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CHARLIE PARKERSON: If you were applying 200 and 400 ppm of N twice a week did you take soluble salt readings, and what were they?

JIM KLETT: I measured soluble salts at the end of the experiment; using the soil-paste method. They were all 15+ which is very high.

MYCORRHIZAE AND PLANT GROWTH

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It has long been assumed that soil borne fungi adversely affect nursery crops. Stem and root rots, dampening off, etc., are common fungal problems to the nurseryman. However, there are groups of soil-borne fungal organisms which are beneficial to plants. Mycorrhizal fungi are capable of forming a symbiotic relationship with plant roots. This plant-fungal association is called mycorrhiza and literally means "fungus root"; *myco* meaning fungus and *rhiza* meaning root. The coexistence established between the root and fungus is generally beneficial to both organisms. However, there are exceptions or variations to this general definition ranging from fungal parasitism to total dependence of the plant on the mycorrhizal fungus. Mycorrhizal fungi can also exhibit specificity ranging from many plant-host associations to a single plant-host. They are naturally occuring fungi and 80 to 90% of all plants are reported to have a mycorrhizal association(s).

There is overwhelming evidence that many plants, including some of our most important nursery crops, could not survive without mycorrhizae. Most mycorrhizal associations occur naturally, and with a few exceptions, the nurseryman is quite often unaware of existing mycorrhizal benefits. Slow growth or poor field survival of a particular plant is often assumed to be characteristic or attributed to poor cultural practices rather than to the absence of mycorrhizal fungi.

Benefits of a mycorrhizal fungus can be species specific. A classic example is *Rhizoctonia* spp. which are beneficial fungi to orchids, but are serious pathogens on other hosts. In addition, there is evidence that mycorrhizal fungi are ecologically

adapted to specific soil environments. The ectomycorrhizal fungus *Thelephora terrestris* Ehrh. ex. Fr. occurs naturally in conifer forests of the Southeast. Consequently, conifer seedlings produced in the Southeast are generally infected with *T. terrestris* from spores blown into seedbeds. However, when *T. terrestris* mycorrhizal conifer seedlings were outplanted onto a drastically disturbed site in Kentucky, only 2% survived compared to nearly 50% survival for conifer seedlings infected by the ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker and Couch (4).

Mycorrhizal types. Mycorrhizae can be divided into three classes; ecto-, endo- and ectendo- mycorrhizae. Ectomycorrhizae occur on feeder roots of both gymnosperms (especially *Pinaceae*) and certain angiosperms (willows, oaks, birches, hickories, walnuts). Ectomycorrhizae are characterized by swollen short roots and specific branching characteristics ranging from monopodical (nonforked) to forked or even multiforked (ramiform) configurations. Ectomycorrhizae may exhibit more than one type of branching pattern on the same plant. I have found monopodial, "Y" shaped, and ramiform branching patterns on *Pisolithus tinctorius* mycorrhizae of yellow birch (*Betula alleghaniensis*) seedlings. Ectomycorrhizal roots can vary in length from 1 to 2 mm, as on many species of pine, up to 10 mm or more.

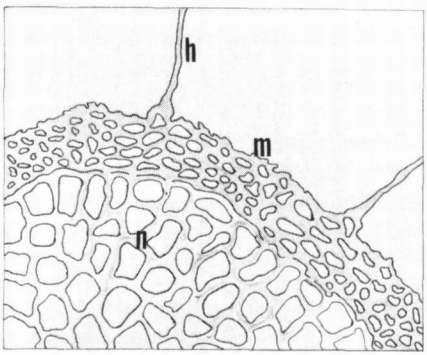

Figure 1. Diagram of root cross section with ectomycorrhizal infection. m, fungal mantle; n, Hartig net in cortex; h, hyphal strand.

Root hairs are lacking in ectomycorrhizae and have been replaced with profuse development of fungal hyphae which may or may not completely surround the entire feeder root forming a sheath or mantle (Figure 1). This fungal mantle prevents feeder roots from coming into immediate contact with the surrounding soil and imparts the mycorrhizal root a characteristic color ranging from white, brown, yellow, black, blue or combinations thereof. These fungal or hyphal strands radiate

outward from the root surfaces, distances of 15 to 20 ft or more. The hyphal strands appear to substitute for root hairs increasing the root surface area and function in water and nutrient uptake. Fungal strands also penetrate internally into the root cortical region forming a network of intercellular hyphal strands. These interconnecting fungal strands are collectively referred to as a Hartig-net and appear to replace the middle lamella, a pectin-like material which cements the root cells together. Hartig-net development in all mycorrhizal infections is restricted to the cortical region.

Ectomycorrhizal fungi can produce aerial fruiting bodies which release billions of wind blown spores. The fruiting structures are often called "puffballs" since large clouds of spores are released when mature fruiting bodies are opened. Aerial spore disemination greatly enhances the spread of ectomycorrhizal fungi in nursery soils.

Endomycorrhizae are found on many agronomic and nursery crops. Their presence may go undetected since only a loose network of hyphal strands may appear on the surface of feeder roots. Hyphal strands of endomycorrhizae generally penetrate through the epidermis into cortical cells, hence the prefix endo- is used (Figure 2). These penetrating hyphal strands can coil around forming shrub-like structures called arbuscules and/or produce thin-wall, spherical to oval structures called vesicles. Thick-walled spores are also produced on or near the root surface or inside cortical cells. Generally, endomycorrhizal fungi do not drastically modify the morphological features of a root as do ectomycorrhizal fungi. Endomycorrhizal fungi generally do not produce aerial fruiting bodies, consequently, their dissemination in nursery soils is more restricted than ectomycorrhizal fungi.

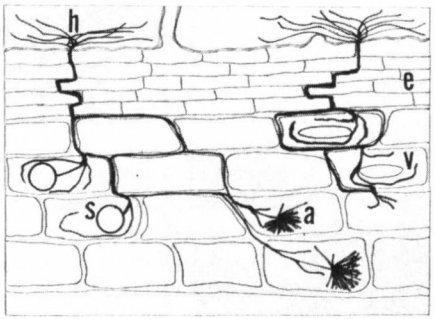

Figure 2. Diagram of root longitudinal section with ectomycorrhizal infection. h, hyphal strands; s, spores; a, arbuscules; v, vesicles; e, epidermal region.

A third class of mycorrhizae found on pine species has been classified as an ectendo-type. The taxonomic classification of ectendo-mycorrhizae is still in doubt, however, they appear to exhibit characteristics of both ecto- and endomycorrhizae (8). Ectendomycorrhizae may or may not develop a fungal mantle, but both inter- and intracellular hyphal strands occur in the cortical region of the root (Figure 3). Very little is known about the species of fungi involved. They have been found in nursery soils and appear to be associated with species which are normally ectomycorrhizal plants (7).

Mycorrhizal fungi have been associated with increased plant growth, rooting of cuttings, nutrient and water uptake, and disease resistance (1,3,5,6). The remainder of this paper is a preliminary report on the effects of the ectomycorrhizal fungus *Pisolithus tinctorius* on swamp chestnut oak (*Quercus prinus*).

Many oaks species are characteristically slow growing. Although growth of oak seedlings has been accelerated by repeated fertilizer applications, the added production costs can often outweigh the benefits. Cultural techniques which increase height growth of oak seedlings while reducing production costs would be a substantial benefit to the nursery industry. Tailoring oak seedlings grown in fumigated seedbeds with a specific mycorrhizal fungus may be a possible mechanism of increasing plant height growth. The objective of this study was to determine the interaction of the mycorrhizal fungus *P. tinctorius* with swamp chestnut oak seedlings.

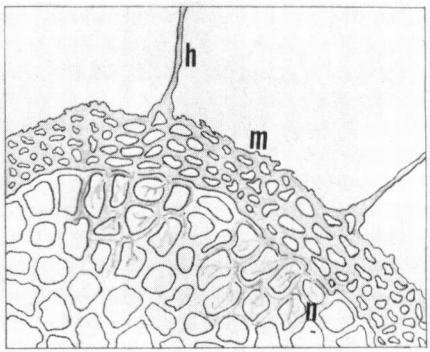

Figure 3. Diagram of root cross section showing ectendomycorrhizal infection and penetration of hyphal strands into cortical cells. m, fungal mantle; n, Hartig net; h, hyphal strand.

MATERIALS AND METHODS

In the fall of 1976, swamp chestnut oak seed was collected and immediately planted in 10 cm pots containing sterilized

Weblite.[1] Seedlings were grown for 4 months at prevailing greenhouse temperatures (20 to 25°C day/20° night), and an extended photoperiod of 18 hours. Seedlings were fertilized daily with a 15-15-15 water soluble fertilizer at a rate of 200 ppm N and once a week with a Hoaglands micronutrient solution (2).

An isolate of *P. tinctorius* was obtained from sporocarp tissue following procedures of Miller.[2] The isolate was subcultured in 9 cm Petri plates on a modified Melin-Norkrans agar medium (6). After 3 to 4 weeks of dark incubation at 21°C ± 2°C, 8 mm discs were removed from the periphery of fungal colonies and used for mass innoculation of 3.75 liter jars. Mass production of mycorrhizal inoculum using a peat-vermiculite substrate was accomplished following the procedures of Marx (5).

After 3 months the *P. tinctorius* inoculum was removed from the jars, passed through a 5 mm mesh screen, collected in cheesecloth, and leached with 8 liters of cool tap water. Excessive water was squeezed from the inoculum. The inoculum was placed in plastic bags, and stored at 3°C for 24 hours before use.

Twenty uniform swamp chestnut oak seedlings were repotted into 15 cm pots containing a 1:10 (V/V) mixture of *P. tinctorius* inoculum and sterilized Weblite. No additional fertilizer was added after repotting. All seedlings were grown in the greenhouse as previously described. Initial and final height growth measurements were recorded and seedlings were inspected monthly for mycorrhizal formation.

RESULTS

Visible mycorrhizal formation occurred between the third and fourth month after seedling inoculation. I believe that this is the first report of *P. tinctorius* mycorrhizal formation on swamp chestnut oak. However, only 9 of the original 20 inoculated seedlings exhibited mycorrhizal development at the last inspection date, November 30, 1977. By the fifth month, hyphal strands penetrated the entire container medium of infected seedlings. Sporocarp production also occurred within 5 months in 6 of the 9 containers with mycorrhizal plants.

The mycorrhizal fungus was responsible in nearly doubling plant height growth. The total average increase in plant height growth of mycorrhizal plants was 306% compared to 164% for nonmycorrhizal plants (Table 1).

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[1] Weblite is a soil-less commercial nursery mix manufactured by Weblite Corporation, P.O. Box 12887, Roanoke, VA 24029.

[2] Personal communication from O.K. Miller, Dept. of Botany, Va. Poly. Inst., Blacksburg, VA.

Table 1. Effect of *Pisolithus tinctorius* on height growth of swamp chestnut oak 5 months after inoculation.[y]

	Average % increase in plant height[z]	Range of % increase in plant height
Infected	306	203 - 397
Noninfected	164	131 - 185

[y] Seedlings inoculated when 4 months old.
[z] Significant at 1% level within column.

Mycorrhizae on swamp chestnut oak completely changed root morphology and was characterized by both profuse monopodial and short ramiform and branching patterns (Figure 4, 5). The fungal mantle on ramiform mycorrhizal roots completely covered the entire root, whereas the fungal mantle on monopodial mycorrhizal roots was restricted to the apical, non-suberized portion of long roots. Ramiform short roots were between 0.3 and 0.8 mm in length. Root hairs were absent on mycorrhizal roots. In addition, all mycorrhizal roots were 2 to 3 times larger in diameter than nonmycorrhizal roots.

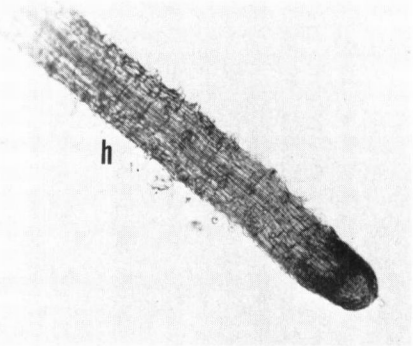

Figure 4. Nonmycorrhizal root tip of swamp chestnut oak. h, root hairs. 100X.

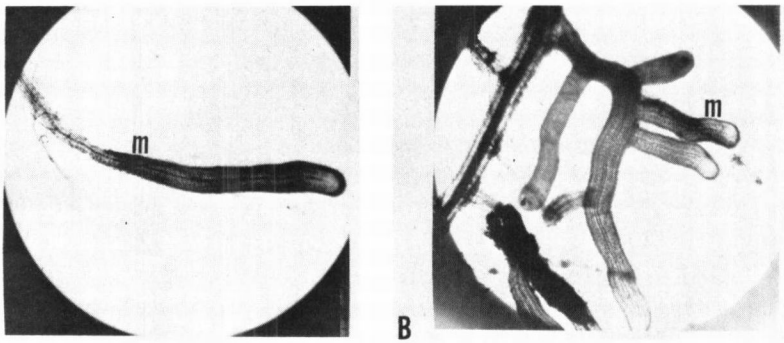

Figure 5. Mycorrhizal branching patterns of swamp chestnut oak. A, monopodial; B, ramiform. Note the presence of the fungal mantle (m) completely covering the root tip in A and the entire ramiform branched root in B. 60X.

DISCUSSION AND CONCLUSION

Plant production is becoming an exacting science with constant changes occurring in supplies, governmental regulations, financing, marketing strategies, as well as cultural practices. In recent years, new cultural practices have sought to change the "natural biological system" in order to increase plant production. Advancements in nursery production practices such as, large scale field fumigation and use of soil-less propagation and container media have eliminated many soil-borne pathogens as well as beneficial mycorrhizal fungi. Reintroduction of specific mycorrhizal fungi into sterile nursery soils or container media may be a functional method of increasing plant growth. In order to maximize mycorrhizal infections and potential benefits, the interaction of mycorrhizal fungi with individual nursery crops and production practices such as fertilization, irrigation, pest control, container media, etc. must be thoroughly investigated.

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MARTIN MEYER: How do you inoculate your plants with mycorrhizae?

DALE MARONEK: We have been trying several different methods. The ectomycorrhizae produce spores in puff balls. Using spores we get a very low percent of infection. We are now growing our inoculum in culture jars in the lab following the procedures worked out by the Southeastern Forest Experiment Station. This provides vegetative mycelia with which we

can inoculate the container media at a ratio of 1 part inoculum to 1 part container mix. For endo-mycorrhizae, the mycelium is grown on roots of an alternate host such as sorghum, we grind up the sorghum plant, take a spore count and use that as the inoculum.

JIM CROSS: Have you run any experiments where the container mix has an optimum or high level of nutrients in it before inoculation with the mycorrhizae?

DALE MARONEK: We feel that one advantage of mycorrhizae is that you don't have to use as high a level of fertilizer. In some cases, high fertilization rates seem to suppress the development of mycorrhizae, in other cases too little fertilizer will also restrict development of the fungi.

BRUCE BRIGGS: Do you have any idea of how the mycorrhizae influence root initiation?

DALE MARONEK: Fungi in general are known to produce plant hormones. Many produce auxins and we are currently looking at the production of cytokinins that are produces from some of the mycorrhizae that we are working with. Not all produce the cytokinins but some do.

BILL ALMY: Are they affected by fungicides normally used in nurseries?

DALE MARONEK: Some are and some are not. We have been trying to provide small amounts of fungicides to some of the mycorrhizae to condition them to this situation and prevent their complete destruction if this fungicide is applied to the plants growing in the soil.

VOICE: How fast does the mycorrhizae move through the soil?

DALE MARONEK: After 2 years on a plot that is 25 feet square we can find mycorrhizae totally permeating the corner areas of the plot. We feel it could easily move 5 to 10 feet per year.

CHARLIE PARKERSON: Isn't that only in the presence of a root?

DALE MARONEK: Yes, the roots of a host plant must be present in the soil but the hyphae may extend 20-30 ft from the host root.