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I am submitting herewith a thesis written by Victor Lavann Ford entitled "Endomycorrhizal Infection of Containerized Yellow-Poplar Seedlings." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

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ENDOMYCORRHIZAL INFECTION OF CONTAINERIZED YELLOW-POPLAR SEEDLINGS

A Thesis

Presented for the

Master of Science

Degree

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Victor Lavann Ford

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ABSTRACT

Yellow-poplar, an important hardwood in the Southern Appalachians, can be infected with the endomycorrhizal fungi, *Glomus masseae* (Nicol. and Gerede.) Gerdemann and Trappe and *Glomus fasuculatus* (Thaxter) Gerdemann and Trappe. Objectives were to determine (1) the effects of various mycorrhizal inocula on seedling growth, (2) the developmental patterns of seedling root infection and corresponding seedling growth, (3) a correlation between fungal structures and seedling growth rates (4) the technology of seedling propagation in Spencer-Lemaire rootrainers.

Sterilized growth medium in rootrainers was amended with inocula of *Glomus mosseae, Glomus fasciculatus, Glomus masseae* plus *Glomus fasciculatus*, soil from a natural yellow-poplar stand, or no inocula at all. Two to three week old yellow-poplar seedlings were planted in the respective media in rootrainers and grown in greenhouse culture. The plants were harvested at 3, 5, 7, 9, 12 and 16 weeks after planting and measured for root length, shoot length, stem diameter at the root collar and oven-dry weight. Total length of the plant and root to shoot ratio was also analyzed. The roots were preserved and later stained to quantify the amount of free hyphae, arbuscules, vesicles and hyphal coiling. All seedling roots from mycorrhizal treatments had fungal structures developing. No fungal structures were found in the uninoculated controls.

Seedlings exposed to the uninoculated medium had the lowest growth rate for all growth variables except root length. The seedlings exposed to the natural inoculum were intermediate between the control

seedlings and those exposed to the mixture of *Glomus mosseae* and *Glomus fasciculatus*. The seedlings exposed to the single inocula of *Glomus mosseae* and *Glomus fasciculatus* were the largest. There appeared to be some interaction between the endophytes when mixed in treatments, thereby leading to a loss of efficiency in the mycorrhizal relationship.

Each acceleration growth corresponded to an increase of all fungal structures except vesicles. Vesicles were most abundant in the natural inoculum seedlings. There were no statistically significant correlations between any fungal structure and any growth variable.

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CHAPTER I

INTRODUCTION

Yellow-poplar (*Liriodendron tulipifera* L.) is a common and important timber species on fertile, moist sites in the Southern Appalachians. This species is particularly abundant in moist, well-drained, loose textured soils. It does not favor excessively dry sites (Olson, 1969). Using a series of pot culture experiments, Clark (1964) demonstrated that yellow-poplar did not favor old field or compacted soils. In order to maintain good growth rates and form, adequate soil moisture, nutrients and favorable soil texture must be maintained (Buckner, 1972); slope, aspect and elevation are environmental factors that determine the position of yellow-poplar in natural stands (Autch, 1935; Minckler, 1941).

Yellow-poplar is a valuable species because its silvical characteristics facilitate silvicultural manipulation, it has straight growth habit, and the wood has qualities that make it suitable and valuable for pulp, veneer, lumber and composite board. The importance of yellow-poplar in reforestation programs can only increase as the technology develops to increase the success of outplanting of this species.

Successionally pure yellow-poplar stands are temporary. The species is a pioneer on sites disturbed by agriculture, clearcutting, fire and other natural disturbances such as disease and insects. Natural regeneration is assured with adequate moisture and seed supplies

(Renshaw and Doolittle, 1958). Yellow-poplar will outgrow competing species rapidly as seedlings and saplings, therefore its intolerance to shade is not a problem. Yellow-poplar is unique among eastern hardwoods in being both a member of the climax forest and a pioneer on good sites. Buckner and McCraken (1978) stated that windthrow of mature trees was a primary mechanism of opening the canopy enough to allow the species to reproduce itself in a climax forest. Once established, the species is dominant in the immediate stand and grows until it becomes established as a dominate species in the mature canopy.

High quality sites available for reforestation are scarce. These sites are maintained in agricultural crop production or existing forest communities. Much of the land available for reforestation has been disturbed, primarily by agriculture, strip mines or fire. These disturbed lands are marginal for successful survival and growth for yellow-poplar due to the exposed subsoil (Loftus, 1971 and 1974). Stands disturbed by insect epidemics do not have the added problems of soil distrubance. Usually there is a stress factor that affects the physiology of the tree leading to the lowered resistance to insect attack. Belanger, et al. (1977) found that southern pine beetle (*Dendroctonus frontalis* Zimm.) attacked pines that were in moisture stress and were growing slowly in heavily stocked stands. In the Southern Appalachians, the southern pine beetle becomes epidemic on native Virginia and shortleaf pines every 5 to 9 years (Anderson, 1960).

Utilizing bark beetle sites for reforestation with yellow-poplar represents two advantages. The probability of yellow-poplar survival on

these sites is good because the sites were not disturbed as much as a strip mine or an abandoned field. Secondly, the presence of an angiosperm on such a site would eliminate the future affects of the southern pine beetle. However, past attempts at planting yellow-poplar on old field sites or these minimally disturbed sites has proved to be unsuccessful in too many cases (Loftus, 1974). Technology must be developed to increase the survival and subsequent growth of yellow-poplar on adverse sites if it is to compete successfully with the pine as a preferred species for out planting.

Mycorrhizae presents some definite advantages to seedlings on adverse sites for several reasons (Harley, 1969). Seedling vigor is enhanced through increased root absorptive capacity; Bowen et al. (1974) found that mycorrhizal roots on *Pinus radiata* and *Araucaria cunninghamii* absorbed more zinc and phosphorus on adverse sites than nonmycorrhizal plants. The possibility of preferential absorption rates for other nutrients also exist. Increased absorptive capacity has been suggested as a means of absorbing more water and thereby creating greater osmotic potential through mycorrhizal associations (Uhlig, 1973). Secondly, mycorrhizal fungi on seedling roots minimize the potential invasion by root pathogenic fungi, probably through increased antibiotics, changes in the rhizosphere compostion and establishing a mechanical barrier minimizing pathogen contact with the root (Sinclair and Stack, 1975; Marx, 1972).

Marx (1975) obtained significantly higher survival and growth rates with various species of pine infected with mycorrhizae when

outplanted on strip-mines. Other plants have also been helped by mycorrhizal infections (Daft, 1975), for example, four-wing saltbush, *Altriplex canescens* (Pursh) Nutt., (Aldon, 1975); and red maple, *Acer rubrum* L., (Daft and Hacskylo, 1977). The theme of reforestation on adverse sites using mycorrhizae has taken a new prospective by using containerized seedlings and an appropriate mycorrhizal fungus to taylor a seedling for outplanting on adverse sites (Marx and Barnett, 1974).

Statement of Objectives

These experiments were designed to accomplish the following objectives:

1. To determine the effects of various types of fungal inocula on seedling survival and growth. Inocula used were cultures of *Glomus mosseae* (Nicol. and Gerde.) Gerdeman and Trappe, *Glomus fasciculatus* (Thaxter) Gerdemann and Trappe, and natural inoculum from a yellow-poplar stand at the University of Tennessee arboretum in Oak Ridge, Tennessee.
2. To determine the developmental patterns of seedling root infection and corresponding seedling growth. Sequential analysis of root tissue correlated with seedling growth was used to determine the effect of infection on seedling growth.
3. To correlate different fungal structures with seedling growth stages. The presence of hyphae, vesicles and arbuscles were correlated with seedling growth.

4. To determine the technology of yellow-poplar seedling propagation in Spencer-Lemaire roottrainers.

CHAPTER II

LITERATURE REVIEW

Description of Mycorrhizae

Definition and Types. Mycorrhizae are compound organs made up of a seed plant and a fungal hyphae arranged in an orderly fashion (Boyce, 1961). Three types of mycorrhizae are distinguished by the presence of a fungal sheath or if the hyphae penetrates the cortical cell wall of the root. The types are ectomycorrhizae, endomycorrhizae, and entendomycorrhizae (Harley, 1968).

Ectomycorrhizae occur naturally on many important forest tree families in the world, e.g., *Pinaceae, Fagaceae, Betulaceae, and Salicaceae.* Infection is initiated from spores or hyphae of the fungal symbionts inhabiting the rhizosphere of the feeder roots. Spores and hyphae are stimulated to infect the root by root exudates. The hyphae then grow over the root surface and form the characteristic ectomycorrhizal mantle. Subsequent to mantle development, hyphae penetrate intercellularly through the cortical cells, at times completely replacing the middle lamella to form the Hartig net (Marx, 1975). Ectomycorrhizal fungi include *Gastromycetes, Hymenomycetes and Tuberales.* There are approximately 2100 species of fungi in North America which can form ectomycorrhizae (Marx, 1975).

Endomycorrhizae are formed on most important agronomic crops and many forest genera, e.g., *Acer, Ulmus, Nyssa, Prunus, Liquidambar, Plantanus, Juglans and Fraxinus.* Never forming a mantle or Hartig net,

endomycorrhizae are characterized by a loose network of hyphae extending into the soil from the feeder-root surfaces. The absence of a mantle coupled with the infection producing slight visible morophological change in the root makes determination of endomycorrhizal infection with the unaided eye difficult. Hyphae of endomycorrhizal fungi penetrate the cell walls and progress within the cortical cells of feeder-roots. These infective hyphae may develop specialized nutrient exchanging, haustoria-like structures called arbuscules, which are produced within the cytoplasm of the cortical cells (Figure 1). Vesicles are thin-walled, spherical to ovate structures formed in the cortical cells (Figure 2). Although their function is not exactly understood, it is thought that they are storage organs (Nicolson, 1967). The term "vesicular-arbuscular mycorrhizae" (VAM) differentiates this type of endomycorrhizae from the septate endomycorrhizae of *Orchidaceae* and *Ericales* (Harley, 1968), which do not form these structures. VAM fungi produce large conspicuous spores on the root surface, in the rhizosphere and sometimes in the root tissue. They do not produce a large above ground fruiting body, as most ectomycorrhizal fungi, but they produce single spores of sporocarps containing chlamydospores and/or zygospores produced below the ground. These fungi are dispersed by growing from feeder-root to feeder-root, by moving water and soil, by insects and by animals (Nicolson, 1967).

Taxonomy of the Endogonaceae. VAM fungi belong to the class *Zygomycetes*, the order *Mucorales*, and the family *Endogonaceae*. In the past, all aseptate fungi were placed under the large heterogenous class of *Phycomycetes*. Recently, this large class was deleted and its members

Figure 1. An arbuscle within a cortical cell of a sectioned yellow-poplar root which was embedded in paraffin and stained with methylene blue. Magnification 360 X.



Figure 1

Figure 2. A vesicle within a cortical cell of a whole, cleared yellow-poplar root stained with trypan blue. Magnification 360 X.

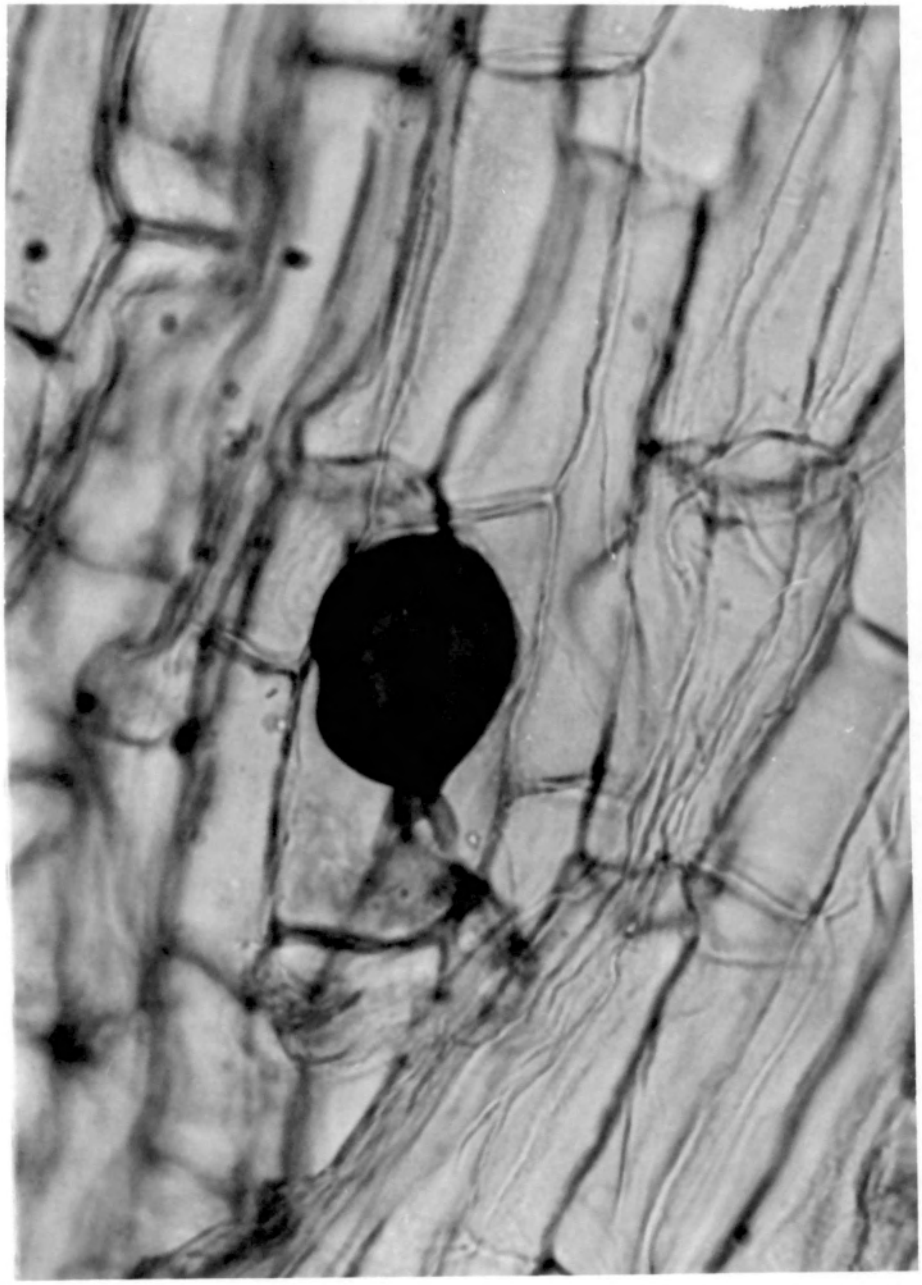


Figure 2

were placed into the *Chytridiomycetes, Hyphochytridiomycetes, Oomycetes,* or *Zygomycetes*. Endogonaceae were placed into the *Zygomycetes* because when sexual union takes place, the gametangia are somewhat equal in shape and size and the resulting union produces a zygospore. The family is *Mucorales* because some members of the family produce sporangia-like structures (Alexopoulous, 1962).

Initally almost all species of the *Endogonaceae* were placed in the large genus *Endogone*. The result was total taxonomic confusion, but as knowledge was accumulated, Gerdemann and Trappe (1974a) divided *Endogonaceae* into seven genera (Table 1). The critera for division were fruiting aggregation, kinds of spores, spore germination and type of mycorrhizae. This classification is flexible enough to accommodate more information on fungal life cycles, biological and chemical relationships.

Characteristics of Glomus. *Glomus* chlamydospores are borne on undifferentiated, nongametangial hyphae. Spores are usually terminal, both inside and outside the host plant, and at times there can be more than one basal attachment to the hyphae. The spore walls are single or double and mature sproes contain oil droplets of variable size. At maturity, the spore contents are separated from attached hyphae by a septum or occluded by spore wall thickening (Gerdemann and Trappe, 1977a). They are all hypogeous and can be collected by wet-sieving and decanting (Gerdemann and Nicolson, 1963), using a truffle fork (Gerdemann and Trappe, 1974b), and isolation in "inoculated pot cultures." Zygospores and sporangia associated with *Glomus* have not been found.

Table 1. Genera of *Endogonaceae*

Genera	Fruiting	Kind of Spores	Spore Germination	Type of Mycorrhizae
Endogone	Sporocarps	Zygospores	Unknown	Ectomycorrhiza or unknown
Gigaspora	Single spores	Azygospores?	Through wall	Arbuscular
Acaulospora	Single spores	Azygospores?	Through wall	Vesicular-arbuscular
Glomus	Sporocarps and single spores	Chlamydospores	Regrowth of attached hyphae	Vesicular-arbuscular
Sclerocystis	Sporocarps	Chlamydospores	Unknown	Vesicular-arbuscular
Glaziella	Sporocarps	Chlamydospores	Unknown	Unknown
Modicella	Sporocarps	Sporangiospores	Through wall	Unknown

Source: J. W. Gerdemann and J. M. Trappe, Taxonomy of the Endogonaceae; *Endomycorrhizas*, 1975.

Benefits from Endomycorrhizae

Need for endomycorrhizae. Whether the fungus or the plant benefits most from the relationship has been argued, but in recent years it has been summarized that both receive benefits and the relationship can be called mutually symbiotic (Harley, 1968). Pirozyniski and Mallock (1975) have hypothesized that evolution of terrestrial plants was made possible through a lichen relationship. Baylis (1970), using New Zealand forest species, summarized that mycorrhizae are needed because of a lack of roothairs, but this is not the case. Ninety-five percent of all plants are mycorrhizal and they will form roothairs in sterile soil in the absence of the fungus. If given enough nutrients, the plants in sterile soil will do quite well (Kormanik, *et al.*, 1977). The mycorrhizal relationship is important in natural conditions where a limiting amount of available nutrients is present. The fungus can exploit nutrients, both organic and inorganic scources, not otherwise available to the root (Noggle and Fritz, 1976). Improved growth of plants with VAM has been obtained in soil, in peat mixed with sand, in sand and in water culture, (Mosse, 1973). Kleinschmidt and Gerdemann (1972) found that citrus seedlings grown in fumigated nursery soils were chlorotic and stunted, but after inoculation with *Glomus mosseae,* the seedlings grew normally. Marx, *et al.* (1971) found similar results with the same species of fungus with several other citrus species. *Liquidambar, Allium, Fragaria, Zea, Trifolium* and *Glycine* infected with *Glomus mosseae* in sterilized soil grew better than the same species in just sterilized soil (Mosse, *et al.*, 1969).

Sweetgum grown in nursery. Sweetgum (*Liquidambar styraciflua* L.) is an economic forest tree species native to the southeastern United States and one species which responds well to mycorrhizal infection. After a marked beneficial effect of mycorrhizal infection on growth and survival of nursery grown sweetgum seedlings was observed, Bryan and Ruehle (1976) concluded that the mycorrhizal association was required for optimum growth. Dependence of sweetgum on mycorrhizae may be the cause of past failure with sweetgum in fumigated nursery beds, ultimately requiring a second growing season before these seedlings can be planted. Bryan and Kormanik (1977a) produced 1-0 seedlings as large as standard 2-0 seedlings using *G. mosseae* as the endophyte. They suggested that after fumigation, a cover crop of inoculated sorghum, *Sorghum vulgare* var. *roxburghi* (Stepf.) Haines, be cultivated to build up the endophyte population before seeding sweetgum. High fertility levels and genetically improved seedlings cannot overcome the symbiotic effect of mycorrhizae on sweetgum (Kormanik, *et al.*, 1977b).

Phosphorus uptake. Gerdemann (1964) first suggested that improved growth of plants inoculated with VAM might be due to increased uptake of phosphorous. This has lead to subsequent research in which various techniques, such as varying the amount and form of phosphorus, radioactive tracers and gel electrophoresis, have been applied to determine the extent of and physiology of phosphorus transfer from fungus to plant. Yellow-poplar and sweetgum inoculated with *Glomus fasciculatus* absorbed significantly more P-32 than uninoculated controls (Gray and Gerdemann, 1967). Hattingh, *et al.* (1973), using the same isotope on onion

inoculated with *G. mosseae* or *G. fasciculatus*, found that there was no difference in phosphorus absorbed between fungal treatments, but controls had significantly lower levels of the nutrient. They also found that if the extra-matrical hyphae were severed from the root, the roots behaved as if they were nonmycorrhizal. Azcon, *et al.* (1976) found that bacteria in conjunction with endomycorrhizae on onion (*Allium* cepa L.) improved phosphorus uptake in soils where phosphorus was supplied by insoluble rock phosphate. Using gel electrophoresis Ginaninazzi-Pearson and Ginaninazzi (1976) found indications of a new enzyme associated with inoculated onions, but it was absent in the uninoculated controls. It was presumed that this enzyme was associated with phosphorus uptake and transfer.

Other nutrient uptake. There has been little work done with potassium, nitrogen and the micronutrients concerning mycorrhizae. Ho and Trappe (1975) found that species of *Glomus* were capable of reducing nitrate to nitrite. Expanding on this theme, Haines and Best (1976) found the same thing using *G. mosseae*, but they also found that ammonia could be reduced to nitrite and that nitrite could not be reduced any farther. Mycorrhizal maize and clover absorbed significantly more sulfur isotope than their nonmycorrhizal comparisons (Gray and Gerdemann, 1973). Benson and Covey (1976) found that mycorrhizal apple seedlings grew better than uninfected seedlings in zinc deficient soils and responded better to zinc fertilization. A higher potassium, iron and copper content and a low manganese content was found in mycorrhizal apples (Mosse, 1957). Ross and Harper (1970) found that mycorrhizal soybeans had high quantities

of phosphorus, nitrogen, calcium, copper and manganese. Gerdemann (1965) found that maize had low concentrations of potassium, magnesium, boron and manganese. Thus, the effect of accumulation of certain nutrients in the mycorrhizal to nonmycorrhizal state may indicate that certain VAM fungi to be host specific (Mosse, 1974).

Function of arbuscules. Nutrients are believed to be transferred from the fungus to the root through the arbuscules. These are specialized hyphal structures and consist of fine, dichotomously-branched, tree-like filaments which frequently occupy the entire lumen of the cell. Diameter of the filaments is less than one micron (Gerdemann, 1968). Under nutritional stress, the morphology of arbuscules may be altered, for example when available phosphorus is limited, a heavy infection of arbuscules are formed while at high phosphate levels few arbuscules are formed (Sanders and Tinker, 1973). Woolhouse (1975) suggested that arbuscule formation was related to phosphate concentration and could be summarized in three stages: (1) the host plant sends a "signal" that phosphate is limited, (2) the fungus responds by penetrating the root and forming arbuscules and (3) the host plant modifies the fungus such that phosphate flows from the fungus to the host plant. The arbuscules are then digested and the contents are absorbed by the host cells (Kaspari, 1973). However, when P-32 was added to the host plant, Cox and Tinker (1976) concluded that phosphate transfer must be across membranes.

Uptake of water. Endomycorrhizae have been shown to increase water uptake. Using soybeans, Safir, *et al.* (1971) explained the difference between mycorrhizal and nonmycorrhizal plants in four ways: (1) the external hyphae increased the total surface area of the root system, (2) the hyphae which penetrated the root cortex provided a path of least resistance to water movement across the epidermal cells and cortical cells of the root, (3) the hyphae increased nutrient uptake which decreased the internal resistance of water transport within the root, (4) the mycorrhizal relationship increased root growth so there was a larger root system.

Resistance of water transport into mycorrhizal plants was 40 percent lower than uninoculated controls and addition of nutrients solutions to these controls eliminated the difference (Safir, *et al.*, 1972). It was thought that the lowered resistance in infected roots growing in soil with low levels of nutrients probably resulted from the improved nutrient status of the plant. It has been found that the increased amount of vascular tissue in *Lycopersicon* (tomato), Zea (maize), and *Petunia* resulted in the greater uptake of phosphorus (Daft and Okusanya, 1973).

VAM Infection of Yellow-Poplar

The endomycorrhizal infection of yellow-poplar with *Glomus* species resembled the classical Parisian type of growth habit (Gallaud, 1905). The beginning stages of infection were the same as any VAM plant. The fungus produced an appressorium and penetrated the epidermis directly, and the hyphae grew through the cortex intracellularly and very few intercellular hyphae were formed. This was contrasted to the Arum type

(Gallaud, 1905), common to most grasses, which grew intercellularly before penetrating the cortical cell.

Gerdemann (1965) found as the Parisian type of infection progressed through the root, it produced complex coils of hyphae in the cortical cells (Figure 3). The fungus did not penetrate the endodermis and the layer of cells nearest to the endodermis was usually free of infection. The arbucules were confined almost entirely to the two layers of cortical cells nearest to the inner most cortical cell layer. They were formed in cells otherwise free of hyphae as terminal branches from coiled hyphae of adjacent cells. Vesicles were usually intercellular and tended to be somewhat small and rounded in *G. fasciculatus*. The vesicles were borne terminally, somehwat different from the classic Parisian infection type (Gerdemann, 1965).

Very little work has been done with growth benefits of yellow-poplar infected with VAM. Clark (1963, 1964, 1969) concluded:

1. Sterlized soil infested with an endophyte in pure culture increased growth over sterile soil and sterile soil amended with duff from a natural yellow-poplar stand;
2. That microorganisms were essential for growth and development of yellow-poplar;
3. That *Glomus* spores can cause mycorrhizal infection of yellow-poplar.

Establishment of Yellow-Poplar

With competition eliminated and the right site conditions, yellow-poplar can be established on a wide variety of sites using

Figure 3. Hyphal coiling within a cortical cell of a sectioned yellow-poplar root which was embedded in paraffin and stained with methylene blue. Magnification 360 X.

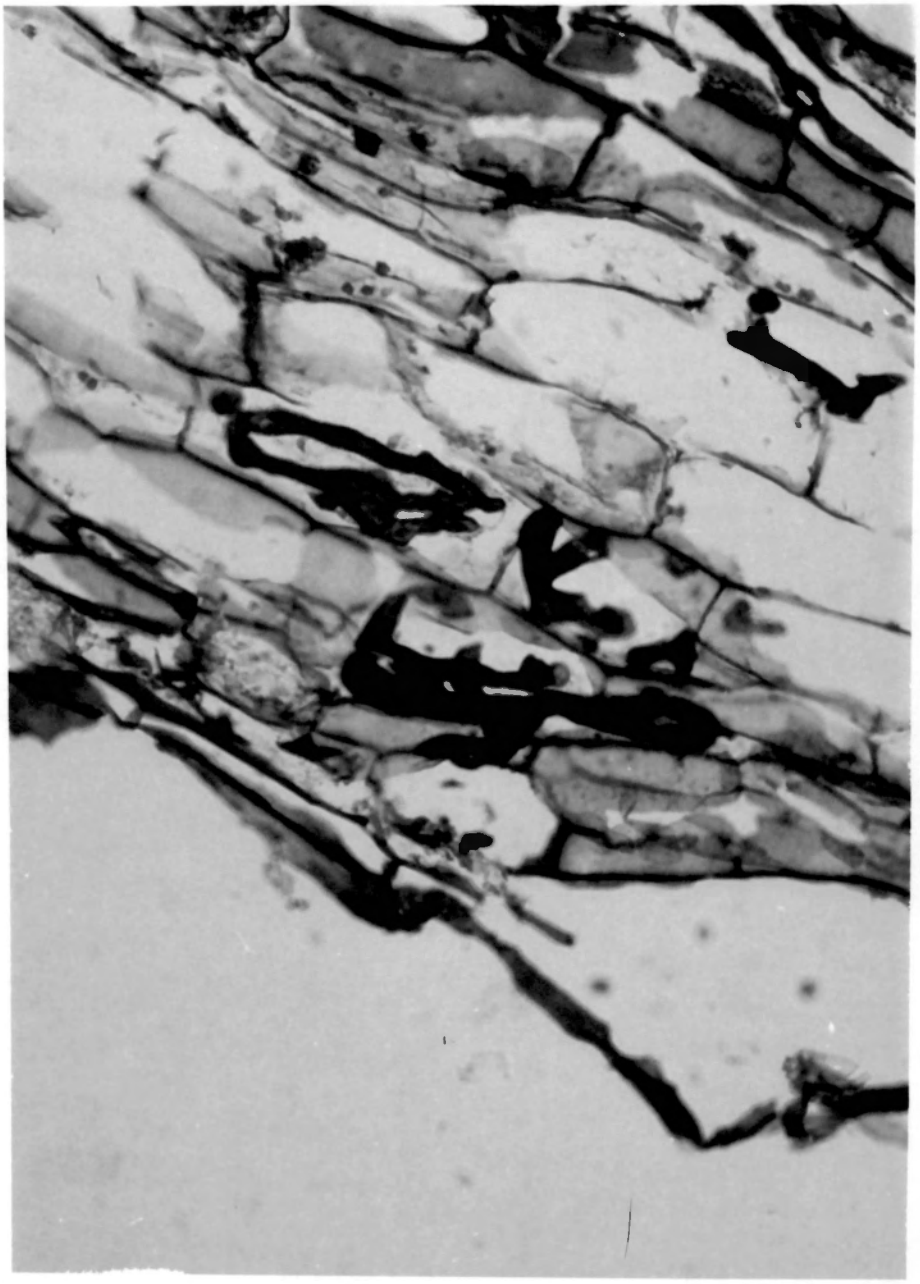


Figure 3

standard nursery, bare-rooted, genetically superior seedlings with standard planting techniques. Russell (1977) stated that the reason yellow-poplar outplanting fails most of the time was that inferior seedlings were planted on poorly prepared and marginal sites. To establish yellow-poplar, adequate site preparation is essential. McGee (1977) found that vigorous mechanical and chemical site preparation must be done to insure economic and biological growth of the species. The reasons were that yellow-poplar was extremely shade intolerant and it possessed a rapidly growing and deeply penetrating juvenile taproot, as well as widespreading lateral roots (Toumey, 1929). If competition or environmental barriers are eliminated, yellow-poplar will exploit the soil for needed nutrients and water with result being a larger more vigorous plant. Seedlings can outgrow much of the herbaceous vegetation but sprout competition is too much for the young seedlings and must be eliminated.

CHAPTER III

METHODS AND PROCEDURES

Growth Conditions

Seed and seed-flat preparation. Yellow-poplar seeds were collected from McMinn County, Tennessee by Tennessee Division of Forestry personnel and stratified 90 days at 5°C. Before planting, the seeds were surfaced sterilized for 3 minutes in a 0.5 percent solution of sodium hypochlorite. After sterilization, the seeds were thoroughly rinsed in tap water and drained

The germination medium, made from equal parts of perlite and masonry sand, was sterilized with methyl bromide after it was placed in seed-flats (33.56 cm x 58.42 cm x 7.62 cm). Seeds were distributed evenly and covered with sand. Seed flats were illuminated and warmed by 150 watt lamps. The temperature of the medium was maintained between 20°C and 30°C to insure prompt germination.

Mycorrhizae preparation. Soil cultures of *Glomus mosseae* and *Glomus fasciculatus* were obtained from the Institute of Mycorrhizal Research and Development, U. S. Forest Service, Athens, Georgia. Cultures of the fungi were maintained in the greenhouse on roots of *Sorghum vulgare* var. *roxburghi*.Portions of the culture soil including sorghum roots containing the various fungal structures served as source of inoculum for this study. Endophytes in soil from a yellow-poplar stand at the University of Tennessee arboretum served as inoculum for the natural treatment.

Rootrainer preparation and planting. Due to their space efficiency, ease of harvesting the entire root system and facilitating reforestation programs, Spencer-Lemaire rootrainers were used in this study. The "Super 45" (951 cm x 6.35 cm x 22.85 cm) is a book type, polystyrene container with three cavities. Ten rootrainers were packaged together into a compact bundle (Figure 4). Each bundle was one replication with six plants one for each of six harvests in each of the five treatments. There were eight replications and there were two duplications of the experiment.

Growth medium was a 1:1:1 mixture of fine pine bark, masonry sand and silt loam soil. The pH was 6.9, the phosphorus concentration was 5.44 parts per million (ppm), and the potassium concentration was 326.4 ppm. The neutral pH assured optimum conditions for the fungi (Green, *et al.*, 1976). Pine bark increased medium drainage and the mixture provided adequate nutrition and water retention. The well-mixed medium was sterilized using methyl bromide, Dow MC-33, under black plastic. The medium was treated for 48 hours and subsequently aired for 72 hours. Endomycorrhizal inoculum was mixed with the sterile medium (200 ml:9500 ml) and after a glass wool plug was placed in the bottom of each rootrainer the individual treatment media were poured into each rootrainer. Treatments were assigned randomly by rootrainers and 2 to 3 weeks old yellow-poplar seedlings were transplanted. Only healthy seedlings, showing no symptoms of damping-off and approximately equal size were used. Filtered leachate of all fungal treatments combined was added to the controls to standarize the nonmycorrhizal microflora of the rhizosphere.

Figure 4. Experiment arranged in the greenhouse bench including seedflats, bundled rootrainers, heating lamps and artificial fluorescent lighting.

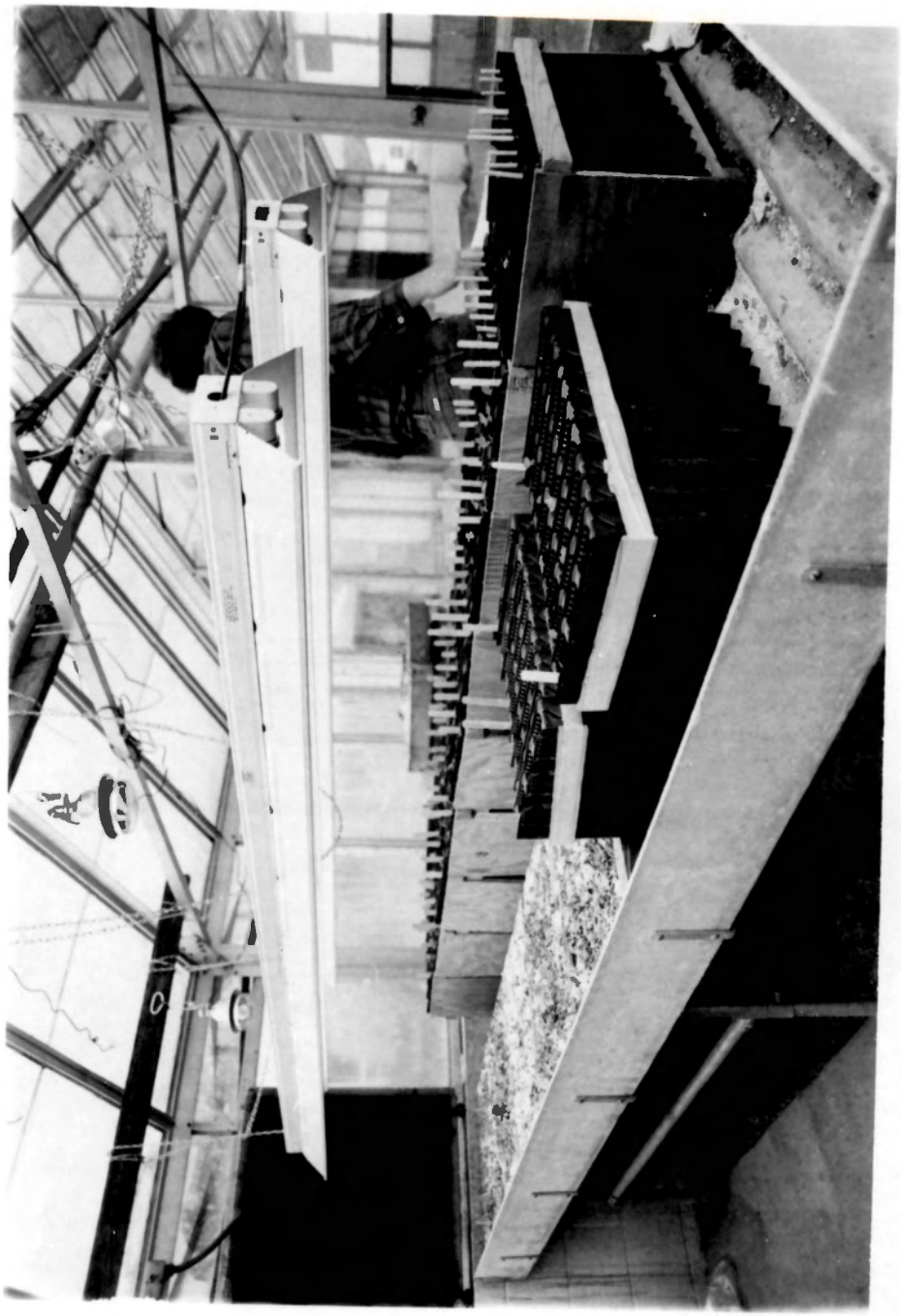


Figure 4

Greenhouse technique. Gro-Lux, commercial fluorescent lights for plant growth, were used to extend the photoperiod to 16 hours (Figure 4) the lights ran continuously from 6:00 a.m. to 10:00 p.m. Water was appliec as needed with one-half strength Rapid-Gro added every 3 weeks. Benomyl was applied foliarly at 8 weeks to control powdery mildew (Nesheim and Linn, 1969).

Data Collection

Harvest. Harvests were made at 3, 5, 7, 9, 12 and 16 week intervals One seedling per treatment per bundle was removed for measurements of shoot length, root length, stem diameter at the root collar and oven-dry weight. After measurements, roots were placed in culture tubes filled with formalin-alcohol-acetic adic (FAA) to preserve the tissues.

Staining. Seedling roots were stained using the method described by Phillips and Hayman (1970). After the FAA was decanted, whole roots were heated in a water bath at 90° C with 10 percent KOH added to the culture tubes for 30 minutes to remove the cytoplasm and the nuclei from the cortical cells. Roots were bleached in an ammonia-peroxide solution (1.0 ml commercial cleaner, 10.0 ml hydrogen peroxide and 200.0 ml distilled water) at room temperature. After bleaching, the roots were rinsed in distilled water and acidified in 1 percent HCl. The roots were stained by heating at 90° C for 10 minutes in 0.05 percent trypan blue in lactophenol. As the roots were difficult to store and handle in lactophenol, glycerine would have been better for storage.

Scoring. Approximately 1 centimeter segments were cut from each root and mounted temporarily in lactophenol. Fifty microscopic fields (area: 3 square mm, magnification 100 X) were taken from each root, using 8 to 10 root sections (Barnhill, 1977). Free hyphae, intracellular hyphal coils, arbuscules, vesicles and total infection were scored in order to quantify each in the whole root system. The fields were tallied on the basis of presence or absence of each fungal structure until 50 fields were completed. If 50 fields were not scored, the percentage of the number of fields scored was used.

Statistical Analysis

Single degree of freedom orthogonal contrasts were used to compare selected effects. These were used not only for all growth variables and fungal structure percentages, but also for root to shoot ratios and total length of the plants.

Unequal subsample sizes due to mortality were taken into account by using the Type III partial sum of squares in the data analysis produced by SAS 76 (Barr *et al.*, 1976). The means were adjusted by Harvey's least squares analysis (1960).

CHAPTER IV

RESULTS

Statement of Variables Measured

Growth variables were chosen to achieve general expression of seedling performance. Root length (RL), shoot length (SL) and total plant length (TOT) were used to determine any benefits the treatments had on elongation of the seedling. Root to shoot ratio (RSR), computed by dividing the length of the root by the length of the shoot, was used to determine the balance of the plant. Root collar diameter (RCD) has a direct relationship to the plantability of the seedling and oven-dry weight (DW) is a direct measure of above-ground biomass.

Fungal structures were scored to determine a general pattern of progressive infection of each structure through the time of this experiment and ultimately to find any correlation between percentage of infection of these structures to the growth variables. Free hyphae (H), because of their direct bearing on the amount of hyphae in the soil were scored. Vesicles (V) are thought to be storage organs of glycogens and oils and were counted to determine the relationship these structures had on the developing plant. Hyphal coiling (HC) and arbuscules (A), thought to be directly involved with exchanging of nutrients, were scored to determine if these structures had any effect on growth of the seedling. Total infection (I), which was a composite of all the fungal structures together, was used to determine if all structures combined had any effect on the growth variables measured.

Statement of Biological Results

Root length. Figure 5 presents the relationship of RL responses to time of development with various VAM inocula. The growth form is sigmoid with growth rate becoming exponential at 5 weeks and slowing at 12 weeks. All treatments growth forms were similar with no statistical difference between any of the treatments.

Shoot length. Figure 6 presents the relationship of SL and time of development after exposure to different VAM treatments. The growth of the seedlings exposed started slow but accelerated between 9 and 12 weeks, with still a larger acceleration between 12 and 16 weeks. The seedlings exposed to the natural inoculum did not start growth acceleration until 12 weeks. The growth of the uninoculated seedlings also accelerated their growth between 12 and 16 weeks, but the acceleration was not as great as the mycorrhizal seedlings.

Total length. Figure 7 shows the response of total plant length to the mycorrhizal treatments after time of exposure. TOT reflects both the combined growth rates of the root system and the shoot. Only at 16 weeks were any group of seedlings significantly different from each other for this variable. The control seedlings were smaller than the inoculated seedlings. The growth forms were neither a sigmoid curve or an exponential curve but a combination of the two.

Root to shoot ratio. Figure 8 shows the RSR response for the control and mycorrhizal seedlings after exposure to the treatments. There was a steady increase for all *Glomus* treatments until 9 weeks when the

Figure 5. Root length of yellow-poplar seedlings grown for varying periods in a greenhouse after exposure to different mycorrhizal inocula.

CRANES CREST

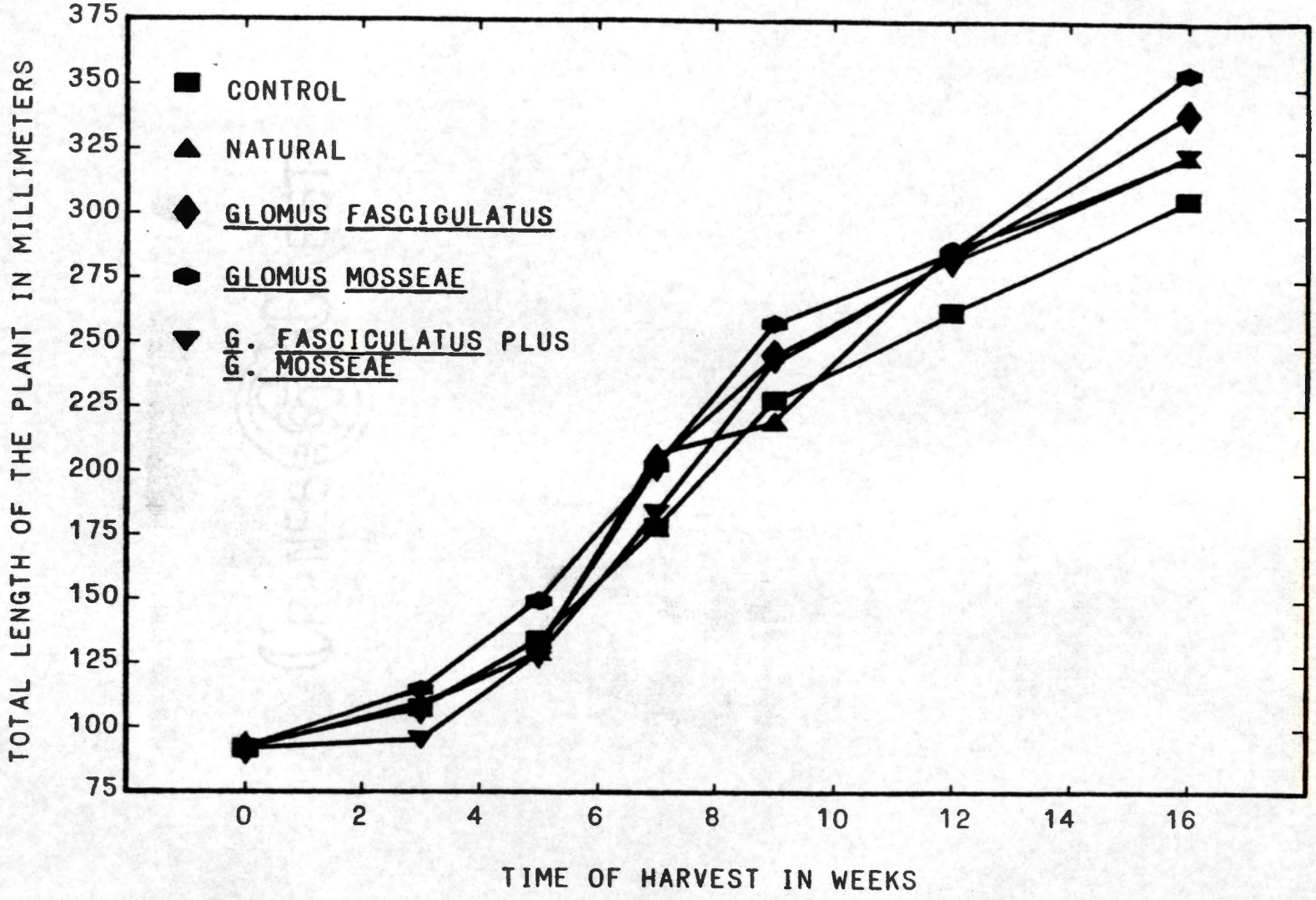

TOTAL LENGTH OF THE PLANT IN MILLIMETERS
375
350
325
300
275
250
225
200
175
150
125
100
75
CONTROL
NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS G. MOSSEAE
0
2
4
6
8
10
12
14
16
TIME OF HARVEST IN WEEKS

Figure 5

Figure 6. Shoot length of yellow-poplar seedlings grown for varying periods in a greenhouse after exposure to different mycorrhizal inocula.

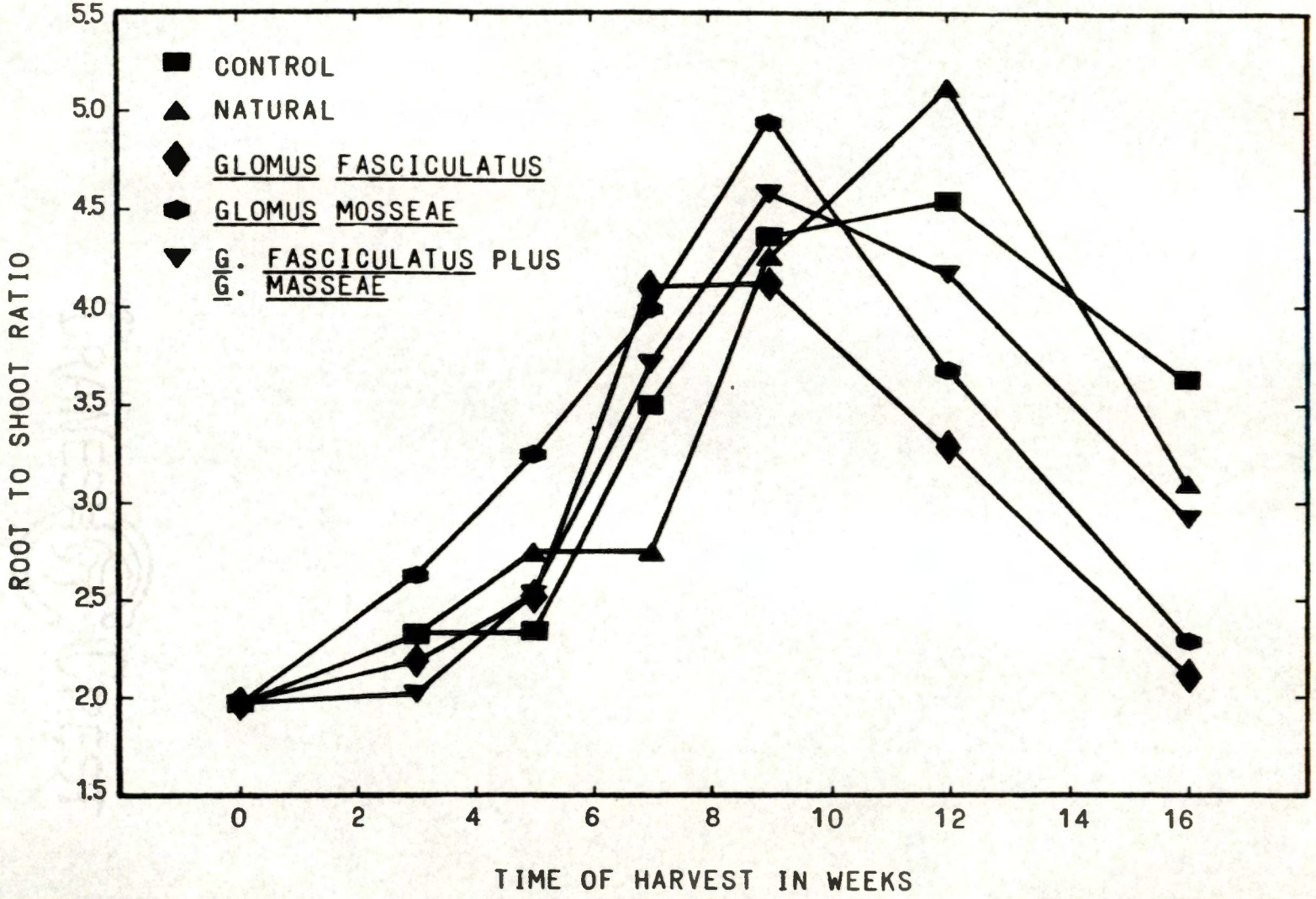

CONTROL
NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS G. MASSEAE
ROOT TO SHOOT RATIO
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0
2
4
6
8
10
12
14
16
TIME OF HARVEST IN WEEKS

Figure 6

Figure 7. Total length of yellow-poplar seedlings grown for varying periods in a greenhouse after exposure to different mycorrhizal inocula.

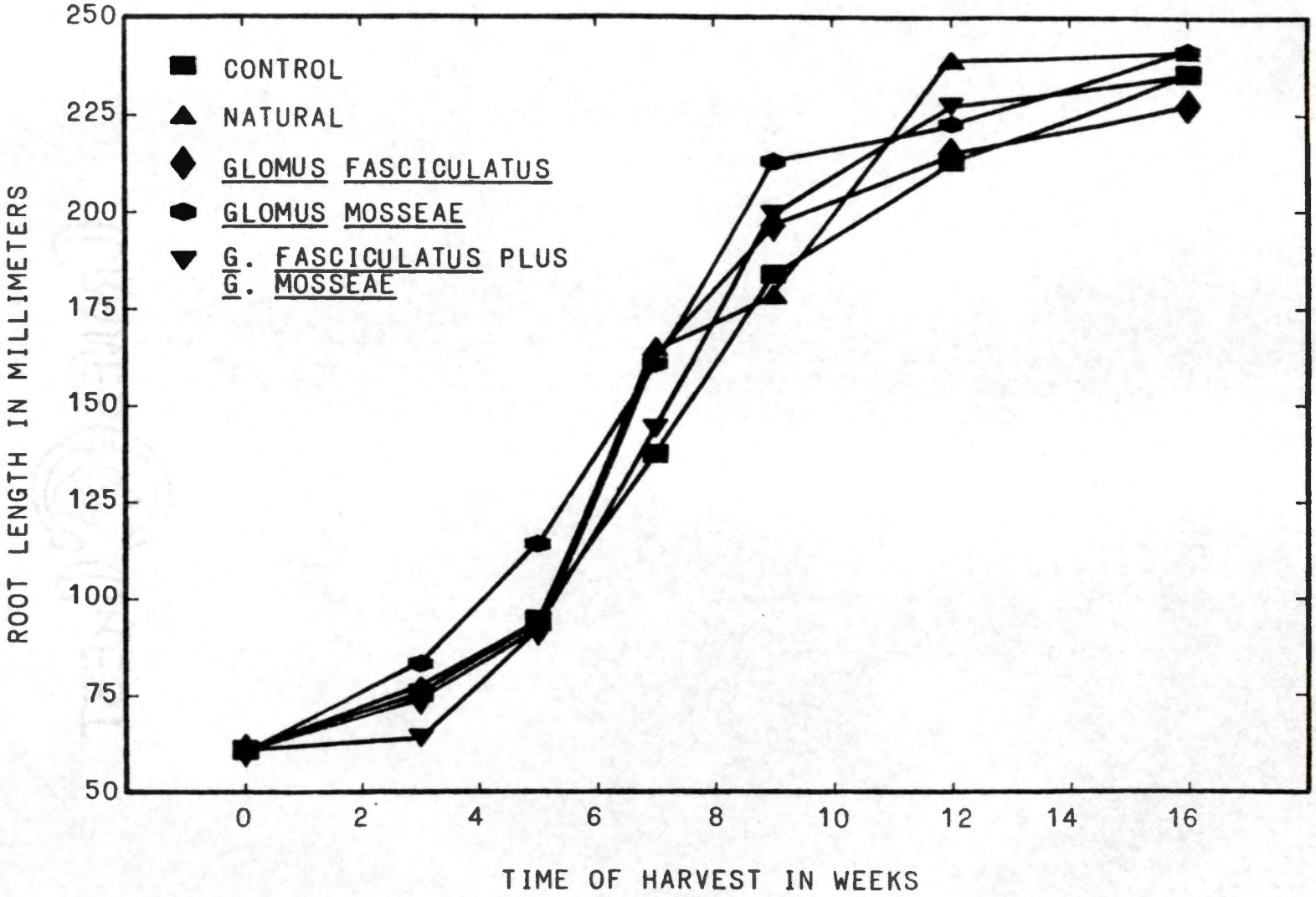

CONTROL
NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS G. MOSSEAE
ROOT LENGTH IN MILLIMETERS
250
225
200
175
150
125
100
75
50
0
2
4
6
8
10
12
14
16
TIME OF HARVEST IN WEEKS

Figure 7

Figure 8. Root to shoot ratio of yellow-poplar seedlings grown for varying periods in a greenhouse after exposure to mycorrhizal inocula.

CRANESBILL CREST

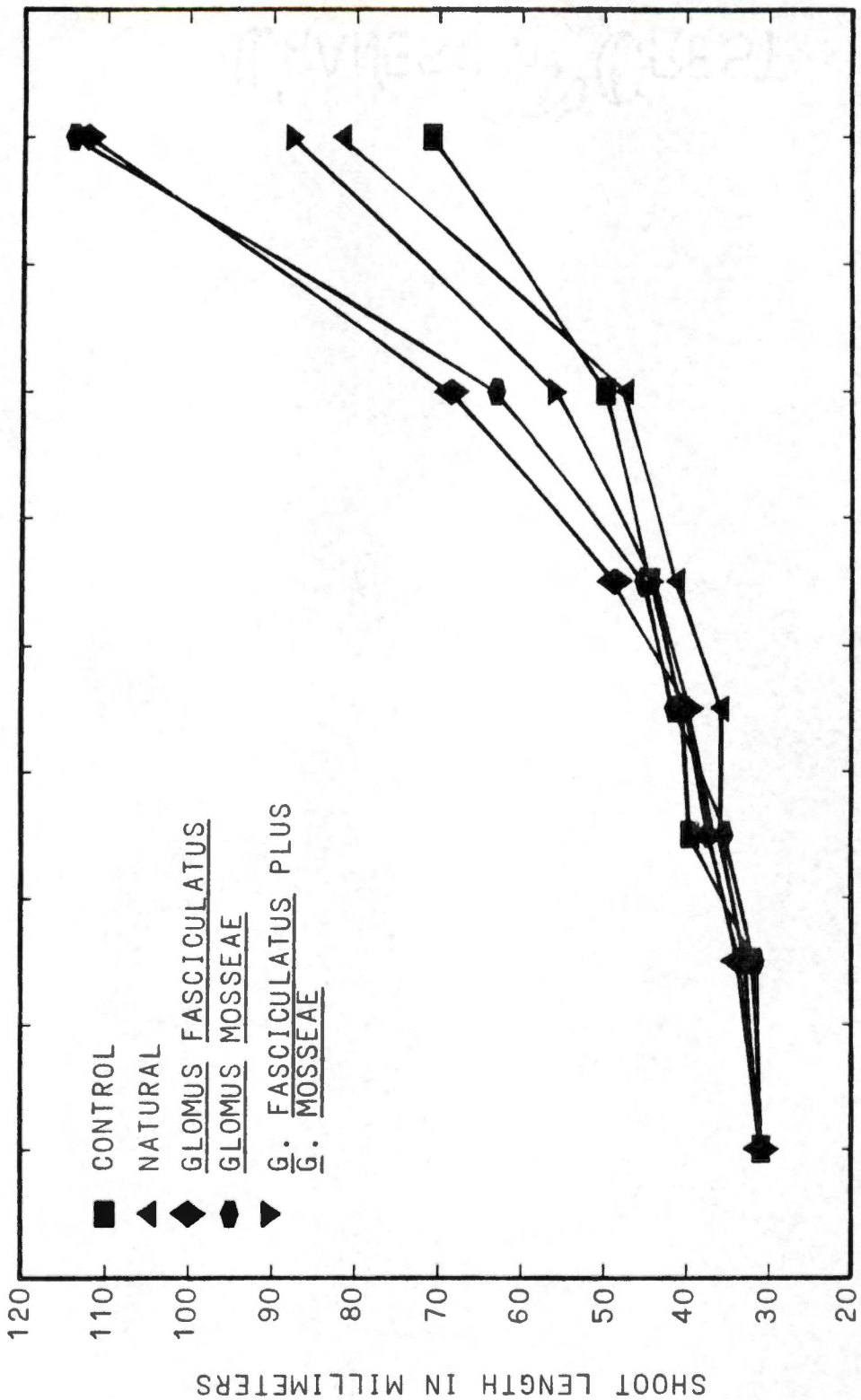

CONTROL
NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS
G. MOSSEAE
SHOOT LENGTH IN MILLIMETERS
TIME OF HARVEST IN WEEKS
120
110
100
90
80
70
60
50
40
30
20
0
2
4
6
8
10
12
14
16

Figure 8

RSR peaked. At 12 weeks, the control seedlings and those exposed to the natural inoculum achieved their maximum RSR. At 16 weeks, the seedlings inoculated with the natural endophytes had a root to shoot ratio less than the control seedlings and the seedlings grown in the mixture of *G. mosseae* and *G. fasciculatus* followed. The seedlings exposed to each organism separately had the lowest RSR and there was no difference between the seedlings grown in these treatments.

Root colar diameter. Figure 9 shows the development of stem diameter at the root color after planting the seedling in the mycorrhizae media. Growth slowly increased for all seedlings until the ninth week when the single culture treatments accelerated stem diameter growth. After 12 weeks, the seedlings grown in the mixed *Glomus* treatment displayed accelerated growth, but the diameter growth of the seedlings that grew in the natural and control media grew at a slow, constant rate.

Oven-dry weight. Figure 10 presents seedling top dry weight accumulation after exposure to the various VAM treatments. Rate for all seedlings was nearly constant until 9 weeks, when all inoculated seedlings began to increase their growth rate. The seedlings grown with the *Glomus* inocula had largest biomass accujulation. The growth of seedlings cultured in the medium with natural endophytes accelerated their growth between 12 and 16 weeks and during the same period of time the growth of nonmycorrhizal seedlings accelerated only slightly.

Fungal structures. Figures 11, 12, 13 and 14 present the relationship of I, H, A and HC respectively to time after exposure to various VAM

Figure 9. Stem diameter at the root collar of yellow-poplar seedlings grown for varying periods in a greenhouse after exposure to different mycorrhizal inocula.

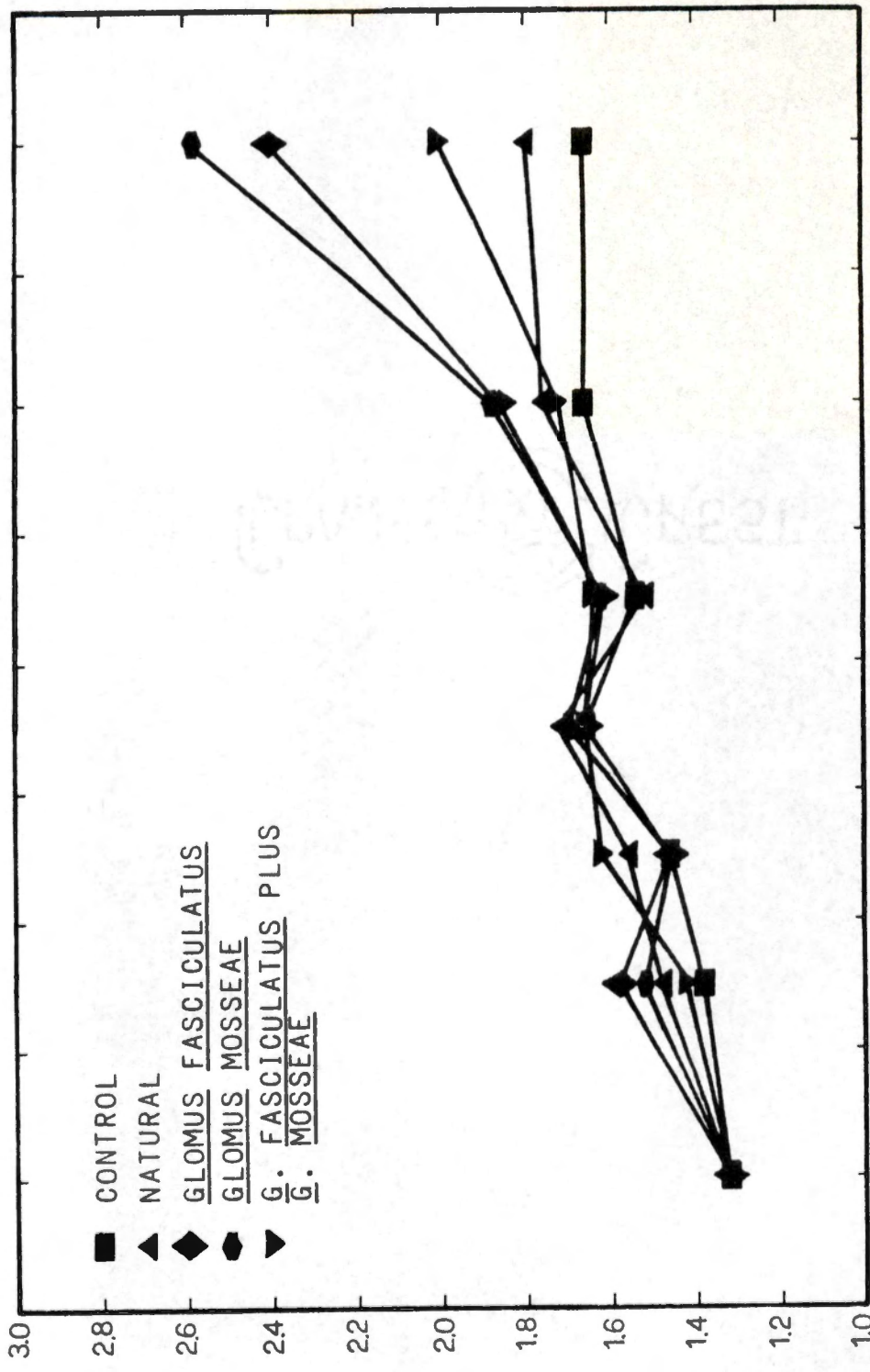

CONTROL
NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS
G. MOSSEAE
ROOT COLLAR DIAMETER IN MILLIMETERS
TIME OF HARVEST IN WEEKS
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0
2
4
6
8
10
12
14
16

Figure 9

Figure 10. Oven-dry weight of yellow-poplar seedlings grown for varying periods in a greenhouse after exposure to different mycorrhizal.

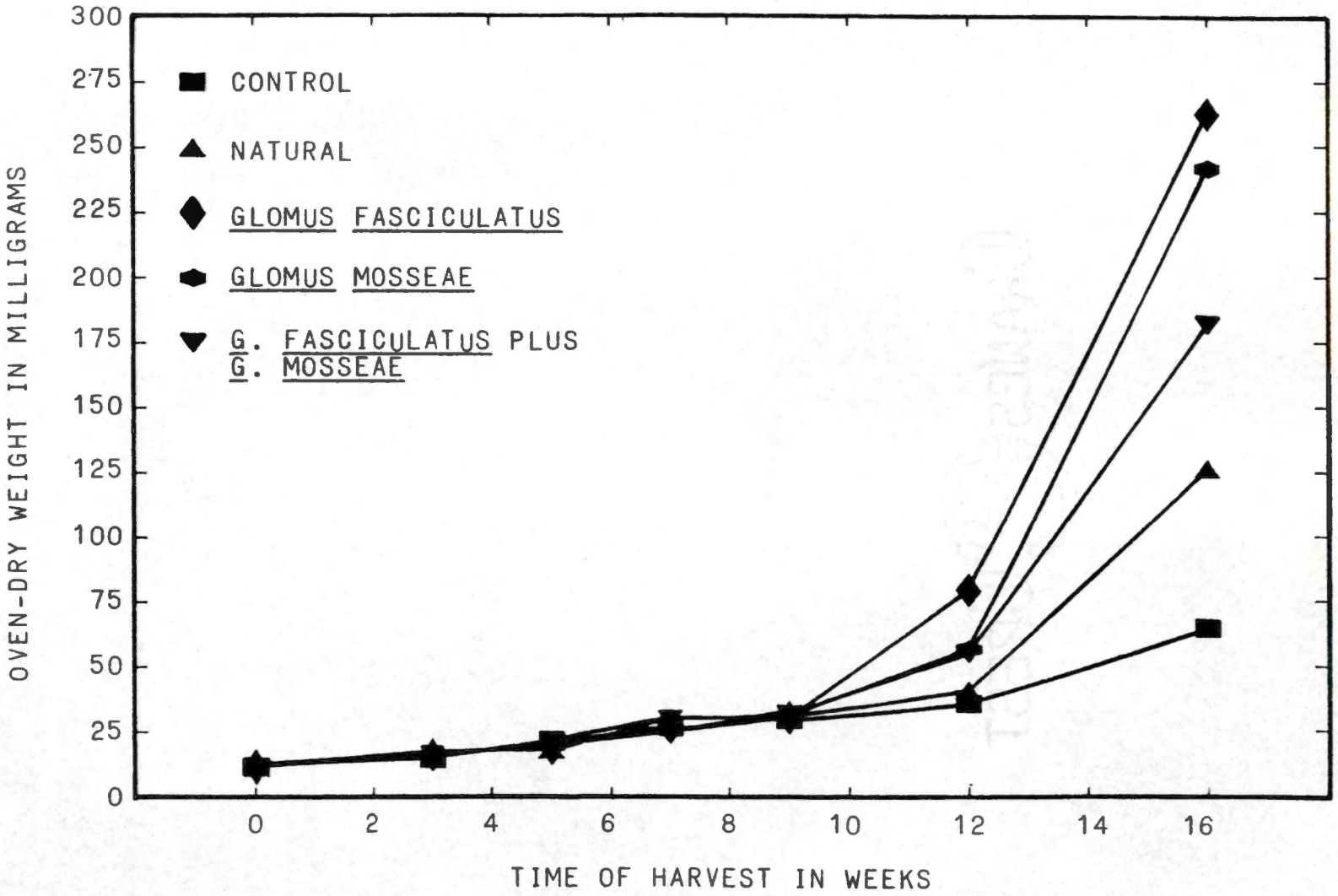

300
275
250
225
200
175
150
125
100
75
50
25
0
OVEN-DRY WEIGHT IN MILLIGRAMS
CONTROL
NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS G. MOSSEAE
0
2
4
6
8
10
12
14
16
TIME OF HARVEST IN WEEKS

Figure 10

Figure 11. Percent total infection of yellow-poplar seedlings grown for varying periods in a greenhouse after exposure to different mycorrhizal inocula.

CRANES CREST

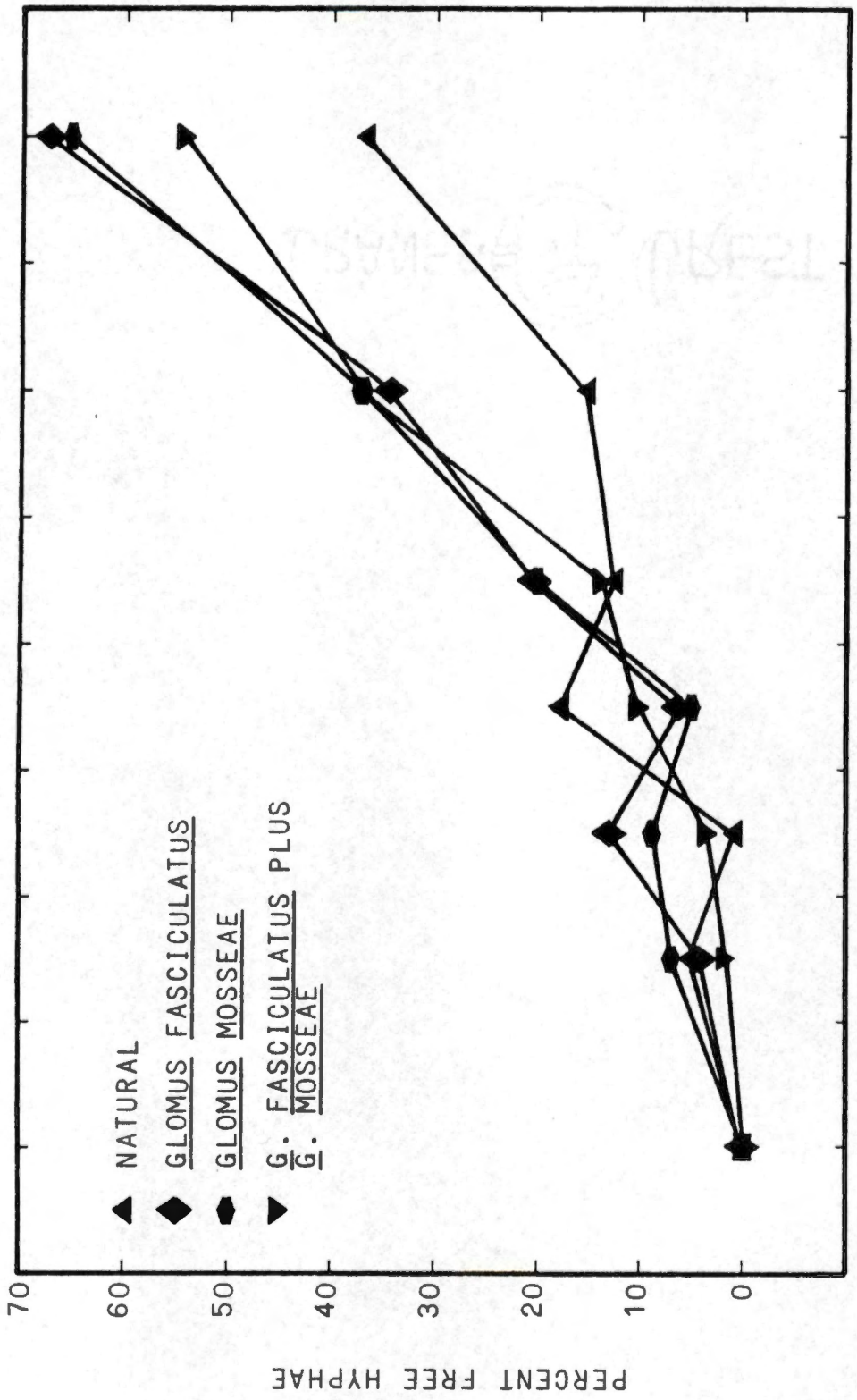

PERCENT FREE HYPHAE
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
TIME OF HARVEST IN WEEKS
NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS
G. MOSSEAE

Figure 11

Figure 12. Percent free hyphae infection of roots of yellow-poplar seedlings grown for varying periods in a greenhouse after exposure to different mycorrhizal inocula.

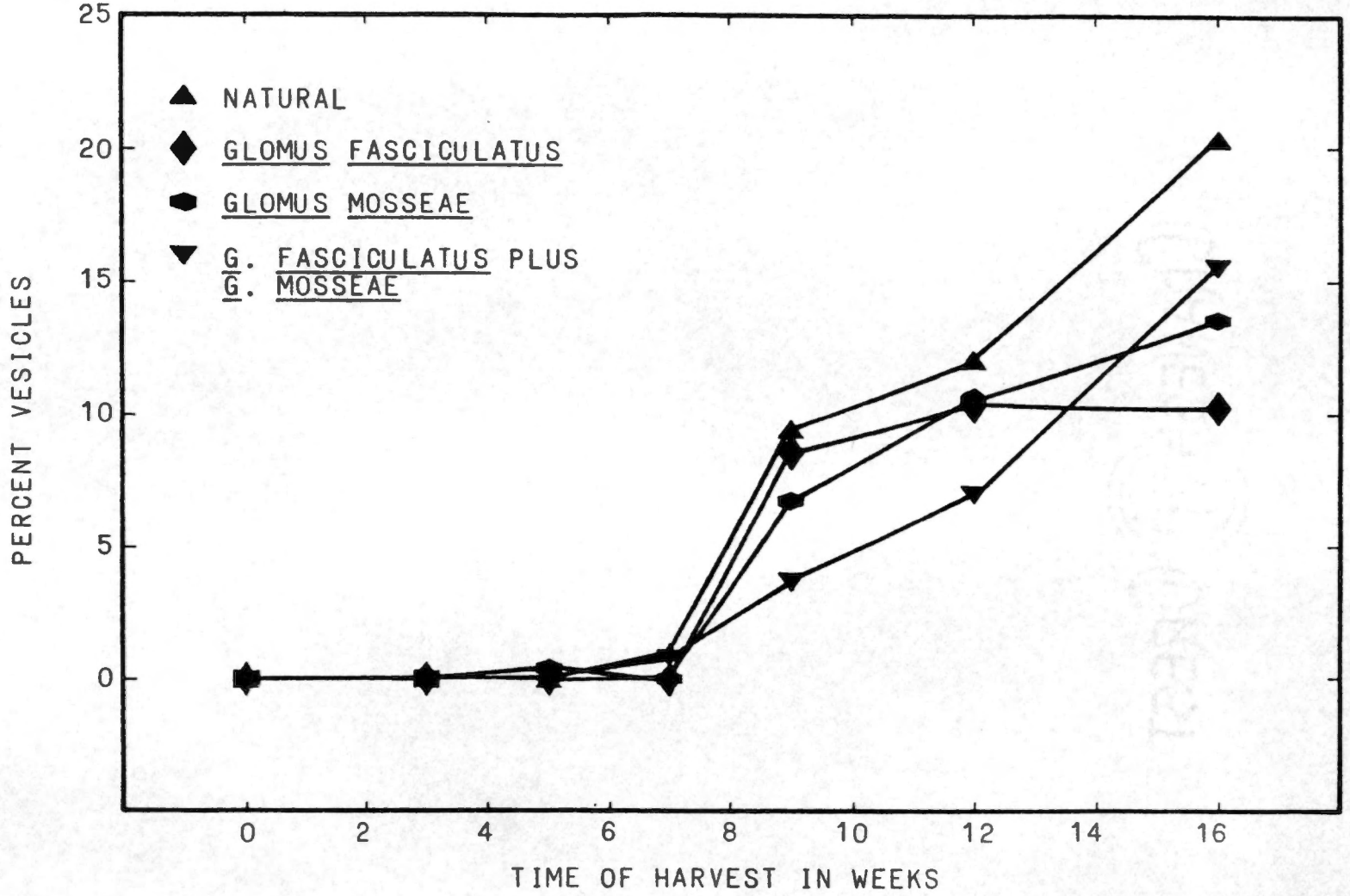

NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS G. MOSSEAE
PERCENT VESICLES
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
TIME OF HARVEST IN WEEKS

Figure 12

Figure 13. Percent arbuscule infection of yellow-poplar seedlings grown for varying periods in a greenhouse after exposure to different mycorrhizal inocula.

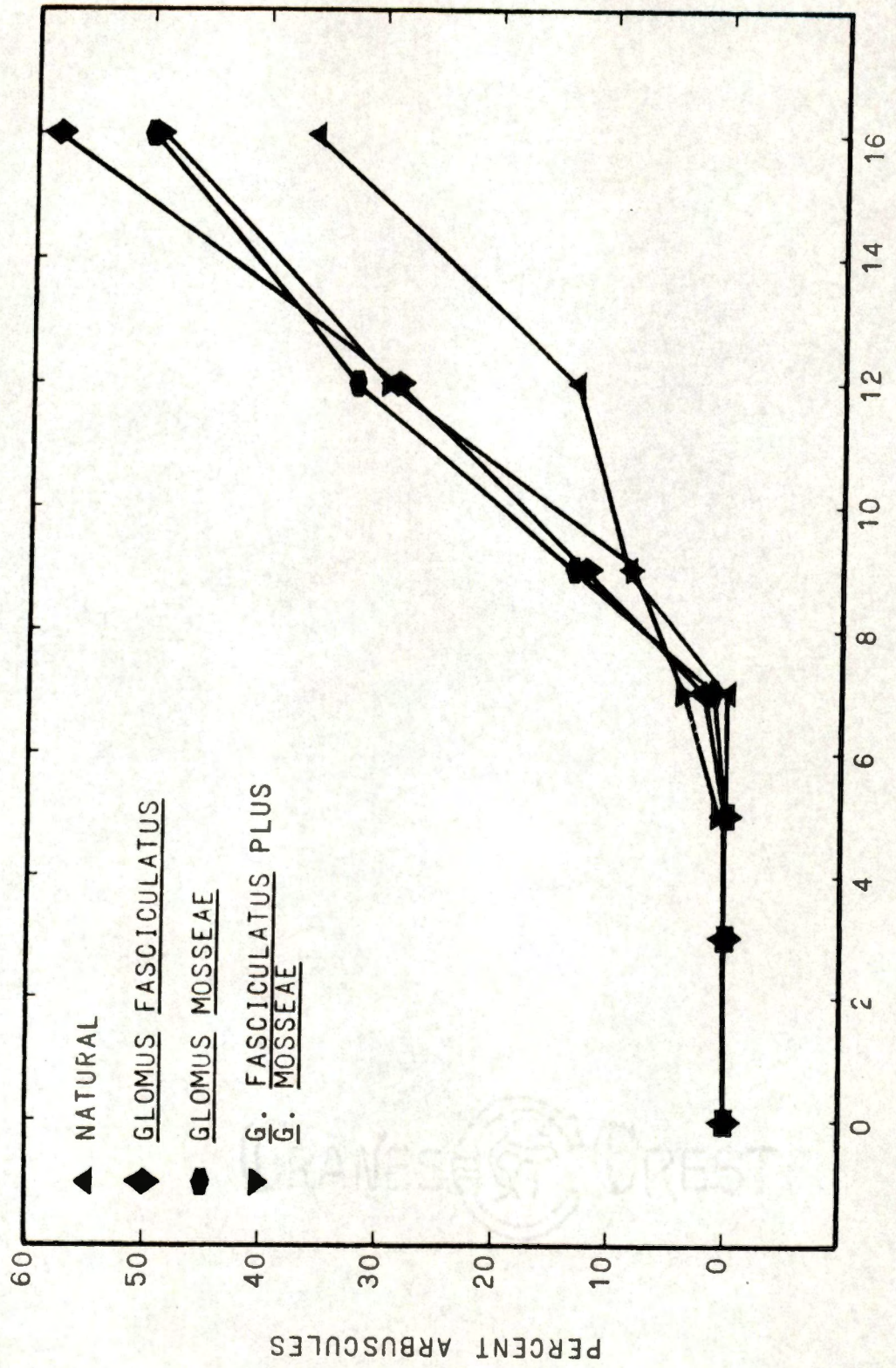

NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS
G. MOSSEAE
PERCENT ARBUSCULES
0
10
20
30
40
50
60
0
2
4
6
8
10
12
14
16
TIME OF HARVEST IN WEEKS

Figure 13

Figure 14. Hyphal coiling of yellow-poplar seedlings grown for varying periods of time after exposure to different mycorrhizal inocula.

CRANESE CREST

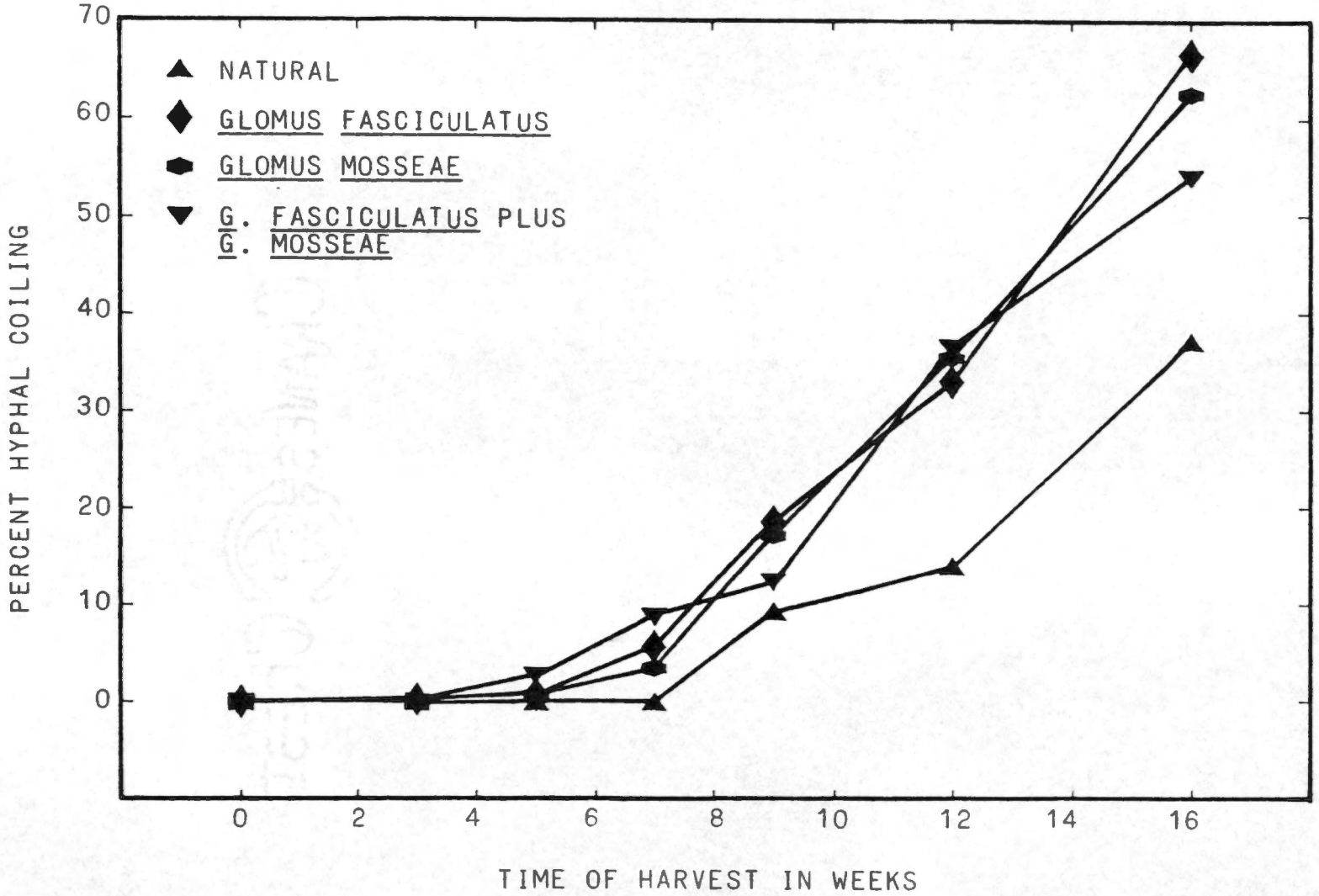

NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS G. MOSSEAE
PERCENT HYPHAL COILING
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
TIME OF HARVEST IN WEEKS

Figure 14

treatments. Each fungal structure had similar abundance of infection for each treatment. All seedlings were infected at a slow, steady rate until 7 weeks when the *Glomus* inocula had an increase in fungal structures which lasted throughout the remainder of the experiment. The VAM fungi rate of infection for the seedlings exposed to the natural treatment had increased until 12 weeks for these variables. At that time, these fungal structures had an increase of abundance comparable to the *Glomus* cultures.

Figure 15 shows the developmental pattern of vesicular infection after exposure to the mycorrhizal treatments. The infection rate for V was very different than the rest of the fungal variables. The seedlings exposed to the *Glomus* inocula did not have any vesicles until between 9 and 12 weeks. Although not significantly, except at 7 weeks, the roots of the seedlings exposed to the natural endophytes contained more vesicles than the roots of the seedlings exposed to all the *Glomus* inocula. The roots of the seedlings exposed to the mixture of *G. mosseae* and *G. fasciculatus* had slightly more vesicles than the roots grown in the single cultures of each endophyte.

Both linear and curvilinear regressions were used for analysis of data and no significant correlation could be determined between the percentage of any fungal structure and any growth variable.

Figure 15. Percent vesicle infection of yellow-poplar seedlings grown for varying periods of time in a greenhouse after exposure to different mycorrhizal inocula.

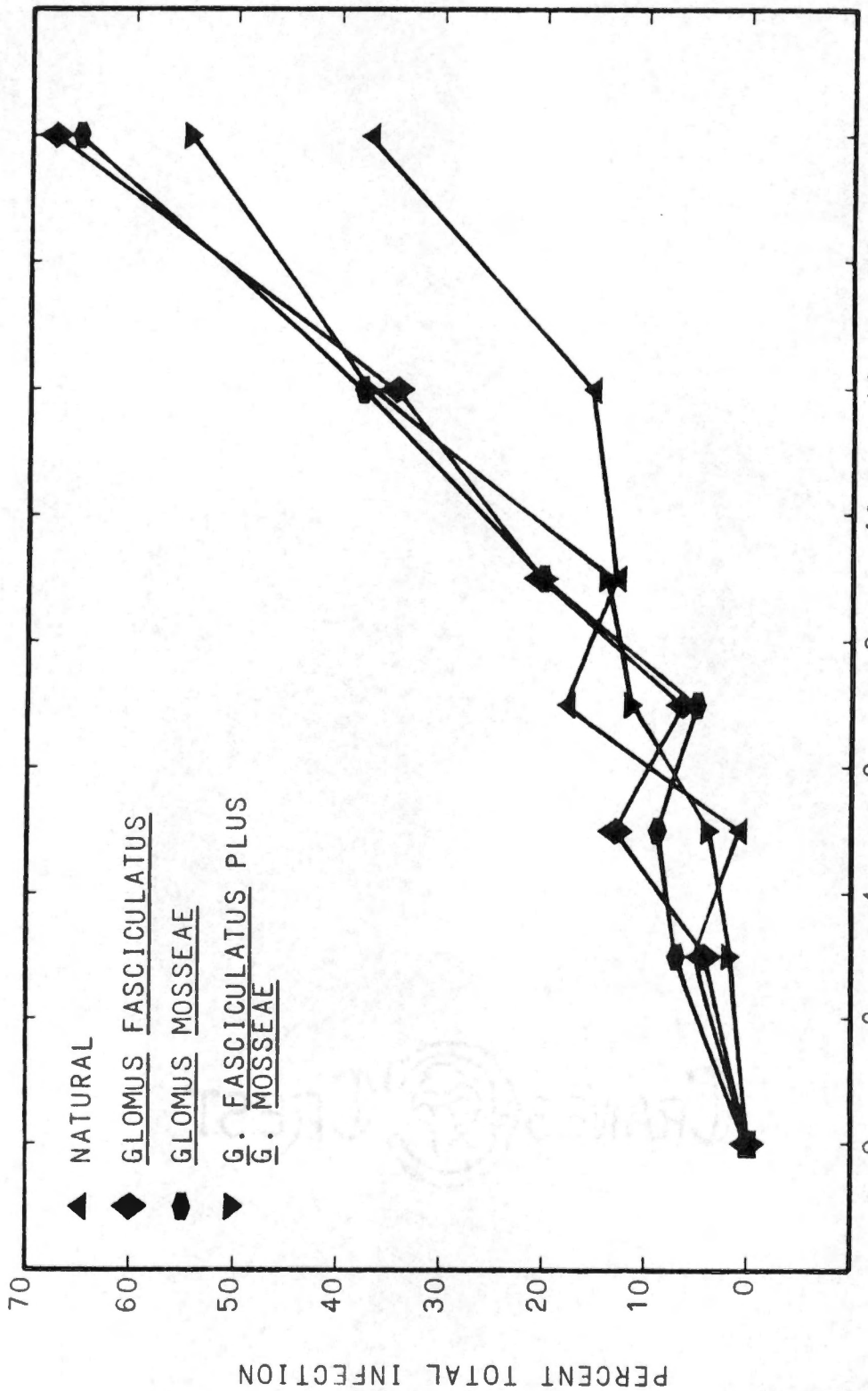

NATURAL
GLOMUS FASCICULATUS
GLOMUS MOSSEAE
G. FASCICULATUS PLUS
G. MOSSEAE
PERCENT TOTAL INFECTION
0
10
20
30
40
50
60
70
0
2
4
6
8
10
12
14
16
TIME OF HARVEST IN WEEKS

Figure 15

CHAPTER V

DISCUSSION

Growth and Development

For individual cells, organs or a collection of organisms growth is potentially unlimited and it begins as an exponential function. Mutual interactions within an individual impose limitations on growth and the rate decreases, leaving a sigmoid function (Leopold and Kriedemann, 1975).

The growth variables measured in this experiment had an exponential phase of development. Only longitudinal root growth decreased resulting in a sigmoid growth curve. The fungal infection did not increase exponentially, but did have a point in time where the rate of infection rapidly increased.

Root growth. Root length during the 16 week duration of this experiment reflected a sigmoid growth relationship. The limited space of the rootrainer imposed growth limitations, such that the early exponential rate slowed into a sigmoid pattern. Root biomass would have been a better indication of root growth, but it was impossible to obtain root biomass estimates and make mycorrhizal determinations on the same tissue.

Roots are dependent on leaves for carbohydrates and certain other growth substances. Harley (1969) thought that mycorrhizal fungi also acted as a sink for carbohydrates and the fungi also required other growth substances for optimum growth. He also stated that mycorrhizal fungi supplied hormones to regulate root growth, as in the short roots of ectomycorrhizae.

Shoot growth. Exponential shoot growth occurred during this study between 12 and 16 weeks for seedlings grown in single cultures of *G. mosseae* and *G. fasciculatus*. In seedlings grown in the natural inoculated medium amended with soil and medium with combined *Glomus* spp. began an exponental growth pattern at 16 weeks. Those seedlings growing in the medium without mycorrhizal inoculum did not begin exponential growth, but maintained a constant, slower growth rate.

Shoot growth is dependent on sufficient quantities of phosphorus, nitrogen and potassium, which are required for structural formation of important proteins and assimilation of structural components of the cell wall. Gerdemann (1965) stated that plants with VAM removed larger quantities of nutrients from the soil. Due to the availability of these nutrients, shoot growth was stimulated at an earlier age and in turn yielded a larger seedling. Bryan and Kormanik (1977) found that 1-0 sweetgum seedlings infected with VAM fungi were larger than standard 2-0 seedlings that were not inoculated with VAM. These noninfected sweetgum seedlings were stunted, and had a purpulish cast to their leaves. In this study, the yellow-poplar seedlings did not exhibit any color contrast between infected and noninfected seedlings, but noninfected seedlings were stunted.

Root to shoot ratio. There are various complex physiological interactions between the root and the shoot systems which form a highly sensitive control system by which the plant is able to adjust the root to shoot proportions according to the environment in which it is growing (Luckwill, 1960). There are three major factors that influence the root

to shoot ratio: (1) soil moisture, (2) nitrogen supply and (3) light intensity. Dry soils, high light intensity and low nitrogen levels lead to a larger root mass, thereby creating a high root to shoot ratio. Conversly, a moist soil, low light intensity and high nitrogen levels will create a disproportionally lower root to shoot. Luckwill (1960) concluded that plants adapt their growth in such a way that it is not the root to shoot ratio that is constant, rather a ratio of nitrate intake to carbohydrate synthesis. The balance of nitrate intake to carbohydrate synthesis is essential so that proteins can be manufactured and healthy growth can be maintained.

The balance of root to shoot is an important consideration for nursery production of seedlings destined for outplanting. A newly transplanted seedling must have nutrients and water. A seedling that has low root to shoot ratio will not have enough root system to amply supply the shoot.

Root dry weight to shoot dry weight is the preferred indication of balance (Evans, 1972). This study only took into account elongation due to the fact that root biomass and mycorrhizae analysis was impossible on the same tissue. By using a ratio of root elongation to shoot elongation, a balance of the root to shoot would be close to the biomass balance until the root reached linear capacity of the rootrainer.

There was a peak at 9 weeks in the ratio of root to shoot in seedlings exposed to the media containing single cultures of *G. mosseae* and *G. fasciculatus*, and after this time there was a sharp drop in both groups of treatments. The seedlings grown in the media containing natural endophytes and the mixed *Glomus* spp. appeared to peak between 9 and 12

weeks with a sharp decline in root to shoot ratio. The uninoculated seedlings peaked at 12 weeks and the decline was not as sharp.

The peak suggested that the mycorrhizae was not effective in supplying nutrients until the drop in RSR. The sharp drop in the inoculated plants suggested at this time the mycorrhizal relationship was in full swing and the efficiency of the endophytes were greatly improving. The ranking of the root to shoot at 16 weeks was an indication of the efficiency of each source of inocula by supporting more shoot development with less root.

Total length of the plant was just another measurement of the relationship between root to shoot. The addition of any information from this variable was marginal.

Shoot biomass accumulation. Dry weight of the shoot is an equilivance of photosynthetic efficiency and accumulated biomass. Biomass accumulation is a function of net photosynthate. When the plant is utilizing carbohydrates for respiration instead of assimilating structure, biomass is lost.

The benefits of mycorrhizae provide an enriched nutrient base so that plants can fulfill their growth potential (Kormanik, *et al.*, 1977a). Without the benefits of mycorrhizae, the plant must use more photosynthate to procure the needed nutrients and water to maintain metabolism. A yellow-poplar without mycorrhizae is inefficient.

The seedlings grown in *Glomus* inocula starts exponential growth earlier than the seedlings exposed to natural soil or uninoculated seedlings. Among the *Glomus*-infected seedlings, the single cultures were

almost identical, but exceeded the seedlings grown in the combination of the two species of fungi. Seedlings exposed to the *Glomus*-amended media had larger and more numerous leaves to produce more photosynthate. With more leaf area, the plants were able to grow more.

Diameter growth. Stem diameter at the root colar did not accelerate growth until after 12 weeks. At this time, only the seedlings exposed to the *Glomus* treatments accelerated diameter growth and the seedlings exposed to a single inoculum responded better than seedlings to the mixed inoculum. The seedlings grown in the natural treatment and with no mycorrhizae did not accelerate growth but grew at a slow pace.

Diameter growth had a lower priority than height growth even at an early age (Leopold and Kriedemann, 1975). In the treatments with more photosynthate available, diameter growth increases. Along with carbohydrates produced, there are the needed nutrients for protein synthesi and regulatory substances for a rapidly growing cambium. In the treatments without mycorrhizae or with a less efficient mycorrhizal condition, excess nutrients and carbohydrates are not taken up for growth.

For seedling production, diameter growth is a function of the plantibility of the seedling. Seedlings are graded by the size of the diameter, the larger the diameter the better the seedling quality.

Arbuscules, free hyphae, hyphal coiling and total infection development. All fungual structures, except vesicles, increased in a gradual, nonexponential pattern, but there was still an acceleration in frequency of infection between 7 and 9 weeks. There appeared to be an increase in

frequency of these structures before the seedlings accelerated their growth.

There was a lack of significant correlation of fungal structures to growth variables. This lack may be due to imperfections of the sampling technique. Each field was scored on a presence or absence basis. If scored by a quantitification system, each field would yield a closer approximation of the amount of each structure in each root system. Techniques which would employ chemical means of quantitifing infection of the whole root would yield better results, but individual analysis of fungal structures would have been impossible. Still, there is a trend present in these data which suggests when observing the curves that a relationship between the increase of infection and the acceleration of growth structures.

Vesicles. The vesicles had a different pattern of infection than other fungal structures. Seedlings exposed to the soil with natural inoculum had more vesicles than the seedlings exposed to the *Glomus* inocula. There are many species of VAM fungi when infecting yellow-poplar could produce more vesicles than *G. mosseae* or *G. fasciculatus.*

Mosse (1973) thought that vesicles are more like propagules rather than storage organs. Adverse conditions could stimulate vesicle production similar to species of fungi product resting spores when conditions become unfavorable. Lack of information on the structure and function of vesicles make determining the cause of vesicle production difficult.

Nutrition and the Efficiency of VAM

When the plant growth is substandard, malnutrition is one of the first factors to consider. Plants require ample, but not excessive amounts of 16 different nutrients (Treshow, 1970). Nutrients form the structural components of the plant, the nucleic acids directing plant processes and the enzymes regulating metabolic activities. Nutrients are also necessary for maintaining osmotic balance and absorption of ions. Mycorrhizae are essential for increased uptake of these essential nutrients (Kormanik, *et al.*, 1977a).

In this study, yellow-poplar seedlings infected with mycorrhizal fungi were larger in all growth variables and thus, appeared to be receiving more nutrients than the nonmycorrhizal seedlings. Previous studies have shown that sweetgum exhibited a significant need for mycorrhizae (Bryan and Ruehle, 1976) and that yellow-poplar needed mycorrhizae for optimum growth (Clark, 1963). Marx and Barnett (1974) showed that ectomycorrhizae were needed for growth of containerized pine seedlings. This study presents similar evidence for endomycorrhizae infection of containerized yellow-poplar seedlings.

These seedlings inoculated with *G. mosseae* and G. fasciculatus grew equally well to each other and superior to the seedlings exposed to the other treatments. Schoknecht and Hattingh (1976) found no difference in the amount of nutrients contained in the root cells of onion infected with *G. mosseae* or *G. fasciculatus*. Azcon, *et al.* (1976) found under the same conditions that there was no difference in the amount of phosphorus of the whole onion with either endophyte.

The mixture of *G. mosseae* and *G. fasciculatus* yielded lower growth rates than the seedlings exposed to individual cultures due to some interaction between the two fungi, so that the mycorrhizal relationship is less efficient. The seedlings subjected to the mixed *Glomus* inoculum showed no significant difference in percentage of fungal structures, thus it seems that the endophytes reaction to one another does not effect the amount of infection. This result does seem to hold for every case. Barnhill (1977) found that a combined inoculum of the two endophytes produced lower infection rates than if the seedlings were grown in single culture of each endophyte. Antibiotics can play a role in affecting fungi in the same medium. Marx (1972) found that ectomycorrhizae fungi produce antibiotics that prevent growth of pathogenic fungi, but no such evidence has been presented with endomycorrhizal fungi.

Seedlings exposed to the natural endophytes were intermediate between the control seedlings and the seedlings exposed to the mixed *Glomus* treatment. There appeared to have been two reasons why natural soil inoculum impeded growth of yellow-poplar: (1) interaction between two or more endophytes and (2) introduction of parasites of the VAM fungi and yellow-poplar. Clark (1963) found that sterilized soil and macerated yellow-poplar roots stimulated yellow-poplar seedling growth over unsterilized soil from a yellow-poplar stand. Ross and Ruttencutter (1977) found two hyperparasitic fungi, a *Phlyctochytrium* sp. and a *Pythium*-like species, which were parasitic on *Glomus macrocarpus* var. *geosporus* and *Gigaspora gigantea*.

It was a possibility that along with the endophytes, these hyperparasites were introduced. By parasitizing the VAM fungi, the mycorrhizal relationship was rendered less efficient and resulted in impeded growth of yellow-poplar seedlings. In addition to hyperparasites of VAM fungi, parasites of yellow-poplar roots can be introduced leading to an adverse growth environment. Mematodes and various other fungi besides VAM fungi were observed on the roots of the seedlings which received the natural treatment.

Bryan and Kormanik (1977) found that sweetgum responded better from inoculum from a natural sweetgum stand than from a inoculum of *G. mosseae*. The endophytes were not identified in the natural treatment and there must not have been any effective paratism on the VAM fungi or the seedling that would lead to loss of growth.

CHAPTER VI

CONCLUSIONS

The conclusions drawn from this study are:

1. The source of incoculum does have an effect on yellow-poplar seedling growth. Seedlings that grew in single cultures of *Glomus mosseae* and *G. fasciculatus* produced the best results but there was no difference between them. The combination of the two fungi produced the next largest growth variables, followed by the natural inoculum and lastly, the uninoculated controls. No source of inocula had any effect on the root length of the plant.
2. All seedling growth variables changed exponentially, except root length which flattened and resulted in a sigmoid corve. All fungal structures had the same infection curve except for the vesicles, which had greater fluctuation.
3. There was no correlation between percentage of fungal structures scored and any growth variable.
4. Yellow-poplar seedlings can be grown in Spencer-Lemaire rootrainers. The media used was sufficient to achieve adequant mycorrhizal infection and suitable growth conditions for yellow-poplar.

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APPENDIX

Table 2. Treatment Means of Harvests

Harvest	TOT	RSR	RL	SL	RCD	DW	H	V	A	HC	I
Pre-plant	91.7	1.97	60.8	30.9	13.2	11.70	00.00	00.00	00.00	00.00	00.00
3 Week											
CO	107.6	2.33	75.5	32.1	13.8	14.28	00.00	00.00	00.00	00.00	00.00
NA	110.4	2.32	77.4	33.0	14.8	16.61	05.22	00.00	00.00	00.02	05.22
GF	107.7	2.19	74.1	33.7	15.8	15.91	04.20	00.00	00.00	00.24	04.21
GM	115.0	2.63	83.2	31.8	15.2	15.17	07.00	00.00	00.00	00.03	07.00
MF	95.4	2.02	63.8	31.6	14.2	15.88	01.70	00.00	00.00	00.02	01.70
5 Week											
CO	134.2	2.34	94.6	39.5	14.6	20.96	00.00	00.00	00.00	00.00	00.00
NA	129.9	2.75	93.9	35.9	15.6	17.98	01.00	00.00	00.00	00.00	01.00
GF	129.8	2.52	92.4	37.4	14.6	18.54	13.00	00.00	00.00	00.76	13.00
GM	149.7	3.25	114.3	35.4	14.6	20.21	08.90	00.41	00.00	00.73	08.90
MF	128.7	2.53	93.0	36.7	16.2	21.68	03.50	00.00	00.55	02.70	03.80
7 Week											
CO	178.4	3.50	137.6	40.7	16.6	26.51	00.00	00.00	00.00	00.00	00.00
NA	207.7	2.75	164.3	35.9	17.2	30.12	17.89	01.00	00.00	00.00	17.89
GF	203.1	4.10	163.1	40.0	16.6	26.51	06.30	00.00	01.87	05.73	06.46
GM	202.4	3.99	160.8	41.8	17.0	25.20	05.04	00.00	01.13	03.50	05.05
MF	184.1	3.71	144.1	39.8	16.6	29.78	10.14	00.79	03.69	08.85	11.35
9 Week											
CO	228.1	4.36	183.8	44.3	15.4	29.41	00.00	00.00	00.00	00.00	00.00
NA	220.1	4.26	178.8	41.3	15.2	30.54	12.68	09.45	08.65	09.34	12.91
GF	245.3	4.12	196.6	48.7	16.2	30.20	20.39	08.57	12.37	18.65	20.39
GM	258.1	4.94	212.9	45.2	16.2	31.09	20.07	06.74	13.30	17.29	20.07
MF	243.2	4.58	199.3	43.9	16.4	31.96	13.77	03.70	08.05	12.45	13.82
12 Week											
CO	262.3	4.54	212.6	49.7	16.6	35.61	00.00	00.00	00.00	00.00	00.00
NA	286.4	5.12	238.9	47.5	17.6	40.59	15.42	12.04	13.28	14.06	15.42
GF	283.2	3.29	214.7	68.5	18.6	79.40	34.42	10.34	28.77	33.06	34.51
GM	285.7	3.68	222.5	63.1	18.8	56.63	37.02	10.55	32.25	35.46	37.70
MF	282.7	4.17	227.0	55.7	17.2	54.99	36.88	06.99	29.40	36.54	36.99
16 Week											
CO	305.9	3.63	235.1	70.8	16.6	64.74	00.00	00.00	00.00	00.00	00.00
NA	323.2	3.10	241.4	81.8	18.0	126.72	36.90	20.31	36.07	37.16	37.20
GF	339.5	2.11	227.2	112.3	24.0	262.46	67.40	10.25	58.15	66.68	67.81
GM	335.5	2.29	241.5	114.0	25.8	241.54	65.35	13.56	50.03	62.56	65.35
MF	323.0	2.92	235.5	87.5	20.0	181.82	54.22	15.55	48.98	53.93	54.35

Legend:

Variables

- TOT - Total length of plant in millimeters
- RSR - Root to shoot ratio
- RL - Root length in millimeters
- SL - Shoot length in millimeters
- RCD - Root collar diameter in 1/128 of an inch (0.19 mm)
- DW - Oven-dry weight in milligrams
- H - Percent free hyphae
- V - Percent vesicles
- A - Percent arbuscules
- HC - Hyphal coiling
- I - Percent total infection

Treatments

- CO - Uninoculated control
- NA - Natural soil
- GF - *Glomus fasciculatus*
- GM- - *Glomus mosseae*
- MF - *G. mosseae* plus *G. fasciculatus*

Table 3. Orthogonal Comparisons

Harvest	TOT	RSR	RL	SL	RCD	DW	H	V	HC	A	I
3 Week											
C1	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*
C2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C3	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	**
C4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5 Week											
C1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C2	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS
C3	NS	NS	NS	NS	*	NS	NS	NS	*	**	NS
C4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
7 Week											
C1	NS	NS	NS	NS	NS	*	*	NS	*	NS	*
C2	NS	NS	NS	NS	NS	*	**	****	*	***	**
C3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
9 Week											
C1	NS	NS	NS	NS	NS	NS	***	*	***	**	***
C2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
12 Week											
C1	NS	NS	NS	*	*	****	****	****	****	****	****
C2	NS	**	NS	***	NS	**	**	NS	***	**	**
C3	NS	*	NS	*	NS	NS	NS	NS	NS	NS	NS
C4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
16 Week											
C1	*	****	NS	***	**	**	****	*	****	****	****
C2	NS	**	NS	**	**	*	***	NS	***	NS	***
C3	NS	**	NS	**	*	NS	NS	NS	NS	NS	NS
C4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Legend: Variables

TOT - Total length of the plant in millimeters
RSR - Root to shoot ratio
RL - Root to length in millimeters
SL - Shoot length in millimeters
RCD - Root collar diameter in 1/128 of an inch (0.19 mm)
DW - Oven-dry weight in milligrams
H - Percent free hyphae
V - Percent vesicles
HC - Percent hyphal coiling
I - Percent total infection

Table 3 (Continued)

Legend: Comparisons

C1 - Control verses the rest
C2 - Natural verses *Glomus* inocula
C3 - Mixed inoculum verses single inocula
C4 - *G. mosseae* versus *G. fasciculatus*

Legend: Significance

* - Probability greater than 0.05
** - Probability greater than 0.01
*** - Probability greater than 0.001
**** - Probability greater than 0.0001
NS - Non-significant

VITA

Victor Lavann Ford was born in Johnson City, Tennessee on October 18, 1955. He attended elementary school at Boones Creek in Washington County, Tennessee and graduated from Central High School in Sullivan County, Tennessee in May 1973. The following September, he entered East Tennessee State University in pre-forestry and transferred to the University of Tennessee at Knoxville in September 1975. In June 1977, he received his Bachelor of Science degree in Forestry with a resource management option. In the same month, he started graduate school in the Department of Forestry, Wildlife and Fisheries at the University of Tennessee and accepted a teaching assistantship with primary duties in Introduction to Forestry and Wood Technology Laboratory. He completed the requirements for his degree in September 1978 and received his Master of Science degree in December 1978.

The author is a member of Gamma Beta Phi, Xi Sigma Pi, Society of American Foresters and U. T. Amateur Radio Club. Mr. Ford will be working on his Doctor of Philosophy degree in Forestry at Virginia Polytechnic Institute and State University starting September 1978.

He is married to the former Rhonda Bombailey of Kingsport, Tennessee.