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Endomycorrhizal colonization of yellow-poplar seedlings

Mary Ann Barnhill

To the Graduate Council:

I am submitting herewith a thesis written by Mary Ann Barnhill entitled "Endomycorrhizal colonization of yellow-poplar seedlings." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

Ronald L. Hay, Major Professor

We have read this thesis and recommend its acceptance:

Edward Buckner, James Hilty

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Accepted for the Council:

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Vice Chancellor
Graduate Studies and Research

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ENDOMYCORRHIZAL COLONIZATION OF YELLOW-POPLAR SEEDLINGS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Mary Ann Barnhill

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ABSTRACT

Objectives were to determine (1) whether two species of endomycorrhizae exhibited similar degrees of colonization in nursery grown yellow-poplar seedlings, (2) whether all seedlings within treated flats were equally colonized, and (3) whether the two fungi acted synergistically when present in combination.

Sterilized growth medium was infested with spores and hyphae of *Glomus mosseae*, *Glomus fasciculatus*, or *G. mosseae* + *G. fasciculatus*. Approximately 150 yellow-poplar seeds from each of five seed sources were sown in each treated and control flat. After 12 weeks, five seedlings from each seed source were harvested and processed for microscopic observation. Fifty microscopic fields ($3mm^2$) from each root were analyzed for the presence of intracellular hyphal coils, vesicles and arbuscules. None were found in the control. Fungal structures were observed in seedling roots in all treatments. However, colonization was considerably greater with *G mosseae* as the only symbiont. Colonization was less extensive when *G. mosseae* was mixed with *G. fasciculatus*. The degree of colonization within roots from the same treatment varied from 0 - 64%.

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INTRODUCTION

Because of its economic value, good growth form, and ease of silvicultural manipulation, yellow-poplar has considerable potential for use in reforestation. However, this species is quite demanding in its requirements for soil moisture, texture, and nutrients (Buckner, 1972); profitable outplantings have been restricted to sites providing optimum growth conditions. Presently, such sites are limited for reforestation purposes because they support agricultural crops or pastures, or they may have been totally disturbed by man's activity, such as surface mining. Yellow-poplar seedlings better adapted to thrive on marginal sites could greatly expand reforestation potential for this important species. Since reforestation is a most critical and costly phase of timber production, reasonable methods must also be developed to produce quality yellow-poplar seedlings for outplanting.

Endomycorrhizal associations have been shown to be particularly beneficial to the survival of several plant species experiencing adverse soil and environmental conditions. Abundant endomycorrhizae have been observed on the roots of a variety of herbaceous plants in anthracite and bituminous coal spoils in this country and in Scotland (Daft and Hacskaylo, 1976; Daft, et al., 1975; Daft and Nicolson, 1974), and it has been suggested that the endophytes were essential for the survival of plants on these coal spoils. Endomycorrhizae have also been shown to significantly increase growth of four-wing saltbush [*Atriplex canescens* (Pursh) Nutt.] on eroded sites in New Mexico

(Williams, et al., 1974). Inoculation of seedlings with specific fungal symbionts may serve as a method for "tailoring" seedlings for outplanting. Marx and Bryan (1975) have reported that sycamore seedlings inoculated with *Glomus mosseae* (Nicolson and Gerdemann; Gerdemann and Trappe) prior to outplanting on surface mined clay spoils had significantly improved survival as compared to non-mycorrhizal seedlings. In addition to providing possible benefits to seedlings outplanted on adverse sites, endomycorrhizae may be an essential requirement in the propagation of hardwood seedlings in a nursery environment, since quality hardwood planting stock which naturally form endomycorrhizae are difficult to propagate in nurseries after soil fumigation (Marx and Bryan, 1975).

Limited work has been done on the endomycorrhizal associations in greenhouse propagated yellow-poplar seedlings. Although studies have suggested that improved growth results from infestation of germination media with forest soil containing spores of endomycorrhizae (Clark, 1963; 1964; 1969), basic experiments have not yet been conducted to determine the relative degree of infectivity of specific symbionts in seedlings of this commercially important species. Before inoculation of yellow-poplar seedlings with endomycorrhizae can be seriously considered as a method of tailoring seedlings for outplantings to adverse sites, basic information on fungal colonization in this species must be obtained.

The purposes of this study were to determine (1) whether the two *Glomus* species (*G. mosseae* and *Glomus fasciculatus* (Thaxter) (Gerdemann and Trappe) had similar degrees of colonization in yellow-poplar seedlings, (2) whether all seedlings within inoculated flats were equally

colonized, (3) whether the two fungi acted synergistically when present in combination and (4) how seedling root morphology was modified by colonization.

CHAPTER I

REVIEW OF THE LITERATURE

The term "mycorrhizae" was first used by Frank (1886) to describe a symbiotic relationship between fungi and the roots of plants (Nicolson, 1967). The association is beneficial to both host and fungus, and has been described as an example of "physiologically well-balanced reciprocal parasitism" (Hacskaylo, 1972). In mycorrhizal plants root morphology is altered, however the host plant is not damaged, provided the fungal infection remains in the root cortex (Gerdmann, 1974). The types of infective fungi, the kinds of fungal structures produced, and the relationships of these structures to the host plant roots are the distinguishing characteristics for three classes of mycorrhizae.

Endomycorrhizae (vesicular-arbuscular, or VA mycorrhizae) are formed by aseptate fungi which belong to one of several species of *Endogonaceae*. Hyphal structures produced by these fungi form a loose network in the soil and penetrate into the root cortex. Within the cortical cells the hyphae form intracellular coils, arbuscules, and large, swollen vesicles (Mosse, 1973a). Ectomycorrhizae are formed primarily by species of *Basidiomycetes*. These symbionts produce a dense hyphal mantle which encloses the infected rootlet. In contrast, ectomycorrhizal hyphae penetrate the root cortex between external cells (intercellular hyphae) forming a Hartig net. Strands of hyphae extending into the soil comprise the water and nutrient absorbing surfaces of a mycorrhizal plant (Harley, 1969). Ectoendomycorrhizae, the third

type of mycorrhizal structure, have features of both ecto- and endomycorrhizae (Marx, 1975).

I. HISTORICAL CONSIDERATIONS

Early studies of endomycorrhizae primarily dealt with descriptions of the anatomy and occurrence of these symbionts, and the attempts to culture various fungal species. Vesicles were first described by Janse in 1896 as special fungal organs observed in 75 plants investigated in Java. Galland introduced the term arbuscule in 1905 to describe haustorium-like structures in roots of infected plants (Butler, 1939). Butler also credits Peyronel with one of the most important early studies of the distribution of endotrophic mycorrhizae in field and woodland flower plants. In this study, endophytes were observed in 132 of 151 plants in 36 natural orders. Nicolson (1967) recently summarized some early VA mycorrhizae studies.

Limited research on the effects of endomycorrhizae and plant growth was conducted prior to 1960, however, much has been done with these specific endophytes during the last 15 years. Mosse (1973a) suggested that several factors might account for the recent increase in endomycorrhizae popularity, e.g., (1) availability of purified Endogone spores or sporocarps for inoculation of host plants to provide a more sophisticated system for evaluation of the effects of endomycorrhizae on plant growth, (2) the descriptions of improved methods for visualization of the fungal structures, and (3) the attention of several reviews on the importance and potentialities of mycorrhizae.

Occurrence in plant species. Endomycorrhizae are found in the Bryophytes, Pteridophytes, Gymnosperms, and Angiosperms (Gerdemann, 1968); they occur on more plant species than any other mycorrhizal type. These fungal symbionts are found in the roots of most cultivated crops: grasses, herbs, shrubs, and a majority of angiosperm forest and shade trees. Marx (1975a) noted that many economically important angiosperms including *Platanus, Ulmus, Populus, Acer, Liquidambar, Fraxinus, Juglans,* and *Liriodendron* normally form endomycorrhizae. Endomycorrhizae occur in most genera of Gymnosperms, including the *Cupressaceae* (*Thuja, Cupressus,* and *Juniperus*) and *Taxodiaceae* (*Sequoia* and *Taxodium*) (Hacskaylo, 1972); and Gerdemann (1968) stated that these fungi are formed in most families with the following exceptions: (1) families that are ectomycorrhizal, primarily *Pinaceae, Betulaceae,* and *Fagaceae*; (2) families that are endomycorrhizal with septate endophytes, such as *Orchidaceae* and *Ericaceae*; and (3) groups that have been reported to be non-mycorrhizal, primarily families in the order *Centrospermae,* and the families *Crucifereae, Fumariaceae, Cyperaceae, Commelinaceae, Urticaceae,* and *Polygonaceae*. More recent studies have shown that the *Centrospermae* in the family *Chenopodiaceae*, and several species in the *Cyperaceae* and *Cruciferae* are endomycorrhizal (Ross and Harper, 1973; Mejstrik, 1972; Williams and Alden, 1975). Both ectomycorrhizae and endomycorrhizae occur in a few plant families including the *Salicaceae, Juglandaceae, Tiliaceae, Myrtaceae,* and *Fagaceae* (Gerdemann, 1965).

Geographic distribution. Endomycorrhizae occur worldwide, in areas ranging from the tropics to the arctic; there are few natural

forest communities that do not contain mycorrhizal species. Ectomycorrhizae on trees are most common in cool regions, ecto- and endomycorrhizae are common in the temperate regions, and endomycorrhizae are most frequent in the tropics (Meyer, 1973).

Taxonomy of *Endogonaceae*. Vesicular-arbuscular mycorrhizae are formed by certain species of *Endogonaceae*, a family of fungi in the *Mucorales* (Gerdemann and Trappe, 1974). Until recently little was known about the *Endogonaceae*, particularly in relation to life cycles and taxonomic relationships. Recently, Gerdemann and Trappe (1975) noted that *Endogonaceae* are the most common soil-borne fungi. In 1974, they revised the genus *Endogone* (see Table I). According to them, seven genera (*Glomus, Sclerocystis, Gigaspora, Acaulospora, Glaziella, Modicella,* and *Endogone*) commonly form endomycorrhizae. *Glomus* may be sporocarpic or not, with chlamydospores generally formed terminally on a single undifferentiated hyphae. Chlamydospores of *Sclerocystis* borne in sporocarps are arranged in a single layer around a central plexus of sterile hyphae. *Gigaspora* species bear the spores at the tip of a single large suspensor-like cell from which a slender hyphae usually projects to the spore. *Acaulospora* does not form sporocarps, and resting spores are borne laterally on hyphae terminating nearby in a large thin-walled vesicle (Mosse, 1973a; Gerdemann and Trappe, 1974).

Morphology of VA mycorrhizae. Endomycorrhizae produce little change in external root morphology of the host plant. There is an extensive, loose hyphal network that can grow a considerable distance into the soil, but it does not affect external root morphology. The

TABLE I

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.

fragile network is easily destroyed in root excavations. "Beaded" roots on *Acer* were thought to be evidence of mycorrhizal infection, but subsequent studies proved otherwise (Medve, 1971). Endomycorrhizae can produce slight pigmentation in the roots of host plants. However, color is quite variable, and appears to be affected by age and size of the roots; therefore it cannot be considered a definitive infection characteristic.

Root morphological changes caused by vesicular-arbuscular mycorrhizae are readily observable after the cells are cleared and stained (Phillips and Hayman, 1970; Trappe, et al., 1973). Hyphae produce appressoria on epidermal cells or root hairs distally from the meristamatic region. Following infection, hyphae penetrate the epidermal and cortical cells, but never invade the endodermis, stele, or root meristem (Sanders and Tinker, 1973). Hyphae may be intracellular, depending on host species (Gerdemann, 1965). Nonseptate hyphae produced by VA fungi are irregular in shape, and highly variable in diameter and thickness of hyphal walls (Nicolson, 1967). When growing conditions are unfavorable or when the fungus is dying, the hyphae may become septate (Gerdemann, 1968).

Soon after infection, arbuscules are formed within root cortical cells. These specialized hyphal structures consist of fine, dichotomously-branched filaments which may occupy the entire cell lumen. The diameter of the branched filaments is less than one micron (Gerdemann, 1968). Under some nutritional stresses, the morphology of arbuscules may be altered; when available phosphorus is limited, heavy infections of vesicular-arbuscular mycorrhizae develop (Sanders and Tinker, 1973),

while plants grown in high levels of available phosphate develop few arbuscules. Woolhouse (1975) hypothesized that arbuscule formation is related to phosphate concentration and can be summarized in three stages: (1) the host plant sends a signal that indicates phosphate is limited, (2) the fungus receives the signal and responds by penetrating the host cell and forming arbuscules, and (3) the host plant modifies the fungus such that the transport of phosphate is reversed and phosphate flows from the fungus into the host plant. Eventually, the arbuscules are digested and the contents absorbed by the host cells (Kaspari, 1973).

Vesicles are terminal, ovate to spherical, structures produced by VA mycorrhizae. These structures are extremely variable in shape, size and cell wall thickness (Gerdemann, 1968). Often, thick-walled vesicles resemble chlamydospores found in the soil (Gerdemann, 1965). Vesicles form either intracellularly or intercellularly, depending on host species (Gerdemann and Trappe, 1974). For example, *G. fasciculatus* infested yellow-poplar (*Liriodendron tulipfera L.*) and maize (*Zea mays L.*) produced intracellular vesicles in yellow-poplar, and intercellular vesicles in maize (Gerdemann, 1965).

Vesicles are thought to be temporary storage organs and arbuscules may function in the reverse manner to haustoria, releasing materials to host cells (Kormanik, et al., 1977).

II. BENEFITS OF MYCORRHIZAE TO PLANTS

Trees having abundant ectomycorrhizal fungi exhibit: (1) an increased uptake of nutrients and water from the soil, (2) an increased

tolerance to drought, high soil temperatures, and extreme changes in soil pH, and (3) an increased resistance to infections by root pathogens such as *Pythium* and *Phytophthora* (Marx, 1973). Less research has been conducted on the benefits to trees through endomycorrhizal infection; however, it has been suggested that the functions of endomycorrhizae are similar to those of ectomycorrhizae (Williams, et al., 1974; Gerdemann, 1968).

The effects of endomycorrhizal infection on growth and development in agronomic crops are well documented. Studies in maize have shown increased growth in plants inoculated with several *Endogone* species (Gerdemann, 1964; Daft and Nicolson, 1966), as well as in plants inoculated with lyophilized endomycorrhizal roots (Jackson, et al., 1972). An increase in dry weight has been reported in maize plants inoculated with *Endogone* sporocarps (Gerdemann, 1964), *G. fasciculatus* (Gerdemann, 1965), and *Glomus mosseae* (Khan, 1972); plus an increase in dry weight and number of grains per ear has been reported in maize inoculated with *Endogone mosseae* (Khan, 1975b). Increase in the amount of vascular tissues and the development of pollen has been reported in maize inoculated with *Endogone macrocarpa* (Daft and Okusanya, 1973).

Increased growth has also been noted in tobacco and tomato plants (Daft and Nicolson, 1966), onions (Mosse, et al., 1969), soybean plants (Ross and Harper, 1970), and the tropical grass, *Papsalum notatum* (Mosse, 1972). *Endogone* also increased the vascular tissue content of tomato, strawberry and petunia plants; and stimulated flower production in strawberries (Daft and Okusanya, 1973). Studies in wheat have shown that infection with *Endogone* resulted in increased dry weight and

growth (Khan, 1975a), and a three-fold increase in grain yield (Khan, 1975b). Increased growth, reproduction, and number and weight of nodules have been observed in the French Broad bean inoculated with *Endogone macrocarpa* (Daft and El-Giahmi, 1974), while stimulation of nodulation has been observed in herbage legumes treated with *Endogone* spores (Crush, 1974).

Recent studies have also shown that several other species of agronomic crops benefit greatly from endomycorrhizal symbiosis. An increase in the rate of growth and development of root and shoot systems of cotton has been reported in the presence of *Endogone calospora*. Earlier flowering and boll maturation suggest the existence of a physiologically beneficial relationship between the fungus and cotton (Rich and Bird, 1974). Clover has been shown to be highly dependent on infection by mycorrhizal fungi for growth in many soils (Powell, 1976a). A three-year study of the effects of *G. mosseae* and *G. fasciculatus* in potato plants has shown that infection with both endophytes results in greater dry weight of tops, roots, and total plants than uninoculated controls.

The few studies that have been conducted on tree species have shown that endomycorrhizal symbiosis results in several benefits to the host plant. Studies have shown increased growth in apple seedlings and cuttings inoculated with sporocarps of *Endogone* species (Mosse, 1957), in pot grown peach seedlings inoculated with a *Glomus* species (Gilmore, 1971) and in rough lemon inoculated with *G. mosseae* (Marx, et al., 1971; Kleinschmidt and Gerdemann, 1972). Recent evaluations have suggested that endomycorrhizal inoculations have beneficial effects

in several species of forest trees. Greater mean dry weight has been observed in mahogany seedlings inoculated with *Endogone* spores than in uninoculated plants (Redhead, 1975). Greenhouse studies of sweetgum seedlings inoculated with *G. mosseae* showed an 82% growth increase for infected seedlings, compared to controls (Bryan and Ruehle, 1976). Nursery tests with sweetgum, sycamore, white ash, black walnut, black cherry, box elder, sugar maple and red maple seedlings demonstrated that seedlings inoculated with fungi were several times larger than non-inoculated seedlings (Marx and Beattie, 1977). Small scale field studies have also shown that specific endomycorrhizae are extremely important in the early rapid growth of hardwoods. Bryan and Kormanik (1976) inoculated sweetgum seedlings with *G. mosseae* and naturally occurring endomycorrhizae. Without the fungus, the seedlings ceased growth, however, natural inoculum and *G. mosseae* yielded greater height, root collar diameter, and oven dry root and top weight.

Few studies have been published describing the effects of endomycorrhizal inoculation in yellow-poplar. Clark (1963; 1964; 1969) reported that pot grown yellow-poplar seedlings inoculated with field soil containing confirmed endomycorrhizae showed greater height growth and total fresh weight than uninoculated seedlings. Improved growth has also been noted in yellow-poplar seedlings inoculated with *Endogone fasciculatus* (Thaxter) compared to uninoculated controls (Gerdemann, 1965).

III. UPTAKE AND UTILIZATION OF PHOSPHORUS

Plants with endomycorrhizae contain a higher concentration of phosphorus than do non-mycorrhizal plants, and it has been suggested that

the improved growth of the infected host is associated with increased phosphorus uptake by the fungal symbiont (Mosse, 1973a).

Studies with radioactively labeled phosphorus (^{32}P) have suggested that: (1) all endomycorrhizae appear to increase phosphorus uptake but with differing degrees of efficiency, (2) that endomycorrhizae and non-mycorrhizal plants appear to use the same sources of available phosphate from the soil, and (3) that endomycorrhizal plants may be able to use phosphate present in extremely low concentrations in the soil, whereas non-mycorrhizal plants do not. Gray and Gerdemann (1967) observed increased radioactivity in roots of yellow-poplar seedlings grown in soil or nutrient solution containing ^{32}P. That the phosphorus was concentrated in mycorrhizal regions of roots of infected plants was shown in later evaluations of levels of radioactivity in root segments of onions exposed to the radioisotope (Gray and Gerdemann, 1969). Additional studies in onion plants grown in a range of soils labeled with radioactive phosphorus demonstrated that although the mycorrhizal plants took up more phosphate from the soil than uninoculated onions, the specific activity of the absorbed phosphorus was very similar in both infected and uninoculated plants (Hayman and Mosse, 1972a). Apparently both mycorrhizal and non-mycorrhizal plants used the same or similarly labeled sources of phosphate. Work by Sanders and Tinker (1971) support these theories. Powell (1976b) later demonstrated that the specific activity of phosphorus was also the same in plants infected with different species of mycorrhizae.

Mosse, et al. (1973) measured radioactivity of ^{32}P in three plant species with and without mycorrhizae to determine whether the

relative concentrations of phosphorus in the soil affected uptake of this element. In two soils the specific activity of phosphorus taken up by mycorrhizal and non-mycorrhizal *Melinis minutiflora* was similar, indicating that even in very phosphorus deficient soils the non-mycorrhizal plants used the same source of phosphate as the mycorrhizal plants. However, in two other soils, non-mycorrhizal plants of *Centrosema pubescens* and *Papsalum notatum* took up no ^{32}P, whereas mycorrhizal plants of both species contained radioactivity. It was postulated that non-mycorrhizal roots of some plants do not use phosphate present in extremely low concentrations in the soil, but that mycorrhizal roots or fungal hyphae do.

Effect of source and concentration of phosphorus. The effects of source and concentration of phosphate on the degree of mycorrhizal infection have also been studied in detail. Experiments have shown that additions of soluble phosphate to the soil improved growth of mycorrhizal plants less than that of non-mycorrhizal plants (Daft and Nicolson, 1969a; Murdoch, et al., 1967; Khan, 1972; Mosse et al., 1976; Baylis, 1967; Mosse, 1973b; and Ross, 1971). In some instances, an actual decrease in rate of growth has been observed in mycorrhizal plants grown in soil supplemented with phosphate in the soluble form (compared with growth rates of non-infected plants). Reductions in the degree of infection have also been noted in both field and pot experiments in which soluble phosphate was used as the source of phosphorus (Mosse, 1973b; Baylis, 1967; Daft and Nicolson, 1969b). Other studies have shown that the amount of endomycorrhizae infection can generally be

manipulated by differential timing of soluble phosphate application. Daft and Nicolson (1966) reported that mycorrhizal tomatoes responded greatly to small additions of bonemeal, whereas their relative advantage over non-mycorrhizal plants decreased when bonemeal was increased 16- and 32-fold. In this study, relative improvements in growth were greatest with tricalcium phosphate, less with finely ground apatite, and least with more soluble dicalcium phosphate. Improved growth and higher phosphorus content has also been reported in mycorrhizal maize treated with the relatively insoluble rock and tricalcium phosphate (Murdoch, et al., 1967), however, more recent work in which mycorrhizal and non-mycorrhizal soybeans were supplied with phosphate sources of varying availability suggested the principle source of phosphate was the one most readily available (Ross and Gilliam, 1973).

It is now generally accepted that large growth responses can result from the development of vesicular-arbuscular mycorrhizae on the root systems of plants growing in phosphate deficient soils. Improved growth has been noted in mycorrhizal maize (Khan, 1972; Mosse and Hayman, 1971), onions (Hayman and Mosse, 1971; Mosse, 1973b; Mosse, et al., 1969) and wheat (Khan, 1975b).

Mechanisms of phosphorus uptake. Studies with ^{32}P have suggested that the increased uptake of phosphorus by mycorrhizal plants is not the result of alterations of the chemical state of the element by the fungi. However, it is known that phosphate ions have small diffusion coefficients in soils, and that there is a depletion of phosphate in solution immediately around the roots (Lewis and Quirk, 1967). The more actively the roots absorb, the greater this depletion of phosphate will be.

Uptake of phosphorus by the expanded network of external hyphae of mycorrhizal fungi, with subsequent translocation and release to the host, has been suggested as the most likely of the mechanisms for improved phosphorus nutrition of VA mycorrhizal plants (Sanders and Tinker, 1971; Hayman and Mosse, 1972b). Evidence has been presented by Hattingh, et al. (1973) that confirms that the hyphal network of endomycorrhizal fungi enables plants to remove phosphate from a large soil volume, extending beyond the immediate vicinity of the root surface. Onion mycorrhizae had high levels of radioactivity when ^{32}P labeled phosphate was injected into soil 27 mm. from the root surface, whereas non-mycorrhizal roots had little radioactivity. Autoradiography indicated diffusion of ^{32}P in the soil of only 7.5 mm. or less from the point of application. When the hyphae from mycorrhizal roots were severed, mycorrhizal roots did not significantly differ in radioactivity from non-mycorrhizal roots. Schoknecht and Hattingh (1976), using X-ray microanalysis of onion mycorrhizae found that the onion cells with arbuscules contained higher levels of phosphorus than adjacent cells without arbuscules. Thus, the external hyphae of VA fungi absorb phosphate, transfer it through soil and into the root, and it is likely that much of it is released in cortical cells containing arbuscules.

The distribution of the extra absorbing surface of the hyphae is critical. The mycelium must extend outside the phosphorus depletion zone of the root if phosphorus uptake and translocation is to be a gain to the plant (Sanders and Tinker, 1973). The greater the extent to which the hyphae of the mycorrhizal fungus explore the volume of soil surrounding the root, the more extensive should be the phosphorus

uptake zone of that root. In a study designed to compare the zones of phosphorus uptake (distance from the root surface from which plants are able to obtain phosphorus), mycorrhizal and non-mycorrhizal onion seedlings were grown in individual soil chambers in which roots were confined to one side of a barrier. External hyphae of *G. fasciculatus* arising from mycorrhizal roots grew into an adjacent volume of soil. ^{32}P was injected into the soil at one centimeter intervals, up to a distance of eight centimeters from the confined roots. The mycorrhizal fungus extended the phosphate uptake zones to a distance of at least seven centimeters from the root surface, suggesting that mycorrhizal plants have access to phosphate which is considerably beyond the one to two centimeter zone normally assumed to be the region of phosphate depletion (Rhodes and Gerdemann, 1975).

IV. UPTAKE AND UTILIZATION OF OTHER NUTRIENTS

Most studies designed to show the influence of endomycorrhizae on nutrient uptake of higher plants have concentrated on phosphorus, however, endomycorrhizae also absorb other nutrients. Increased nitrogen, potassium, calcium, sodium, magnesium, iron, manganese, copper, zinc, boron, and aluminum have been observed in mycorrhizal plants, but there are variances according to host species. A higher potassium, iron and copper content and a lower manganese content has been noted in mycorrhizal apples (Mosse, 1957), and mycorrhizal soybeans have been shown to accumulate increased quantities of phosphorus, nitrogen, calcium, copper and manganese (Ross and Harper, 1970). Conversely, lower concentrations of potassium, magnesium, boron, and manganese have been observed in mycorrhizal maize (Gerdemann, 1965).

Severe zinc deficiency symptoms in stunted peach seedlings have been eliminated following inoculation with *Glomus* species (Gilmore, 1971). Studies with radioactive sulfur (^{35}S) have shown that mycorrhizal maize and red clover increased rates of sulfur uptake compared to uninoculated plants (Gray and Gerdemann, 1973). Jackson, et al. (1973) found that mycorrhizal soybeans absorbed significantly more strontium (^{90}Sr) from the soil.

V. WATER

The relationship of mycorrhizae to water transport in plant roots has also been investigated. Endomycorrhizae have been shown to decrease the resistance to water transport in soybean plants. Several mechanisms have been postulated to explain the differences in resistance between mycorrhizal and non-mycorrhizal plants: (1) the external hyphae may increase the total surface area in the root system; (2) the hyphae which penetrate the root cortex to the endodermis may provide a low-resistance pathway for movement of water across the epidermal and cortical cells of the root; (3) the hyphae could increase nutrient uptake, which in turn, could decrease the resistance to water transport within roots; or (4) the mycorrhizal infection might increase root growth so that there is a larger root system (Safir, et al., 1971).

Resistance to water transport into the roots of mycorrhizal plants was approximately 40% lower than in non-mycorrhizal controls (Safir, et al., 1972); additions of nutrient solutions to non-mycorrhizal plants essentially eliminated this difference (Safir, et al., 1972). It was suggested that lowered resistance of mycorrhizal roots growing in

soil with low levels of nutrients probably resulted from enhanced nutritional status of plants, brought about by the fungus. Decreased resistance to water transport may also be caused by changes in root morphology. Daft and Okusanya (1973), found that mycorrhizal infection increased the amount of vascular tissue in tomatoes, petunias, and maize, probably as the result of greater phosphorus uptake.

CHAPTER II

MATERIALS AND METHODS

I. PREPARATION OF SOIL AND YELLOW-POPLAR SEEDS

A germination medium of silt loam, sand, and mulch sized pine bark (1:1:1) was thoroughly mixed and treated with methyl bromide for 72 hours under optimum weather conditions. The germination medium was aereated for two days.

Yellow-poplar seeds were stratified in bags of moist peat moss for 90 days at 35 F. Before planting, the seeds were surface sterilized for three minutes in a 0.05% solution of sodium hypochlorite, and subsequently rinsed in sterile distilled water.

II. MYCORRHIZAE PREPARATION

Soil cultures of *Glomus mosseae* (Nicolson and Gerdemann; Gerdemann and Trappe) and *Glomus fasciculatus* (Thaxter) (Gerdemann and Trappe) were obtained from the Institute of Mycorrhizal Research and Development, U. S. Forest Service, Athens, Georgia. Cultures of the two fungi had previously been maintained in the greenhouse on roots of pot grown *Sorghum vulgare* var. *roxburghi* (Stepf.) Haines. Aliquots of the soil containing sorghum roots as well as the specific endophyte served as inoculae for the present study.

III. INOCULATION PROCEDURES

Four germination flats (2' X 4' X 6" deep) were treated with methyl bromide and filled with sterilized medium. The medium was

infested with either 1600 ml. of *G. mosseae* or *G. fasciculatus*, or 3200 ml. of equal parts of the two endophytes. To avoid contamination disposable gloves were used in thoroughly mixing the growth medium and inoculum in each flat.

Yellow-poplar seeds (150) were sown in rows according to seed source in each treatment flat. Seeds were covered with approximately one-fourth inch germination medium and the flats moistened and maintained in the greenhouse for the duration of the experiment. (See Table II.)

IV. PROCEDURE FOR TRANSPLANTING GERMINATED SEEDLINGS (ORIGINAL DESIGN)

In the original experimental design, five replicate seedlings from each treatment and seed source were to have been transplanted into three-gallon containers containing the 1:1:1 growth medium, supplemented with lime (calcium oxide, rate: 750 lbs/acre) and fertilizer (10:10:10, rate, 125 lbs/acre). At the end of the growing season, measurements of root collar diameter and height growth were to have been made, and each seedling was to have been harvested for evaluation of degree of colonization of the roots by endomycorrhizae. Statistical comparisons of the degree of colonization of roots with the two growth parameters were to have been made using a split plot design. Because a few seedlings in each germination flat became infected with *Pythium*, it was necessary to terminate the experiment 12 weeks after the seeds were planted. Thus, it was not possible to obtain estimates of height growth and root collar diameters in replicate seedlings within the various treatments. However, colonization estimates on the various

TABLE II

SOURCES FOR YELLOW-POPLAR SEEDS

Seed Source	Location	Elevation	Percent Filled
1	Paint Creek Campground (Tennessee)	1600'	16.0
2	Courtland Place (Tennessee)	2400'	15.0
4	Below Rich Mountain Look-out Tower (Tennessee)	3600'	25.0
5	Near Hurricane Gap (North Carolina)	2900'	22.0
6	On Road to Hurricane Gap	2200'	16.0

seedlings and observations of the morphological characteristics of the two endophytes were made.

V. PROCEDURES FOR CLEARING ROOTS AND STAINING ENDOMYCORRHIZAE

After 12 weeks, five seedlings were harvested from each treatment flat for evaluation of endomycorrhizal colonization. Seedling root systems were carefully isolated and lifted from the germination medium, rinsed in tap water, and blotted dry. Tissues were fixed in 10.0 ml. of Formol-Alcohol (5.0 ml. formalin, 5.0 ml. glacial acetic acid, and 90.0 ml. 70% ethanol alcohol) (Humason, 1967).

Seedling roots were stained using the method of Phillips and Hayman (1970). Whole roots were heated at 90 C for 30 minutes in 10% KOH to remove cytoplasm and the nuclei from the cortical cells. Roots were immersed for 10 minutes in peroxide ammonia solution (1.0 ml. Parson' Ammonia Water, 10.0 ml. hydrogen peroxide, and 200.0 ml. distilled water) at 20 C until bleached; then rinsed in distilled water, and acidified in 1% HCl. The cleared roots were stained by simmering for 10 minutes in 0.5% trypan blue in lactophenol, and destained in clear lactophenol. Stained fungal structures were distinct within the cortical cells of the cleared roots.

VI. SCORING OF SLIDES

Ten segments, one centimeter long, were cut from each root and mounted on microscope slides in lactophenol mounting medium. Coverslips were sealed with clear fingernail polish (Humason, 1967). To

obtain a quantitative estimate of the degree of endomycorrhizal colonization, 50 microscopic fields (area: 3 square mm., magnification 100X) were scored for the presence of intracellular hyphal coils, arbuscules, vesicles, and spores. Photomicrographs of representative fungal structures were taken on a Zeiss Photoscope, using Kodak high-contrast copy film.

This method provides several advantages in quantifying the degree of colonization in inoculated seedlings. Since fixed roots can be stored for several weeks in Formol-Alcohol prior to staining, seedlings can be harvested sequentially, and processed for microscopic evaluation at later times. The method provides rapid scoring of root segments; colonization is easily seen at low power magnification, and detailed morphology of endomycorrhizal structures is readily observed at 400X. Evaluations of 10 segments from each root allows for a representative assessment of roots that are sparsely colonized.

CHAPTER III

RESULTS

I. GERMINATION OF SEEDLINGS

Approximately 150 yellow-poplar seeds from each of five seed sources were sown in control and mycorrhizal inoculated growth media. Germination first occurred 11 days after planting. All flats were subsequently monitored at two to four day intervals, and the number of seedlings recorded for each flat. Differences in the germination patterns were not analyzed, however both the germination rate and total seedlings varied among seed sources and treatments. Total seedlings germinated in each flat at the end of the twelfth week are shown in Table III.

Approximately 11 weeks after planting, random seedlings in all treatment flats were noted to have constrictions at the root collars; this is the characteristic pathology associated with *Pythium* infection. Because it was not known how such an infection might affect the degree of colonization of roots by mycorrhizae, all seedlings were harvested at 12 weeks, and five apparently healthy seedlings from each treatment were selected and prepared for microscopic study.

II. MYCORRHIZAL COLONIZATION

Ten segments, one centimeter long, from each root were evaluated for endomycorrhizal fungal structures. At a magnification of 100X, spores, intracellular hyphal coils, arbuscules, and vesicles were

TABLE III

GERMINATION OF SEEDLINGS FROM FIVE SEED SOURCES IN GROWTH MEDIUM INOCULATED WITH *GLOMUS MOSSEAE* AND *GLOMUS FASCICULATUS*

Seed Source	Number and Percentage of Germinations in Each Flat*							
	Control		*G. mosseae*		*G. fasciculatus*		Combination	
	No.	%	No.	%	No.	%	No.	%
1	13	8.6	16	10.6	28	18.8	30	20.0
2	20	13.3	5	3.3	15	10.0	19	12.6
4	23	15.5	21	14.0	10	6.6	21	14.0
5	19	12.8	17	11.0	22	14.6	10	6.6
6	10	6.6	25	16.6	10	6.6	9	6.0

*Approximately 150 seeds from each seed source were planted in each treatment flat,

clearly visible in all infected root segments. Quantitative counts of the mycorrhizal colonization in seedling roots revealed no spores, VA bodies, or intracellular hyphal coils in any of the seedlings from the inoculated controls. Endomycorrhizal structures were observed in seedling roots from each of the treatments. However, the types of structures observed and the relative degree of colonization varied according to inoculum treatment.

III. FUNGAL MORPHOLOGY IN COLONIZED SEEDLINGS

G. mosseae. Most seedlings from the *G. mosseae* treatment showed extensive fungal colonization. In Figure 1, a low power magnification of representative sections of three infected roots is compared with a similar segment from an uninoculated seedling. In the control root, the cleared cortical cells (c) appeared translucent, with no definitive internal structure; the vascular bundles (xylem and phloem vessels) stained a deep blue (Figure 1A). In all *G. mosseae* infected seedlings, masses of intensely stained fungal structures were clearly visible. In these whole mount root sections, the mycorrhizal structures appeared to be clustered around the vascular bundle, and frequently were so dense as to obscure the entire central core of the root. Studies of roots sectionized at right angles to the longitudinal axis have shown that the majority of the intracellular hyphal coils, arbuscles, and vesicles are actually located with the second layer of cortical parenchymal cells of mycorrhizal roots (Nedve, 1971). Examination of infected root segments at higher magnifications (Figure 2, A and B) revealed that numerous cortical cells were invaded by intracellular

Figure 1. Low power magnification (400X) of an uninfected root and three roots extensively colonized by *G. mosseae*.

A. Control root, the vascular bundle (x) is darkly stained and the cortical cells (c) show no endomycorrhizal colonization. B, C, and D, segments of roots heavily colonized by *G. mosseae*. Endomycorrhizal structures are darkly stained and prominent in cortical cells adjacent to the vascular bundle.

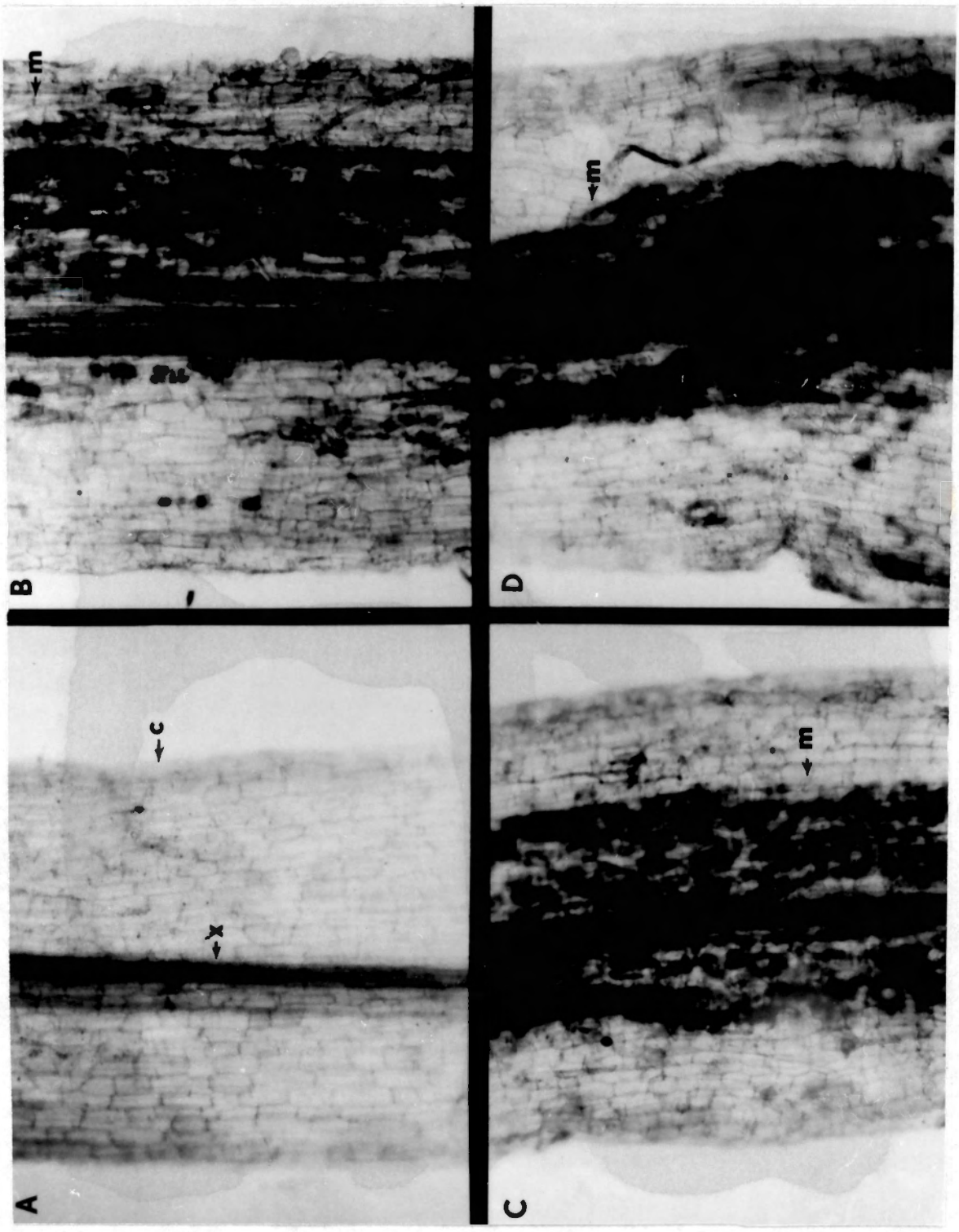

Figure 1

Figure 2. Morphological features of intracellular hyphal coils and arbuscules in *G. mosseae* colonized seedlings.

A and B. Examples of extent of colonization of cortical cells (c) by *G. mosseae*. Numerous arbuscules (a), intracellular hyphae (i) and intercellular hyphae (h) are present (magnification 1000X).

C and D. High power magnification (3000X) of *G. mosseae* arbuscules (a) and intracellular hyphal coils (i). The coiled hypahe have assumed a longitudinal orientation within the cortical cells infected. Arbuscules in all infected cells are mature, with numerous minute bifurcations arising from terminating branches of the hyphal trunk.

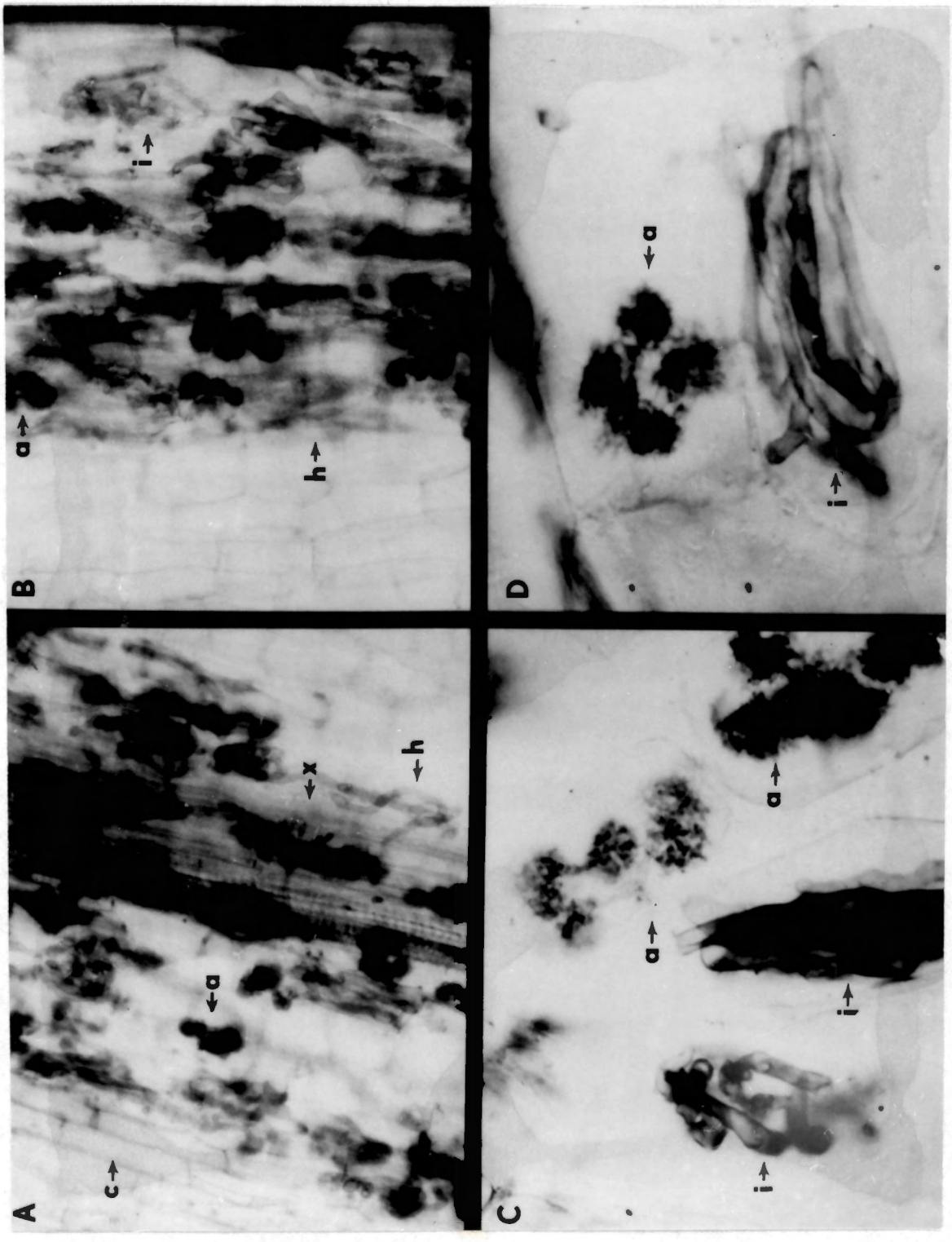

Figure 2

hyphal coils (i), and arbuscules (a). Masses of intercellular hyphal strands (h) were also observed. These were quite variable in size, generally non-septate, and were frequently noted penetrating cortical cells and terminating in vesicles, arbuscules, or coils. The detailed morphological characteristics of the intracellular hyphal structures are shown in Figure 2, C and D, and Figure 3, A-D. The great majority of the arbuscules observed in the *G. mosseae* colonized seedlings occurred singly within the cortical cells and filled the entire cell lumen. Generally, a single hyphal stalk (trunk) was observed penetrating a cortical cell and bifurcating into secondary and tertiary branches. The smallest branches of the tertiary hyphae characteristically terminated in irregular clusters of "stippled" (deteriorated) material. Such arbuscles have been described as the "mature" or "functional" stages of arbuscular differentiation (Kindar and Brown, 1975).

Intracellular hyphal coils were also frequently noted in cortical cells. Although the hyphae were generally quite contorted, the coiling usually assumed a longitudinal orientation within an infected cell (Figures 2D; 3B). Generally, the diameter of the hyphae within a cell was relatively uniform. Vesicles were noted less frequently than arbuscules or intracellular hyphae. These structures were noted to derive terminally from intercellular hyphal strands of varying sizes (Figure 3, C and D). The vesicles frequently filled the entire lumen of the infected cortical cell, and thus appeared as rectangular structures with rounded corners. In all instances the vesicles were deeply stained, with little internal structure.

Figure 3. Morphology of intracellular hyphal coils, arbuscules, and vesicles in *G. mosseae* inoculated seedlings.

A. The small size of the primary hyphal trunk is clearly visible in arbuscules (a) (magnification 3000X).

B. Examples of longitudinal orientation of intracellular hyphae within infected cortical cells (magnification 3000X).

C and D. Darkly stained vesicles (v) arising from the terminal end of intercellular hyphal strand. The vesicles have filled the entire cellular lumen, and assumed the contours of the infected cortical cell (magnification 3000X).

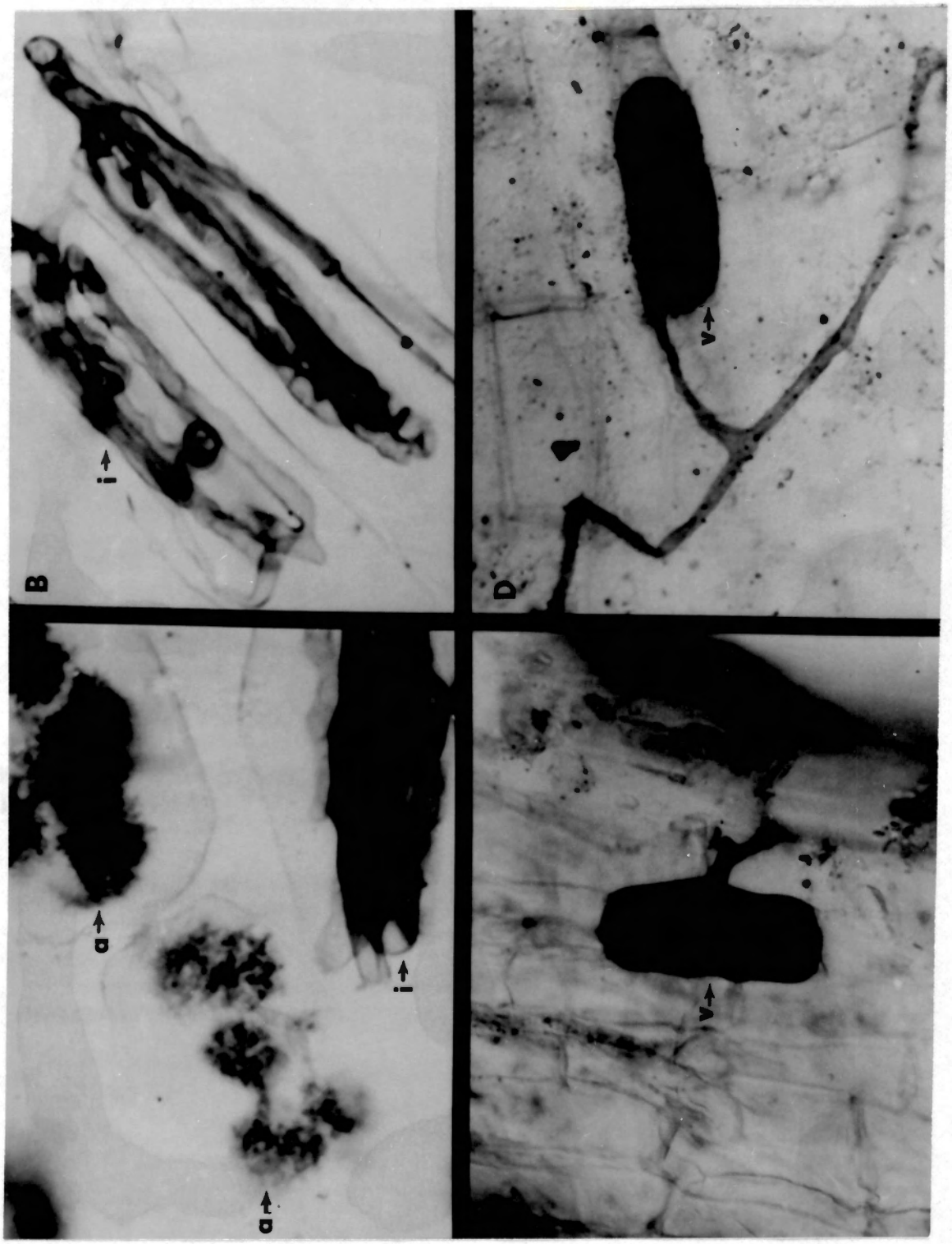

Figure 3

G. fasciculatus. Only a few seedlings treated with *G. fasciculatus* exhibited colonization, and in those seedlings in which mycorrhizal infection occurred, only a few fungal structures were observed. The relative degree of *G. fasciculatus* colonization was quite sparse when compared to that noted in *G. mosseae* (Figure 4A).

In general, the size of the inter- and intracellular hyphae appeared more variable in *G. fasciculatus* than in *G. mosseae*. Several cells with intracellular hyphal coils were noted, but the coiling appeared to be very erratic, often with masses of hyphae twisted within the cortical cells (Figures 4B, 5D). In some instances, the hyphae appeared to follow a course outlined by the cell wall (Figures 4D, 5C). Occasionally septa and lipid droplets were noted in the hyphae strands. The arbuscules in the *G. fasciculatus* seedlings were also more variable in morphology than those observed in *G. mosseae*. A few apparently mature arbuscules were noted, however, most appeared to be less differentiated (Figure 4B, C). Large hyphal trunks were noted penetrating the walls of several cortical cells, and these frequently bifurcated into smaller branches. However, terminal fine branches, indicative of arbuscular disintegration, were not noted in most of the *G. fasciculatus* arbuscules. Vesicles were noted in only one seedling. These structures were ovate, intracellular terminations of a hyphal stalk. The most frequently observed fungal structures in *G. fasciculatus* treated seedlings were spores (Figure 5A). These were spherical, darkly stained structures clustered at the terminal end of a hyphal stalk. In many, internal structures (presumably lipid droplets) could be observed.

Figure 4. Morphology of arbuscules and intracellular hyphal coils in *G. fasciculatus* inoculated seedlings.

A. Low power magnification (400X) of *G. fasciculatus* inoculated seedling. Although intercellular hyphae and arbuscules were present, the infection is not extensive.

B. Arbuscules (a), hyphae (h), and intracellular hyphal coils (i) (magnification 1600X).

C and D. Arbuscules, hyphae, and intracellular hyphal coils (magnification 3000X). The most arbuscules in *G. fasciculatus* infected seedlings were characterized by large hyphae which bifurcated into secondary and tertiary branches. Minute terminal branches were not noted in most *G. fasciculatus* arbuscules. Intracellular hyphae were variable in diameter and contorted within the infected cortical cells.

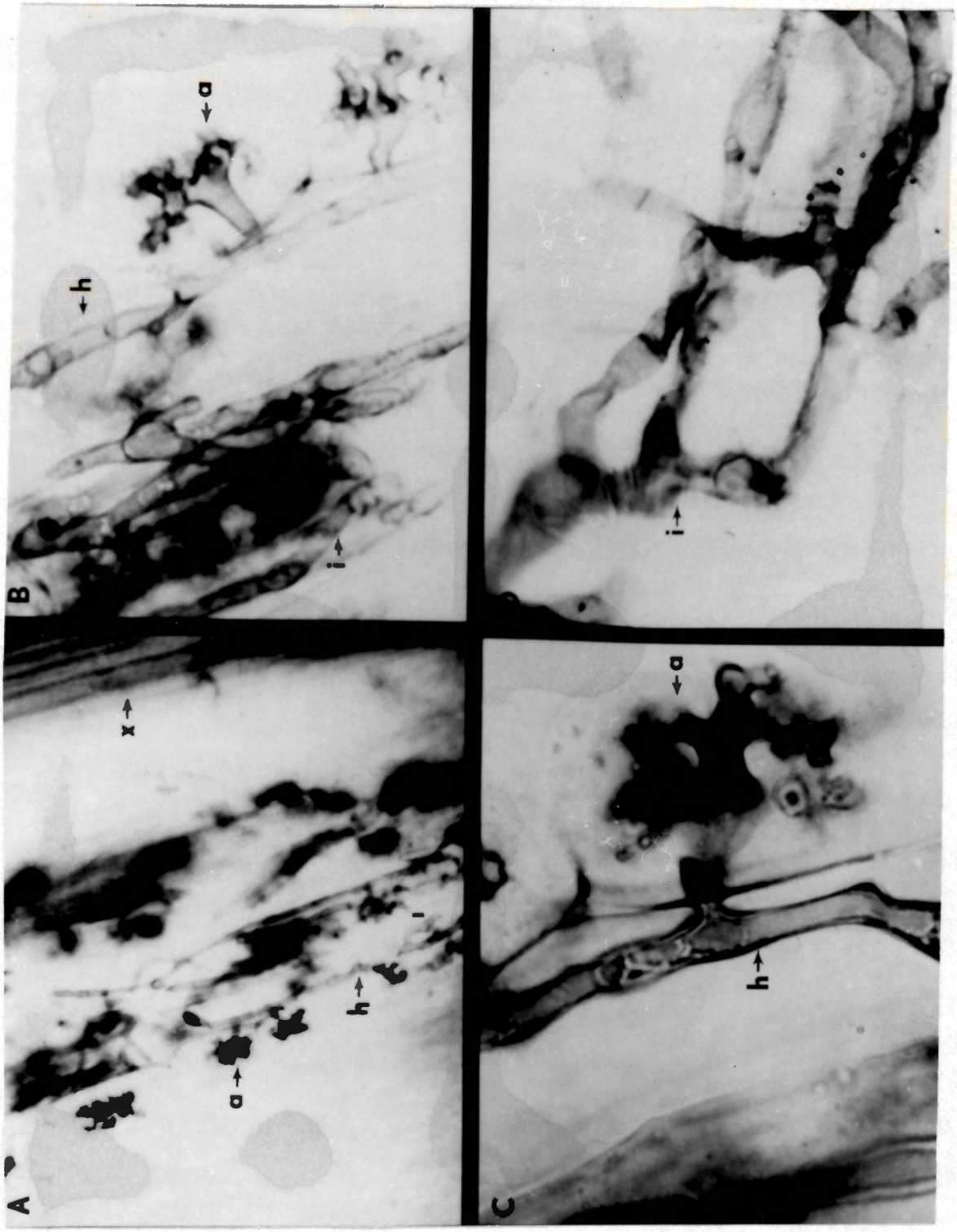

Figure 4

Figure 5. Morphology of spores, vesicles, and intracellular hyphae in *G. fasciculatus* inoculated seedlings (magnification 3000X).

A. Cluster of spores (s) derived from terminal intercellular hyphal strands (h). Lipid droplets can be distinguished in lightly stained structures.

B. Two intercellular vesicles (v) adjacent to the vascular bundle. These structures are ovate, and do not fill the lumen of the infected cells.

C and D. Intracellular hyphal coils (i) and hyphal strands (h). Note variability in diameter of hyphae. Note lack of orientation of hyphae within infected cells. A prominate septa can be seen in a hyphal branch in D.

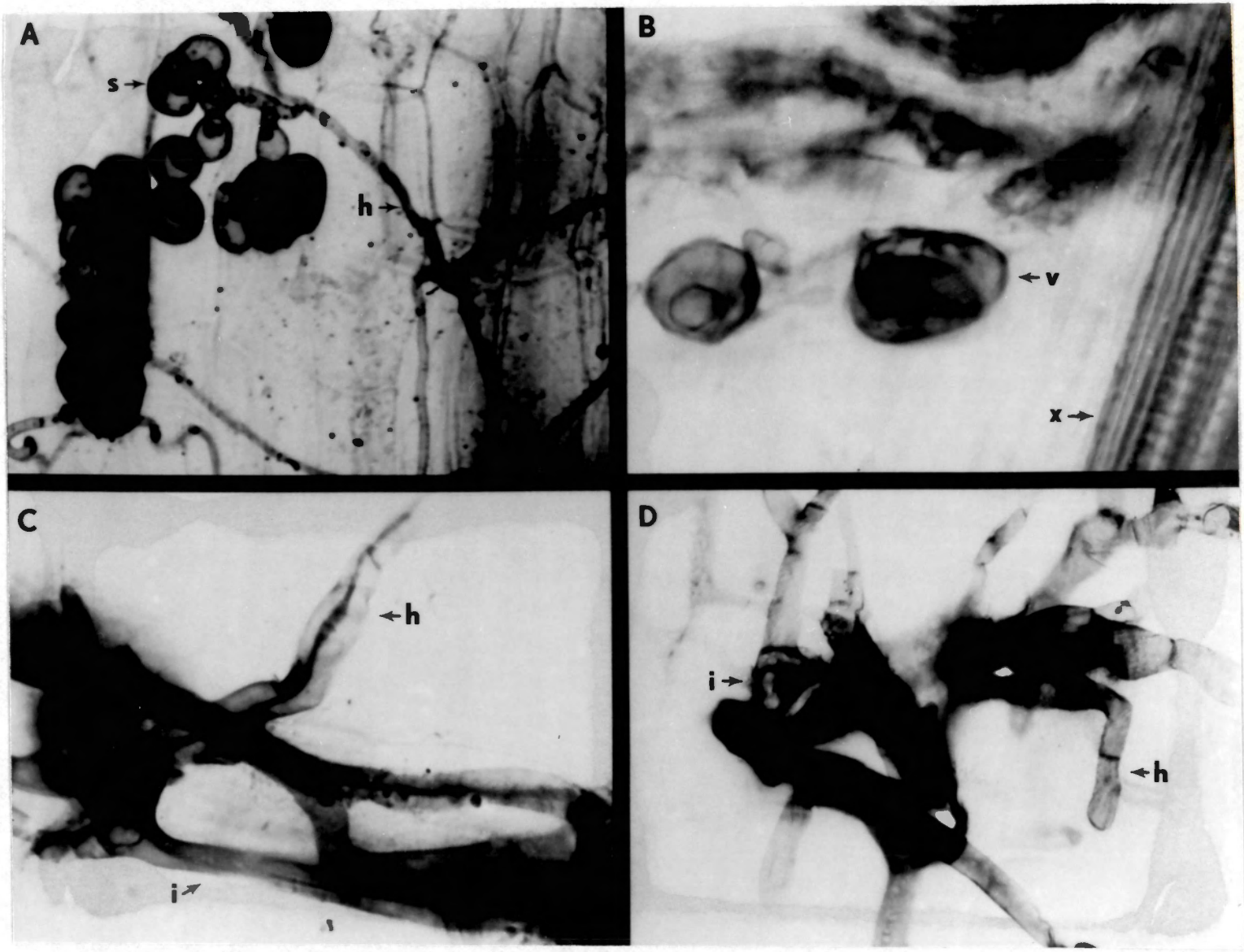

Figure 5

G. mosseae and G. fasciculatus. Very few roots from the combination treatment exhibited colonization, and the number of structures observed was too few to make definitive statements of their specific morphological characteristics. In general, the fungal structures resembled those seen in the *G. mosseae* treatment, more than those in *G. fasciculatus*.

IV. QUANTITATIVE ESTIMATES OF COLONIZATION

G. mosseae treatment. The greatest colonization was observed in seedlings treated with *G. mosseae*. Fungal spores were observed in sections from 23 of 25 roots representing all five seed sources; intracellular hyphal coils were found in 23 roots; and VA bodies in 21 roots (Table IV). Arbuscles were the predominate VA structure in the *G. mosseae* inoculated seedlings. Vesicles were observed, but infrequently (5 of 25 roots). The relative degree of colonization within each root varied considerably. Arbuscles were noted in only one of the 50 fields scored in a seedling from seed source #5 (2% of the root colonized), while 32 fields from a seedling from seed source #1 (64% of the root colonized) contained arbuscules. In heavily infected seedlings, the colonization appeared to be diffuse, and not restricted to specific sections of the root (i.e.--root tip, mid-section, etc.).

Variability was also observed both within replicate seedlings from the same seed source, and among seedlings from different seed sources. Four roots from seed sources #1 and 4 showed the most

TABLE IV

FUNGAL STRUCTURES OBSERVED IN YELLOW-POPLAR SEEDLINGS FROM FIVE SEED SOURCES INOCULATED WITH *GLOMUS MOSSEAE*

Seed Source	Seedling Number	Mycorrhizal Structures: Spores No.	Spores %	Intracellular Hyphal Coils No.	Intracellular Hyphal Coils %	Vesicles/ Arbuscules No.	Vesicles/ Arbuscules %
1	1	13[a]	26[b]	22	44	20	40
1	2	20	40	21	42	21	42
1	3	25	50	26	52	24	48
1	4	13	26	32	64	31	62
1	5	2	4	0	0	0	0
2	1	1	2	3	6	0	0
2	2	1	2	5	10	0	0
2	3	3	6	6	12	2	4
2	4	15	30	14	28	8	16
2	5	17	34	20	40	14	28
4	1	14	28	23	46	21	42
4	2	7	14	19	38	17	34
4	3	22	44	27	54	24	48
4	4	17	34	29	58	23	46
4	5	2	4	0	0	0	0
5	1	13	26	2	4	2	4
5	2	10	20	6	12	5	10
5	3	1	2	4	8	1	2
5	4	15	30	23	46	20	40
5	5	10	20	3	6	3	6
6	1	15	30	9	18	6	12
6	2	9	18	18	36	9	18
6	3	7	14	11	22	10	20
6	4	19	38	7	14	5	10
6	5	14	28	22	44	18	36

[a]Numbers of fields in which mycorrhizal structures were observed (50 microscopic fields evaluated/seedling; magnification 100X, field area 3 mm. square).

[b]Percentage of root colonized.

extensive colonization; however, in both groups, one seedling had no fungal structures other than intercellular hyphae and spores. Roots from seed sources #2 and 5 showed the least amount of infectivity. In general, the number of spores observed was correlated with the relative degree of infection.

G. fasciculatus. Fungal structures were observed in the seedlings inoculated with *G. fasciculatus*; however, the degree of colonization within individual roots was considerably less than that observed in *G. mosseae* (Table V). Intracellular hyphal coils or fungal spores were noted in all but 5 of the 25 treated seedlings, but these were present in only a few of the microscopic fields evaluated from each root (range: 0-10 infected fields/50 fields scored), and in general, only a small number of infected cells were noted per field. Scattered arbuscules were associated with hyphae in 11 infected roots. Only one section with vesicles was observed in the *G. fasciculatus* inoculated seedlings. There were no striking differences in the relative degree of colonization within seedlings from the same treatment, nor among seedlings from the five different sources.

G. mosseae and G. fasciculatus, in combination. Less colonization was noted in the 25 seedlings from the combination endomycorrhizal inoculum than in either the *G. mosseae* or the *G. fasciculatus* treatments alone (Table VI). Spores were present in only 13 roots, and in 15, no hyphal coils or VA bodies were observed. Only a small proportion of the roots were invaded by

TABLE V

FUNGAL STRUCTURES OBSERVED IN YELLOW-POPLAR SEEDLINGS FROM FIVE SEED SOURCES INOCULATED WITH *GLOMUS FASCICULATUS*

Seed Source	Seedlings Number	Mycorrhizal Structures: Spores No.	Spores %	Intracellular Hyphal Coils No.	Intracellular Hyphal Coils %	Vesicles/ Arbuscules No.	Vesicles/ Arbuscules %
1	1	10[a]	20[b]	8	16	8	16
1	2	11	22	8	16	6	12
1	3	2	4	1	2	0	0
1	4	0	0	1	2	0	0
1	5	0	0	1	2	0	0
2	1	4	8	1	2	1	2
2	2	2	4	1	2	1	2
2	3	0	0	0	0	0	0
2	4	8	16	3	6	2	4
2	5	0	0	0	0	0	0
4	1	7	14	2	4	2	4
4	2	0	0	0	0	0	0
4	3	3	6	6	12	2	4
4	4	2	4	1	2	0	0
4	5	0	0	2	4	0	0
5	1	8	16	0	0	0	0
5	2	10	20	0	0	0	0
5	3	1	2	3	6	2	4
5	4	8	16	3	6	0	0
5	5	6	12	4	8	2	4
6	1	0	0	0	0	0	0
6	2	8	16	8	16	5	10
6	3	1	2	4	8	1	2
6	4	0	0	4	8	0	0
6	5	0	0	0	0	0	0

[a]Numbers of fields in which mycorrhizal structures were observed (50 microscopic fields evaluated/seedling; magnification 100X, field area 3 mm. square).

[b]Percentage of root colonized.

TABLE VI

FUNGAL STRUCTURES OBSERVED IN YELLOW-POPLAR SEEDLINGS FROM FIVE SEED SOURCES INOCULATED WITH *GLOMUS MOSSEAE* AND *GLOMUS FASCICULATUS* IN COMBINATION

Seed Source	Seedling Number	Mycorrhizal Structures: Spores #	Spores %	Intracellular Hyphal Coils #	Intracellular Hyphal Coils %	Vesicles/ Arbuscules #	Vesicles/ Arbuscules %
1	1	1[a]	2[b]	0	0	0	0
1	2	4	8	12	24	11	22
1	3	1	2	1	2	0	0
1	4	8	16	3	6	3	6
1	5	3	6	0	0	0	0
2	1	0	0	0	0	0	0
2	2	4	8	0	0	0	0
2	3	6	12	0	0	0	0
2	4	0	0	3	6	2	4
2	5	0	0	1	2	1	2
4	1	0	0	0	0	0	0
4	2	0	0	0	0	0	0
4	3	2	4	0	0	0	0
4	4	0	0	0	0	0	0
4	5	0	0	0	0	0	0
5	1	0	0	0	0	0	0
5	2	1	2	0	0	0	0
5	3	8	16	0	0	0	0
5	4	0	0	3	6	2	4
5	5	0	0	0	0	0	0
6	1	3	6	2	4	1	2
6	2	10	20	0	0	0	0
6	3	0	0	0	0	0	0
6	4	3	6	4	8	4	8
6	5	0	0	2	4	2	4

[a]Number of fields in which mycorrhizal structures were observed (50 microscopic fields evaluated/seedling; magnification 100X, field area 3 mm. square).

[b]Percentage of root colonized.

the fungi in the colonized seedlings. No hyphae, nor VA bodies were observed in roots from seed source #4, and only one in seedlings from seed source #5 contained these structures. In the combination treatment, vesicles were seen in four roots, and arbuscules in nine. The ratio of vesicles to arbuscules in the combination treatment of approximately 1:2 was different than that observed in single treatment with the *G. mosseae* inoculated seedlings (1:4) and in *G. fasciculatus* (0:9).

V. COMPARISON OF COLONIZATION BETWEEN TREATMENTS

Table VII shows the average number of microscopic fields in which mycorrhizal structures were noted in seedlings from each seed source and treatment. Yellow-poplar seedlings from all five seed sources inoculated with *G. mosseae* were more extensively colonized than those inoculated with *G. fasciculatus* or a combination of the two fungi. There may be differences in the relative degree of *G. mosseae* colonization between yellow-poplar seedlings from different seed sources.

TABLE VII

RELATIVE DEGREE OF COLONIZATION IN SEEDLINGS FROM FIVE SEED SOURCES INOCULATED WITH *G. MOSSEAE*, *G. FASICULATA*, OR *G. MOSSEAE* AND *G. FASICULATA**

Fungal Structures	Seed Source 1			2			3			4			5		
	Sp+	Hy	VA	Sp	Hy	VA	Sp	Hy	VA	Sp	Hy	VA	Sp	Hy	VA
Inoculum															
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G. mosseae	14.6	10.1	19.2	7.4	9.6	4.9	12.4	19.6	17.0	9.8	7.6	6.2	12.9	13.4	9.6
G. fasiculata	4.5	4.0	2.9	2.8	1.0	0.9	2.4	2.2	0.9	6.5	2.0	0.9	1.9	3.2	1.2
G. mosseae & *G. fasiculata*	3.4	3.2	2.9	2.0	0.9	0.5	0.4	0	0	1.9	0.9	0.4	3.2	1.5	1.4

*Average number of fields in which fungal structures were observed per fifty fields scored.

+Sp = spores; Hy = Hyphae; VA = vesicles/buscules.

CHAPTER IV

DISCUSSION

I. ENDOMYCORRHIZAL COLONIZATION IN YELLOW-POPLAR

Considerable variability in the degree of colonization was observed within roots from a single treatment. It is not known whether this variability resulted from uneven distribution of the inoculum within the germination medium, or whether subtle differences in light, moisture or pH in the germination flats affected the degree of colonization. Differences in the degree of colonization amoung similarly inoculated seedlings should be considered in future experiments designed to evaluate the effects of endomycorrhizae on parameters such as growth, dry weight, or nutrient uptake.

Extensive infectivity was apparent in 12 weeks in roots inoculated with *G. mosseae*, whereas sparse colonization was noted in *G. fasciculatus* treatments. Since all seedlings in this study were harvested at a single time post-inoculum (12 weeks), it was not possible to determine whether the differences in colonization resulted from a true difference in infectivity, or simply reflected a different rate of spore germination or subsequent fungal growth and development. If spore germination rate is a variable, more extensive colonization may have been possible within *G. fasciculatus*

at a later harvest time. In another study of colonization in yellow-poplar roots by *G. fasciculatus*, extensive arbuscules, vesicles, and intracellular hyphal coils were observed in seedlings harvested 110 days after inoculation (Gerdemann, 1965).

Spores from some *Glomus* species may require specific combinations of soil temperature and pH for maximum germination rate. Schenck, et al. (1975) found that maximum germination of a Washington state isolate of *G. mosseae* occurred at 20 degrees C. In an additional study of the relationship between pH and temperature on the germination of *G. mosseae* spores, Green, et al. (1976) reported that maximum spore germination rates occurred at 25 degrees C and at pH 7.0. It was also noted that a higher pH was required when spores were subjected to lower temperatures. Similar studies of germination requirements for *G. fasciculatus* spores have not been reported; however, it is likely that spore germination in this species is also affected by temperature and pH. These parameters were not monitored in the present study, but it is possible that the prevailing greenhouse conditions may have selectively favored the germination and growth of *G. mosseae*.

In contrast to the findings in the present study, a slightly increased colonization has been observed in apple seedlings inoculated with *G. fasciculatus* as compared to *G. mosseae* (Mosse, 1956). The differences between yellow-poplar and apple seedlings inoculated with these two species of *Glomus* suggest that host specificity may be an important consideration in endomycorrhizal colonization.

In this experiment, the degree of colonization of seedlings inoculated with the two *Glomus* species in combination was also assessed. Less infectivity was noted in this treatment than in seedlings inoculated with either *G. mosseae* or *G. fasciculatus* alone. The reasons for lack of colonization in the combination treatment are not known; perhaps the two endophytes were inhibitory, rather than synergistic.

II. MORPHOLOGY OF ENDOMYCORRHIZAE

Differences in morphology of specific fungal structures were also noted in the *G. mosseae* and *G. fasciculatus* treatments. In general, the intercellular hyphae and intracellular hyphal coils observed in *G. mosseae* were smaller and more uniform in diameter than those observed in *G. fasciculatus*. In both treatments, most of the hyphal strands were aseptate; however, a minor degree of septation was noted in hyphae in *G. fasciculatus*. After penetration of the cortical cells, the *G. mosseae* hyphae characteristically became highly coiled, with the coils assuming a longitudinal orientation with the infected cells. In *G. fasciculatus*, the intracellular coils appeared to be more contorted and to lack specific orientation patterns. The majority of the arbuscules in *G. mosseae* were either mature or in various stages of deterioration. Numerous minute bifurcations were associated with the tertiary branches of the arbuscular trunk, and in many instances these structures appeared to be in the process of disintegration. Considerably

fewer arbuscules were seen in *G. fasciculatus*, and most of these lacked the terminal bifurcations that are associated with mature arbuscules. In addition, the relative size of the primary arbuscular trunk appeared to be larger in *G. fasciculatus*. Considerable variability was observed in the morphological characteristics of the vesicles in *G. mosseae*. In some instances these structures were round to ovate; in other cells, the vesicles appeared to conform to the interior contours of the infected cells. Vesicles were noted in only one seedling in the *G. fasciculatus* treatment; these were intracellular, spherical structures.

General descriptions of the morphological characteristic of fungal structures produced by *G. mosseae* and *G. fasciculatus* cannot be made, since the morphology of VA mycorrhizae is influenced both by the host and the fungal species infecting it (Gerdemann, 1965). Few morphological studies have been conducted on yellow-poplar seedlings infected with these two species of endophytes. Kinden and Brown (1975a; 1975b) described intracellular hyphae, arbuscules, and vesicles in naturally infected yellow-poplar seedlings having spore types characteristic of *G. mosseae*. They also noted that intracellular hyphal development was extensive and that the hyphae assumed both a coiled and linear orientation within infected cortical cells. The hyphae were described as aseptate, except in instances when the hyphae were deteriorating. As in the present study, most arbuscules were noted to be derived from dichotomous branches of the intracellular hyphal trunk which

terminated in minute bifurcations. They described *G. mosseae* vesicles as spherical intracellular structures. A brief morphological description of *G. fasciculatus* has also been described in yellow-poplar seedlings (Gerdemann, 1965). It was noted that hyphal infection and vesicular development by this endophyte was almost entirely intracellular in yellow-poplar, and that arbuscules were formed in cells otherwise free of hyphae. Many arbuscules were in stages of deterioration.

III. SIGNIFICANCE OF FINDINGS

Several findings in this study may have significance in future attempts to propagate yellow-poplar seedlings for outplanting. *G. mosseae* was superior to *G. fasciculatus* in the colonization of roots of yellow-poplar seedlings propogated in the greenhouse. Since this specific endophyte has previously been shown to improve growth and survival of sycamore seedlings outplanted on strip-mined spoils, it is possible that similar beneficial effects might be obtained by inoculation of yellow-poplar nursery stock with this species of endomycorrhizae. The results also indicate that inoculation of seedlings with the two *Glomus* species in combination results in less colonization than that observed in single treatments with *G. mosseae* or *G. fasciculatus*. This observation suggests a single inoculum of endomycorrhizal fungi should be used in the propagation of pot-grown yellow-poplar seedlings. Finally, it was noted that there is considerable variability in the degree of

colonization within seedlings from the same treatment. Since it is often assumed that all treated seedlings are uniformly colonized, this observation could have importance in the interpretation of results in studies designed to correlate plant growth with endomyccorhizal infectivity.

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