of arsenic. Mycorrhizae may also provide tolerance to excessive soil manganese and aluminum (34).

Mycorrhizal fungi also act to increase modulation by symbiotic nitrogen-fixing bacteria such as *Rhizobium (64,69).* Mycorrhizal fungi may stimulate other beneficial rhizosphere organisms as well (1).

Ectomycorrhizal fungi have been reported to provide resistance to plant disease in many plants (48). Although mycorrhizae never confer complete immunity, they often appear to reduce the severity of disease or symptom expression. Resistance of ectomycorrhizae to disease may result from (48):

- mechanical protection by the mantle,
- better plant nutrition,
- production of antibiotics by the mycorrhizal fungus,
- competition for infection sites,
- formation of phytoalexins, and
- alteration of root exudates.

Evidence is accumulating that VA mycorrhizal fungi exert similar effects on plant pathogens. Schenck, et al, (91), has reported mycorrhizal resistance to root-knot nematodes. Schonbeck (93) has examined a variety of foliar and root pathogens on mycorrhizal plants and concluded that root pathogens *(Thielaviopsis, Fusarium,* nematodes, etc.) are usually inhibited by mycorrhizal fungi while foliar pathogens (viruses, rusts, etc.) are often more severe on mycorrhizal plants. Davis, et al. (21,22), and Davis and Menge (20) concluded that the VA mycorrhizal fungus *Glomus fasciculatus* produced little resistance to Phytophthora root rot in citrus and indeed increased Phytophthora root rot in avocado and Verticillium wilt in cotton. VA mycorrhizal effects on disease may result from improved phosphorus nutrition because of the increased absorbing surface of the mycorrhizal hyphae. This effect is magnified when the roots' normal absorbing capacity is reduced because the roots are partially decayed.

There have been reports of mycorrhizal fungi actually reducing growth of some plants (11, 39,13). These parasitic effects are rare and the reason for them is not understood, but they apparently occur in grasses, cereals, and tomatoes at or above optimum soil nutrient levels when the plant is actively regulating mycorrhizal invasion,

MYCORRHIZAE AS SUBSTITUTES FOR FERTILIZERS

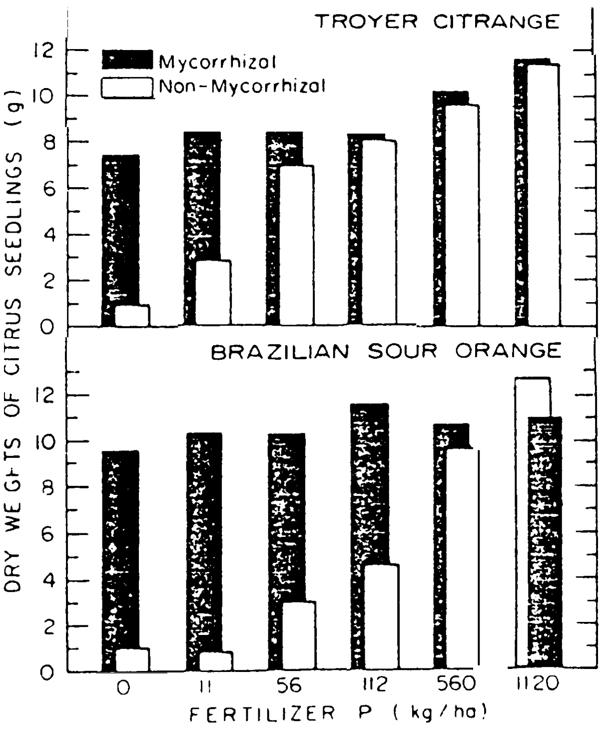
In the past 40 years the use of agricultural fertilizers has more than doubled. Crop yields have risen dramatically as a result. However, because of shortages in some fertilizer supplies and the high cost of energy, the cost of fertilizers has risen tremendously. Agricultural economists indicate that as energy costs rise the most responsive agricultural input is fertilizer. That is, as energy costs rise, fertilizer use will decrease. This response is a dangerous one since chemical fertilizers are said to account for one-third to one-half of the current U.S. agricultural output (47).

Estimates indicate that agriculture uses between 2.6 and 4.4 percent of all U.S. energy use. Fertilizers and their application comprise 30 to 45 percent of the total agricultural energy use. Nitrogen is the main energy user, with phosphorus and potassium accounting for only 16 percent of the fertilizer energy use (47).

Because mycorrhizal fungi increase the efficiency of fertilizer use, they can be thought of as "biotic fertilizers" and can indeed be substituted for substantial amounts of some fertilizers (53,55). Mosse (61) maintains that 75 percent of all phosphorus applied to crops is not used during the first year and reverts to forms unavailable to plants. In soils high in pH, aluminum, or calcium carbonate, nearly 100 percent of the phosphorus fertilizer can be immobilized to nonusable forms via chemical reactions in the soil. Tropical oxisols and ultisols are notorious for their capacity to immobilize phosphorus. Because mycorrhizal plants are better suited to exploiting soil with low amounts of available phosphorus, zinc, and copper, the addition of large amounts of these fertilizers each year may be unnecessary. Menge, et al. (55), compared mycorrhizal citrus seedlings with non-mycorrhizal seedlings that received various amounts of phosphorus fertilizer (figure 4).

Mycorrhizal Troyer citrange that received no fertilizer phosphorus were equal in size to non-mycorrhizal Troyer citrange that received 112 kg phosphorus per hectare, Similarly, mycorrhizal Brazilian sour orange that received no fertilizer phosphorus were equal in size to non-mycorrhizal plants that received 560 kg phosphorus per hectare. Concentrations of phosphorus in non-mycorrhizal Brazilian sour orange leaf tissue were never above 0.05 percent (less than 0.9 percent phosphorus indicates phosphorus deficiency) even when seedlings were fertilized with 1,120 kg phosphorus per hectare. Concentrations of phosphorus in leaves of mycorrhizal Brazilian sour orange were above deficiency levels in all seedlings fertilized with more than 56 kg phosphorus per hectare. Concentration of phosphorus in leaves of mycorrhizal Troyer citrange were never in the deficiency range even when plants were not fertilized with phosphorus.

Figure 4.—Dry Weights of Mycorrhizal and Non-Mycorrhizal Brazilian Sour Orange and Troyer Citrange Seedlings Fertilized With Different Amounts of Phosphorus



Non-mycorrhizal Troyer citrange, on the other hand, required over 56 kg phosphorus per hectare before adequate phosphorus concentrations were restored to the leaves. At 1980 retail costs for triple super-phosphate, it appears that use of mycorrhizal fungi could result in savings of $111 to $558/ha ($45 to $226/acre) in the cost of phosphorus fertilization of citrus in fumigated nursery soil. In one California citrus nursery, it was found that inoculation with mycorrhizal fungi could reduce phosphorus fertilization by two-thirds and save $652/ha ($264/acre). Similar savings in phosphorus fertilizers have been shown by Kormanik, et al. (43), in fumigated forest nurseries in the production of sweetgum.

Mycorrhizal fungi also can be substituted for copper fertilizer in the culture of citrus seedlings (97). Other data has shown that mycorrhizal fungi can be substituted for zinc fertilizer in the greenhouse culture of citrus and even nitrogen fertilization can be reduced by as much as 300 percent in the presence of mycorrhizae (Menge, et al., unpublished data). This nitrogen savings effect is probably due to an increased efficiency of nitrogen use resulting from improved phosphorus nutrition of the plant.

Since mycorrhizal fungi are present in most soils, their unique fertilizer-absorbing abilities are normally already being used by most crops. If mycorrhizal fungi are removed or damaged in any way, then the amount of fertilizer required by a crop increases enormously. This is demonstrated by reports that citrus grown in fumigated soil or in hydroponic solutions often require massive phosphorus applications for adequate growth compared to field grown citrus (55). Citrus in the field can absorb phosphorus from phosphorus-deficient soils more efficiently than either corn or tomatoes, and citrus orchards do not normally require phosphorus fertilization (9), Differences in phosphorus absorption by citrus grown in fumigated soil and citrus grown in nonfumigated soils can be reconciled if mycorrhizal fungi, which are present in nearly all citrus orchards (52), are the equivalent of 100 to 500 pounds phosphorus per acre.

When and if the cost of fertilizer becomes exorbitant, we must devise the most efficient fertilizer supply systems possible—to minimize costs while conserving energy and nonrenewable resources. I submit that mycorrhizal fungi could be one alternative that might increase crop yields and yet reduce fertilizer costs and energy demands.

CURRENT COMMERCIAL USE OF MYCORRHIZAL FUNGI

Although nearly all plants require mycorrhizal fungi for maximum growth, the widespread occurrence of these fungi in nearly all soils limits the immediate needs for inoculation with mycorrhizal fungi. Mycorrhizal fungi are currently commercially usable in only three major agricultural areas: 1) disturbed sites, 2) fumigated soils, 3) greenhouses.

Disturbed Sites

Mycorrhizal fungi have been conclusively shown to improve revegetation of coal spoils, strip mines, waste areas, road sites, and other disturbed areas (18,19,15,49,81). In these stressed sites, mycorrhizal fungi are usually lacking and adding mycorrhizal fungi provides a nutritional advantage to associated plants in addition to providing possible resistance to low pH, heavy metal toxicants, and high temperature,

Fumigated or Chemically Treated Sites

Fumigation with biocides or pesticides such as methyl bromide (56), chloropicrin (72), dazomet (50), 1,3-D (72), vapam (71), and vorlex (71) may destroy or inhibit root infection by mycorrhizal fungi, Application of many soil fungicides such as arasan (71), banzot (95), benomyl (96), botran (71), carbofuran (3), chloramformethane (37), dichlofluanid (37), ethirimol (37), lanstan (71), mylone (71), PCNB (96), sodium azide (3), thiabendazole (37), thiram (96), triademifon (37), tridemorph (37), and vitavax (96) have also been reported to be harmful to mycorrhizal development, Flooding, planting non-my corrhizal crops, or removing topsoil, may also reduce the population of mycorrhizal fungi to a level requiring reinoculation (7,78).

Fumigation with the biocide methyl bromide to remove soil-borne pests is required by regulation for the production of many nursery crops. It is also regularly used in many field agricultural situations. This chemical is extremely toxic to mycorrhizal fungi and most field fumigations are sufficient to destroy the native mycorrhizal inoculum (56). Stunting of crops following fumigation with methyl bromide is common and is due to the destruction of mycorrhizal fungi. Although a relatively small amount of land is treated with this chemical, less than 100,000 acres annually in the United States, stunting following fumigation with methyl bromide has been reported in the United States, Africa, Spain, Peru, Venezuela, and many other countries (52). Crops that are routinely grown in methyl bromide fumigated soils include strawberries, tomatoes, tobacco, nursery crops, tree crop replants, and some vegetable crops. For many of these crops the addition of mycorrhizal fungi following fumigation with methyl bromide is not only recommended but is imperative.

It appears that inoculating methyl bromide fumigated crops is economically possible. The cost for inoculating nursery-grown citrus with mycorrhizal fungi is about $288/acre, while the cost for phosphorus fertilizer alone is $338/acre. Fumigated tomatoes receive $51 worth of phosphorus per acre while the cost for mycorrhizal inoculation is less than $28/acre. Mycorrhizal fungi can provide additional benefits to the crop other than just improved phosphorus nutrition.

For nursery plants grown in methyl bromide fumigated soil, inoculation with mycorrhizal fungi should be imperative for the following reasons:

- the plants grow better (prevents stunting following fumigation);
- there is a decreased need for fertilization, specifically phosphorus, zinc, and copper, resulting in decreased fertilizer cost and energy conservation;
- there is decreased chance for water stress and therefore reduced transplant injury;
- mycorrhizal plants survive better especially if transplanted to fumigated, poorly fertilized, or disturbed soil;
- plants will be inoculated with effective mycorrhizal fungi rather than leaving mycorrhizal infection to chance; and
- mycorrhizal plants may be more resistant or tolerant to some plant diseases.

Greenhouses

Greenhouse culture uses growth media such as pine bark, vermiculite, perlite, builders sand, and peat moss and these are devoid of mycorrhizal fungi. In addition, most greenhouse operators steam, pasteurize, or chemically treat their mixes to eradicate harmful pathogens. Nurserymen have compensated for the absence of beneficial mycorrhizal fungi by applying luxury amounts of fertilizer and water to achieve desired growth. Inoculation of container grown plants to reduce irrigation, fertilizer, and pesticide applications and cost can be done as demonstrated by Chatfield, et al. (10), Linderman (46), and Crews, et al. (12).

COMMERCIAL PRODUCTION AND INOCULATION WITH MYCORRHIZAL FUNGI

Many ectomycorrhizal fungi can be readily cultured on artificial media and inoculum can be grown under standard laboratory conditions (49). Experimentally, sterilized vermiculite and peat moss is frequently saturated with a liquid nutrient medium (49) and is infested with a desirable ectomycorrhizal fungus. Ectomycorrhizal fungi generally grow quite slowly and may take several months to colonize the vermiculite-peat moss mixture. This material can be used on a small scale to inoculate nurseries and greenhouses with mycorrhizal fungi. Abbott Laboratories, North Chicago, Illinois, has produced massive amounts of inoculum of the ectomycorrhizal fungus *Pisolithus tinctorius (86).* Abbott Laboratories produced the peat moss-vermiculite-nutrient solution inoculum under large-scale commercial conditions using commercial fermenters.

Under the direction of D. H. Marx, the U.S. Forestry Service has undertaken a massive testing program using the commercially produced inoculum. The inoculum will be tested in nearly 100 tree nursery test sites throughout the United States. Results will be available within 4 years and will indicate the commercial feasibility of producing and using mycorrhizal inoculum in fumigated tree nurseries.

Ectomycorrhizal inoculum can best be applied in the nursery. Once the trees become infected, the benefits can be transferred to wherever the trees are grown. In the nursery, mycorrhizal inoculum can be distributed by hand and rototilled into the soil before planting seed. Special machinery has already been built and is being used to incorporate ectomycorrhizal inoculum.

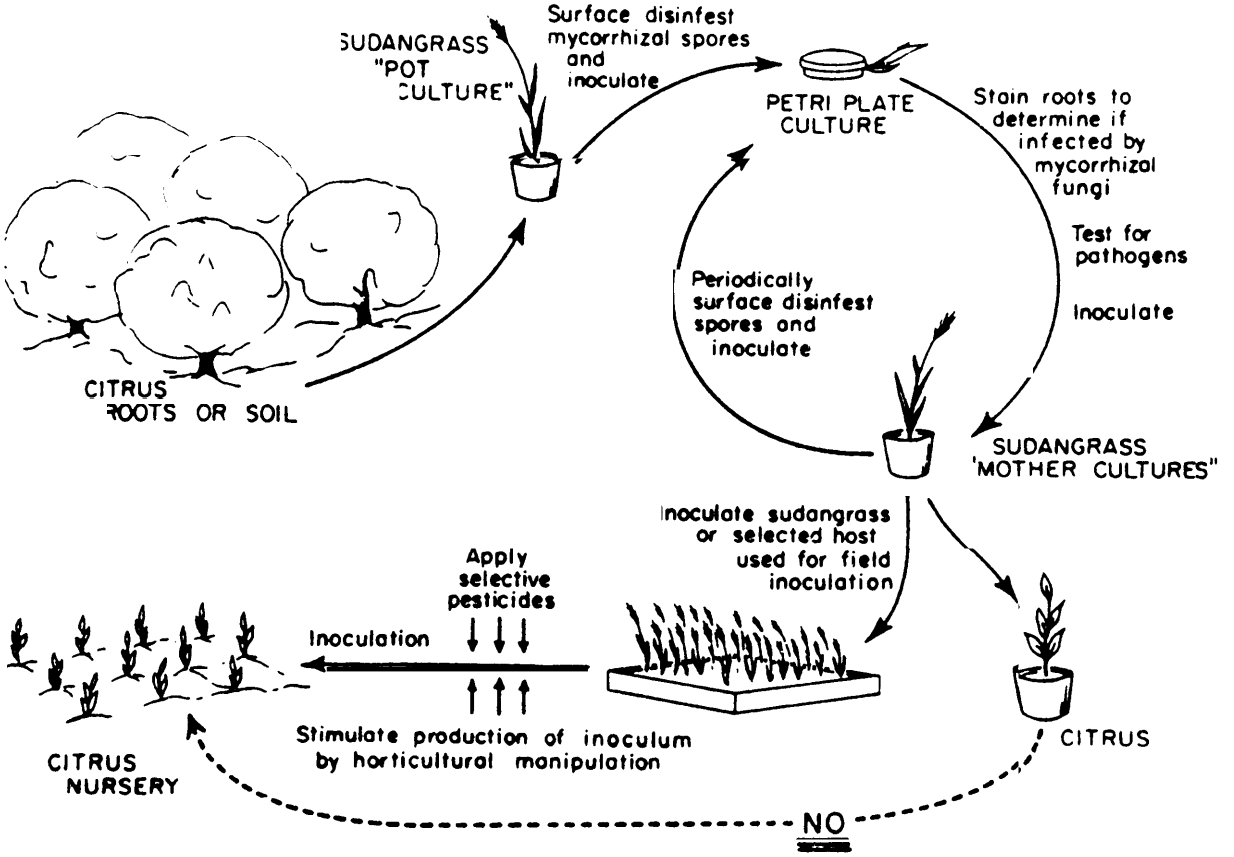
Commercial production of mycorrhizal inoculum for use in sterilized or fumigated soil is being attempted at several locations in the United States. Currently, the only way to produce suitable quantities of a mycorrhizal inoculum is on roots of susceptible host plants.

The possibility of pathogenic organisms contaminating mycorrhizal inoculum is an extremely serious problem when growing VA mycorrhizal inoculum in semi-sterile cultures in the greenhouse. For this reason, many scientists will consider mass production of VA mycorrhizal fungi only if it is done axenically (one organism only).

Realistically, however, not only must these obligate parasites be grown in vitro, but they must produce large quantities of spores in culture which will survive under soil conditions and infect plants in nature. Information gained from the culture of other formerly obligate parasites suggests that the possibility of realizing this goal in the near future is unlikely. Even if mycorrhizal fungi are cultured axenically, mycorrhizal inoculum for field use will probably be produced on the roots of suitable host plants.

With proper safeguards, mycorrhizal inoculum, free of plant pathogens, can be produced on plants in the greenhouse. Figure 5 illustrates a proposed scheme for producing mycorrhizal inoculum [53]. VA mycorrhizal fungi can be isolated by using bits of roots or soil from the field to inoculate roots of "trap plants" growing in sterilized soil in the greenhouse. Sudangrass *(Sorghum vulgare* Pers.) is frequently used, but other plants such as tomato, soybean, corn, and safflower may be equally suitable. The soil used throughout is a low nutrient sand fertilized once per week with one-half the standard Hoagland's solution minus phosphorus. After production of VA mycorrhizal spores in the "pot cultures," the spores can be

Figure 5.—Proposed Scheme for the Commercial Production of Vesicular-Arbuscular Mycorrhizal Inoculum



removed by wet sieving (29), elutriation (25), or centrifugation (85). These spores must be surface disinfested with substances such as chloroamine T or sodium hypochlorite and streptomycin to assure that pathogens do not accompany the spores (68).

These surface disinfested spores are used to inoculate the roots of plants that were germinated and grown under aseptic conditions in growth chambers. The containers illustrated are made from plastic petri plates and filled with the low nutrient sand. After 1 to 4 weeks when the mycorrhizal fungi have infected roots grown under aseptic conditions root pieces can be removed and stained (73) to observe infection, root pieces are carefully removed and used to infect suitable host plants grown in sterilized soil in the greenhouse. Similar root pieces can be removed, examined, and plated on agar to observe pathogenic organisms.

If no pathogens are observed, the greenhouse "pot culture" may be used as a "mother culture" to produce inoculum that will be used in the field. Inoculum should be produced on selected hosts that have no root diseases in common with the host plant for which the inoculum is intended. For instance, inoculum for citrus could be produced on sudangrass but never on citrus. In this way the wide host range of most VA mycorrhizal fungi can be used. As another precaution against propagating pathogens along with mycorrhizal inoculum, the field inoculum should be drenched several times with pesticides chosen to eliminate pathogens known to infect the host for which the inoculum is intended. Mycorrhizal inoculum intended for citrus should be drenched with a nematicide to control the citrus nematode and fungicides to control *Phytophthora* and *Rhizoctonia*. Suggested pesticides are Ethazole and PCNB. PCNB reduces the population of mycorrhizal spores but the other pesticide can actually increase spore production (57). Several other pesticides can be used without harming mycorrhizal fungi (96).

Horticultural practices also could be used at this point to maximize spore production. Eliminating fertilization and slowly reducing the water may be effective in increasing spore production. When spores are mature, plant tops are removed and roots, soil, and spores can be ground up and partially dried (7 to 20 percent moisture content) and stored at 4°C until used. If concentrated spore suspensions are desired, spores can be concentrated by wet sieving (38), elutriation (25), or centrifugation (85) before storage. VA mycorrhizal inoculum can be freeze-dried if desired (38). Inoculum produced in this manner should be consistently infective and yet pathogen free.

Using the method described above, the estimated costs for producing mycorrhizal inoculum are shown in table 1. These figures are derived from production costs of a foliage plant greenhouse and could be reduced considerably

Table 1.—Estimated Cost of Production of Vesicular-Arbuscular Mycorrhizal Inoculum on Sudangrass in 4 Inch Pots

Item	Cost/pot
1. Labor:	
a. to prepare the soil mix	$0.03
b. potting, inoculating, and seeding	0.05
c. moving pots to growing area	0.03
d. pruning	0.02
e. spraying (insecticides and fungicides)	0.03
f. watering	0.02
g. harvesting	0.01
h. grinding and packaging	0.03
i. quality control	0.02
j. maintenance of mother cultures	0.05
Labor cost	$0.29
2. Materials:	
a. pots 4 '	$0.07
b. seed	0.002
c. fertilizer	0.02
d. shipping containers	0.025
e. insecticides and fungicides	0.03
Materials	$0.147
3. Overhead expenses:	
a. heat	$0.08
b. depreciation on greenhouse	0.008
c. depreciation on boilers	0.003
d. maintenance allowance	0.006
e. office supplies	0.002
f. management and office work	0.03
g. return on investment	0.01
h. loss due to undeveloped plants	0.001
i. taxes	0.06
j. laboratory, incubator, etc.	0.04
Total for overhead	$0.24
Total cost $0.677	
Selling price $0.90	
0.18¢/500 spores	

since mycorrhizal inoculum quality is of importance and not plant quality, A reasonably generous estimate of the cost of mycorrhizal production, including technical labor and quality control, together with a small margin of profit, indicates that consumers may pay about 0.180/500 spores of VA mycorrhizal fungi. Such a cost could reasonably be borne by consumers such as greenhouse operators or nurserymen.

A similar method to that outlined above has been patented in England and is being perfected for large-scale commercial use (34). In this method plants are grown in peat blocks that are standing in a shallow nutrient-flow culture. After VA mycorrhizal spores are produced in the peat blocks they are ground up, roots and all, for inoculation. The finished product is not only excellent mycorrhizal inoculum but is light and easy to ship.

Although many methods have been used to inoculate plants with VA mycorrhizal fungi in greenhouse trials, few inoculation methods are acceptable for large-scale commercial inoculation. Several different methods to inoculate corn have been studied and layering inoculum under the seed was superior to seed inoculation or banding the inoculum (38), Hall (30) developed a method for pelleting seed with a mycorrhizal infection and determined that mycorrhizal fungi could survive up to 28 days under these conditions. Menge, et al. (53), found that layering inoculum below the seed and banding inoculum were superior to seed inoculations. Crush and Pattison (14) experimented with several means of inoculating seeds with VA mycorrhizal fungi, but again found that sowing seed above pelleted mychorrhizal inoculum was the most effective method for obtaining mycorrhizal infection. Hattingh and Gerdemann (31) reported growth responses of citrus in a fumigated nursery after inoculating citrus seed with mycorrhizal inoculum. Gaunt (26) inoculated onion and tomato seeds with a VA mycorrhizal fungus and reported that seed inoculated plants grew as well as plants that were inoculated by mixing VA mycorrhizal inoculum into the soil. Commercial applications of mycorrhizal inoculum using fertilizer banding machinery were successfully carried out in citrus nurseries in California (23).

Commercial VA mycorrhizal inoculum is produced using the method described above in two citrus nurseries—Brokaw Nursery, Saticoy California and the Thermal Ranch, Thermal, California. Experimental VA mycorrhizal inoculum is being produced and distributed on a large scale by Abbott Laboratories, North Chicago, Illinois. Other major corporations that are supporting or carrying out research on VA mycorrhizal fungi include Dow Chemical Co., Rohm & Haas Co., Dupont, Monsanto Co., and Ceiba-Geigy Chemical Co.

Plants growing in all soils do not respond favorably to VA mycorrhizal inoculum. If soil nutrition is optimum, mycorrhizal fungi will not enhance growth of plants. A method for detecting which soils require mycorrhizae for maximum production of citrus was devised by Menge, et al. (58). In soils with less than 34 ppm available P (Olson analysis), 12 ppm available Zn, 27 ppm available Mn, or 3 percent organic matter, citrus trees will probably require mycorrhizal fungi for maximum growth. Mycorrhizal inoculations are recommended only in soils with these characteristics, It is estimated that this includes approximately 85 percent of the southern California citrus soils. Similar studies could be done with other crops to determine which soils require mycorrhizal infestation.

POTENTIAL USES FOR MYCORRHIZAL FUNGI

Because mycorrhizal fungi occur on most agronomic crop plants and improve the growth of these plants, the potential use of these fungi as commercial “biotic fertilizers” is enormous. Large-scale field inoculations with mycorrhizal fungi are rare because of limited inoculum, and natural field soils usually contain adequate populations of indigenous mycorrhizal fungi.

Under these conditions, any growth benefit due to mycorrhizal inoculation would depend primarily on the superiority and/or placement of the mycorrhizal inoculum. Beneficial responses under these conditions would be predicted to be far less than the responses obtained in fumigated or partially sterilized soil. However, greenhouse and field experiments in which plants were inoculated with mycorrhizal fungi in nonfumigated soils have demonstrated that growth responses due to mycorrhizal fungi can occur under these circumstances.

In greenhouse experiments, using untreated soil, Mosse and her colleagues (62,63,65,67,70) demonstrated that preinoculation with mycorrhizal fungi could provide the following growth increases:

Crop	*Growth increase*
Centrosema spp.	34 percent
corn	306 percent
Melinis spp.	41-60 percent
onions	48-3155 percent
strawberries	250 percent
Stylosanthes spp.	85-88 percent
sweetgum	45 percent
Viola spp.	527 percent

Other studies have noted similar growth increases in untreated soil:

corn	14 percent (Gerdemann, 1964)
corn	0-53 percent (Jackson, et al., 1972)
mahogany	151 percent (Redhead, 1975)
sudangrass	0-18 percent (Jackson, et al., 1972)
white clover	80-100 percent (Powell, 1977)

In a large-scale field experiment conducted in nonsterile, virgin, infertile fields, wheat preinoculated with a mycorrhizal fungus produced 220 percent more grain than non-mycorrhizal wheat (41). In a similar experiment (40), corn inoculated with a mycorrhizal fungus was 122 percent larger than non-mycorrhizal corn. Hayman (33) reported white clover growth increases in the field due to inoculation with a mycorrhizal fungus. Black and Tinker, in an extremely well-documented field experiment, found that fallow field inoculation with a mycorrhizal fungus increased potato yield 20 percent.

Not all mycorrhizal inoculations in nonsterile soil result in increased growth. Hayman (33) indicated that mycorrhizal fungi did not stimulate growth of white clover at several locations. Powell (75) obtained significant growth increases of white clover after inoculation with mycorrhizal fungi in only three of nine sites. Jackson, et al. (38), indicated that with certain mycorrhizal inoculation methods, growth of corn, sudangrass, and soybeans was not stimulated in nonsterile soil. Mosse (65) obtained significant growth responses of Stylosanthes spp. due to mycorrhizae in 6 of 11 nonsterile soils. Ross and Harper (85) reported no growth stimulation of soybeans in nonsterile soil.

Mosse (65) indicated that the inoculum potential of indigenous mycorrhizal fungi is the major determinant governing growth responses of plants to mycorrhizal fungi in nontreated soil. Powell (75) indicated that many indigenous mycorrhizal fungi are “inefficient” symbionts, and that inoculation by more efficient mycorrhizal fungi will result in growth increases even in nonsterile soil that contain high populations of “inefficient” mycorrhizal fungi. placement of mycorrhizal inoculum is equally important in affecting a plant growth response (38). Certainly, plants infected early in the growing season by mycorrhizal fungi are better than plants that do not become infected until later (82).

Huge expanses of tropical soils (e.g., the Brazilian Cerrado) are either deficient in phosphorus or immobilize phosphorus fertilizers. These marginal agricultural lands could be productive if mycorrhizal fungi, with the ability to efficiently use extremely small quantities of fertilizer, were developed and added to the soil. Cheap but readily available rock phosphate could be added as the phosphorus source. This phosphorus source is a poor fertilizer but releases small quantities of phosphorus for long periods of time. Some mycorrhizal fungi use rock phosphate much better than others and can tremendously improve growth of plants growing in poor soils fertilized with this material (59,66).

Mycorrhizal fungi have been proposed as unstable soil or sand dune stabilizers (96). Finally ectomycorrhizal fungi have been shown to improve rooting of a wide variety of non-host plants and the possibility of using them as a commercial root stimulant has been proposed (45).

CONSTRAINTS ON THE COMMERCIAL USE OF MYCORRHIZAL FUNGI

The current major obstacles to the commercial use of mycorrhizal fungi are:

- the lack of large-scale field experiments under normal agricultural conditions,
- the lack of cost-benefit analysis to determine the economics of mycorrhizal applications, and
- the trend toward excessive fertilization to substitute for the lack of mycorrhizal fungi.

Perhaps the most important deterrent of commercial use of mycorrhizal fungi is the lack of large-scale field tests in a variety of agricultural soils and locations. The program initiated by D. H. Marx and the U.S. Forest Service will correct this deficiency for ectomycorrhizal fungi and within 4 years it will be known if these mycorrhizal fungi will indeed be economically feasible to use on a wide scale in the production of forest trees.

This type of program remains to be established for VA mycorrhizal fungi. Without such data it is difficult to establish a potential market for mycorrhizal inoculum. Without a market there is little incentive for industry to initiate the production of commercial inoculum. Without commercial inoculum it is difficult to carry out large-scale field tests. With the recent establishment of several commercial sources of mycorrhizal inoculum perhaps this cycle will be broken and more field tests will result.

Once large-scale field tests are seen to be successful, light-weight commercial mycorrhizal formulations will develop and new application methods will be devised, Most importantly, from large-scale field tests, cost benefit analysis can be accurately done to determine the economic benefit derived from the use of mycorrhizal fungi. In the end, this will be the determining factor in the commercial application of mycorrhizal fungi. Biological scientists are rarely able to critically assess the economic factors involved in the application of a new technique and I recommend that agricultural economists should be asked to participate in the cost-benefit assessment of VA mycorrhizal inoculation.

Heavy phosphorus fertilization severely inhibits mycorrhizal infections (17,68). More recently, it is becoming evident that heavy nitrogen and zinc applications are also inhibitory to mycorrhizal fungi (32,51). Daily applications of 100 ppm nitrogen under greenhouse conditions have been shown to completely eliminate mycorrhizal infections (J.A. Menge, unpublished data). Many commercial greenhouses add over 200 ppm nitrogen daily to their plants. In greenhouse and fumigated nursery conditions, growers are using excessive fertilization to substitute for the lack of mycorrhizal fungi. Under these conditions, not only do mycorrhizal fungi not benefit their host plants, but it is difficult to successfully establish mycorrhizal infections so that the plants will be mycorrhizal once they leave the supraoptimal fertility regime. As long as fertilizer is relatively available and not excessively expensive, it will take a major educational program to convince many growers to change their standard operating procedures and use mycorrhizal fungi that will not only be cheaper but will conserve fertilizer and energy.

In my opinion, granting agencies such as the National Science Foundation, Rockefeller Foundation, USDA competitive grants, and the Israeli-U.S. granting agency BARD have effectively provided adequate funding for basic mycorrhizal research. The number of scientific papers on mycorrhizal fungi has quadrupled since 1960, which is evidence that there is great interest and money available for basic mycorrhizal research. However, there are few agencies that will fund the final applied steps in a biological commercialization project. Research money for large-scale “applied” or “demonstration” experiments is unavailable. Funding for small-scale pilot projects is also not available. It remains for private industry to pick up the projects from this point, but they have been reluctant to do so. The transition is not going smoothly and seems to be proceeding slowly if at all,

EFFECTS OF MYCORRHIZAL FUNGI ON AGRICULTURE

It is very difficult for a scientist to speculate on the effects of a new procedure on the social and economic structure of an agricultural society. Frequently good ideas do not receive the acclaim they deserve because of prejudices, ignorance, religious preferences, social mores, and other reasons not fully understood by scientists. In my opinion, the effects of mycorrhizal technology would most alter the socioeconomic structure in areas of intensive agriculture. These situations would be more prevalent in agriculture in developed nations. Mycorrhizal fungi are most useful in reclaiming sites disturbed by heavily mechanized industries or soil fumigation. Mycorrhizal fungi can reduce energy and fertilizer and increase the efficiency of crops grown intensively. Therefore, mycorrhizal fungi can be viewed as conservation measures or as substitutes for high energy uses in developed nations.

In less developed nations, growers would have to be educated to the methods of producing, handling, and inoculating living microorganisms. This may be difficult. In countries with a less well-developed agricultural system, mycorrhizal fungi have not been altered and are probably functioning effectively and need not be applied under such conditions. Fertilizer in most underdeveloped countries is probably applied sparingly as manure and therefore mycorrhizal fungi will not result in a great savings either of fertilizer or energy.

If superior strains of mycorrhizal fungi are developed, marginal agricultural land could be made productive. Huge amounts of marginal agricultural land exists in Africa and South America and the proper use of this land may well decide the future of some countries. Increased use of agricultural land will provide for a greater economic base, larger agricultural productivity, and a better way of life for large populations in underdeveloped countries. Educating agriculturists to the importance of mycorrhizal fungi may allow developing countries to avoid the excessive use of energy, fumigants, and fertilizers associated with intensive agriculture.

CONCLUSIONS AND RECOMMENDATIONS

Mycorrhizal fungi may be one alternative that can immediately improve revegetation of disturbed sites, increase crop growth in fumigated soils and greenhouses, and yet reduce fertilizer costs and energy demands. If superior strains of mycorrhizal fungi were developed, they could potentially improve growth of nearly all agronomic crops in a wide variety of soils throughout the world. Both ectomycorrhizal fungi and vesicular-arbuscular mycorrhizal fungi are in commercial production on a small scale. The greatest obstacles to the commercialization of mycorrhizal fungi appears to be: 1) the lack of large-scale field tests under typical agricultural conditions in a variety of locations; 2) adequate cost-benefit analysis to determine the economics of the utilization of mycorrhizal fungi; and 3) a reluctance on the part of growers to switch from an energy dependent, heavy fertilizer system to a new, but cheaper, energy conservative system using mycorrhizal fungi.

Recommendations that could substantially increase the commercial use of’ mycorrhizal fungi (in relative order of importance) are as follows:

1. Improved availability of grant funds for large-scale field applications of mycorrhizal fungi in a wide variety of soils throughout the world. It would be useful to establish several pilot projects in various less developed countries. These pilot projects could produce and distribute mycorrhizal inoculum on a variety of crops growing under different soil conditions. Cost-benefit analysis on such projects could adequately assess the economics of inoculation with mycorrhizal fungi.

2. Funds should be made available to create a worldwide bank of beneficial mycorrhizal fungi. The establishment of such a facility is being investigated by the mycorrhizal community and the National Science Foundation has agreed to entertain a proposal for such a facility. The University of Florida has agreed to supply the facilities as well as substantial operating costs for such an establishment. A second idea would be to add the responsibility for maintaining mycorrhizal cultures to the already established government facility called the American Type Culture Collection which maintains many important fungal cultures.
3. It would be desirable to establish a USDA-supported vesicular-arbuscular mycorrhizal research center that would be responsible for maintaining and coordinating U.S. research on mycorrhizal fungi. This facility would complement the Mycorrhizal Institute in Athens, Georgia, which was created by the Forest Service to coordinate mycorrhizal research on forest trees.
4. A world survey should be conducted to collect and test as many different vesicular-arbuscular mycorrhizal species as possible. The discovery of a superior mycorrhizal strain with a wide host range could tremendously increase agricultural productivity throughout the world.
5. Research is necessary to elucidate the exact role of mycorrhizal fungi play in improving plant growth under stress conditions such as drought, salt, toxic soil materials, or in marginal agricultural lands.
6. Research is necessary to elucidate the genetics of mycorrhizal fungi. Virtually nothing is known on this subject. The ability to breed these organisms could result in tremendously increased agricultural productivity.
7. Efforts should be intensified to grow vesicular-arbuscular mycorrhizal fungi in the laboratory using artificial media. A breakthrough in this area could improve the feasibility of attaining all of the above recommendations, However, since scientists have been trying to artificially culture VA mycorrhizal fungi since 1900, this objective may be difficult to achieve and incentives to work on such a problem are difficult to justify.

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