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TECHNIQUES TO INCREASE SURVIVAL OF
NEW HIGHWAY PLANTINGS USING
MYCORRHIZAL INOCULATION

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PURDUE UNIVERSITY

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Final Report

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PLANTINGS USING MYCORRHIZAL INOCULATION

TO: H. L. Michael, Director
Joint Highway Research Project

September 9, 1982

Project: C-36-48H

FROM: David F. Hamilton
Horticulture

File: 9-5-8

Attached is the Final Report on the portion of the Study concerned with use of mycorrhizae in the HPR Part II Study titled "Techniques to Increase Survival of New Highway Plantings". This Report when accepted would complete the mycorrhizal portion of the Study. The title of this Final Report is as noted above.

The Report is submitted for review and acceptance as fulfillment of objectives of the Study.

Sincerely,

David F. Hamilton/ms

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FINAL REPORT

TECHNIQUES TO INCREASE SURVIVAL OF NEW HIGHWAY
PLANTINGS USING MYCORRHIZAL INOCULATION

by

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and the

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The contents of this report reflect the views of the authors who are responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official views or policies of the Federal Highway Administration. The report does not constitute a standard, specification, or regulation.

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16. Abstract <p>Effects of fertility and endomycorrhizal inoculation on growth of <u>Liriodendron tulipifera</u> L., <u>Acer platanoides</u> L., <u>Forsythia x intermedia</u> Zab., and <u>Lolium perenne</u> L. were determined. Plants were inoculated with endomycorrhizal <u>Glomus fasciculatus</u> and <u>Glomus mosseae</u> and grown in a greenhouse at three fertility levels (0, 2, or 4 g/l Nitrogen) of soil medium. <u>A. platanoides</u>, <u>F. x intermedia</u>, and <u>L. perenne</u> did not respond to mycorrhizal inoculation and grew best at 2 g/l N. Growth of <u>L. tulipifera</u> was increased by mycorrhizal development, and shoots were heaviest at 4 g/l N. Mycorrhizae did not enhance growth of unfertilized plants.</p> <p>The effects of temperature and endomycorrhizal inoculation on growth of <u>L. perenne</u> were determined. Inoculated plants had slight mycorrhizal development, but were not larger than noninoculated plants.</p> <p>The influence of mycorrhizal inoculation on root initiation and growth of cuttings of <u>Ligustrum obtusifolium</u> Var. <u>regelianum</u> (Koehne) Rehd. was determined. Amendment of the rooting medium with inoculum resulted in mycorrhizal development and increased root growth, but did not enhance root initiation.</p> <p>The effects of Benlate fungicide on growth of mycorrhizal and nonmycorrhizal <u>L. tulipifera</u> were determined. Benlate increased growth of noninoculated plants, but decreased growth of inoculated plants.</p> <p>The numbers of mycorrhizal spores in newly disturbed soil and revegetated highway soils were determined. A newly disturbed soil had virtually no mycorrhizal spores, but a soil revegetated with herbaceous plant species and one revegetated with woody and herbaceous species had very high numbers of mycorrhizal spores.</p>			
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ABSTRACT

Effects of fertility and endomycorrhizal inoculation on growth of Liriodendron tulipifera L., Acer platanoides L., Forsythia x intermedia Zab., and Lolium perenne L. were determined. Plants were inoculated with endomycorrhizal Glomus fasciculatus and Glomus mosseae and grown in a greenhouse at three fertility levels (0, 2, or 4 g/l Nitrogen) of soil medium. A. platanoides, F. x intermedia, and L. perenne did not respond to mycorrhizal inoculation and grew best at 2 g/l N. Growth of L. tulipifera was increased by mycorrhizal development, and shoots were heaviest at 4 g/l N. Mycorrhizae did not enhance growth of unfertilized plants.

The effects of temperature and endomycorrhizal inoculation on growth of L. perenne were determined. Inoculated and noninoculated plants were grown at day/night temperature regimes of 20/15°C, 30/25°C, or 40/35°C. Inoculated plants had slight mycorrhizal development, but were not larger than noninoculated plants. Inoculated plants at 40/35°C had less growth than did noninoculated plants. The detrimental effect of inoculation at high temperature is attributed to the presence of active pathogens in the impure inoculum, but no highly compatible mycorrhizal fungi.

The influence of mycorrhizal inoculation on root initiation and growth of cuttings of Ligustrum obtusifolium Var. regelianum (Koehne) Rehd. was determined. Plants were rooted in perlite:peat (1:1, v/v), medium amended with inoculum, or medium amended with pasteurized inoculum. Amendment of the rooting medium with inoculum resulted in mycorrhizal development and increased root growth, but did not enhance root initiation.

The effects of Benlate fungicide on growth of mycorrhizal and nonmycorrhizal L. tulipifera were determined. Inoculated and noninoculated plants were treated with Benlate every two weeks in a drench rate. Inoculated

plants grown without Benlate were heaviest and tallest. Benlate increased growth of noninoculated plants, but decreased growth of inoculated plants.

The numbers of mycorrhizal spores in newly disturbed soil and revegetated highway soils were determined. A newly disturbed soil had virtually no mycorrhizal spores, but a soil revegetated with herbaceous plant species and one revegetated with woody and herbaceous species had very high numbers of mycorrhizal spores.

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HIGHLIGHT SUMMARY

Mycorrhizal development is a critical phenomenon in plants surviving the sub-optimal growing conditions. The mycorrhizal fungi growing in symbiosis with the plants contribute an extensive hyphal network in the soil which greatly increases the surface area available for the uptake of moisture and nutrients. The objectives of this study were to examine for the presence of mycorrhizae on newly disturbed and revegetated highway soils, and to determine the effects of environmental and cultural factors on the growth of mycorrhizally inoculated plants commonly used in highway plantings. Factors examined include the effects of temperature, fertility, and fungicides on inoculated and noninoculated plants.

Highway site soil was found to be virtually void of mycorrhizal spores which could infect plants used to revegetate the area. Because there is no indigenous inoculum source, either inoculation of the site must be conducted or plants which have already developed mycorrhizae should be used to maximize the success of revegetation.

Special considerations are required during plant culture and production to insure mycorrhizal development. Soil pasteurization renders inoculum inviable and should be conducted only prior to mycorrhizal inoculation. Certain fungicides, such as Benlate, should also be avoided because they prevent mycorrhizal formation. Plant and fungal compatibility should be high because at the high temperatures present at highway sites the activity of pathogens present in unpure inoculum could be detrimental to plants. Mycorrhizal inoculation is greatest when some supplemental fertilizers are applied.

This study indicates that mycorrhizal inoculation does promote the growth of some landscape plants important in highway site revegetation. Further research is needed to establish the optimum combinations of more plant and

fungus species, to determine the most effective inoculation techniques, and to continue to study the effects of the severe plant stress imposed by highway sites on mycorrhizal plants. Of major importance are the lack of organic matter and moisture stress resulting from the use of deicing salts.

INTRODUCTION

Symbiotic associations between certain soil fungi and plant roots constitute the relationship termed "mycorrhizae." Mycorrhizal roots are observed in nearly all native stands of plants, in all parts of the world.

There are two major types of mycorrhizae, distinguished by the way in which the fungus attaches itself to the root. One classification is the ectomycorrhizal group, and the other is the endomycorrhizal group. The fungi of the ectomycorrhizal group enter the root and surround the cells, but do not enter these individual cells. Ectomycorrhizae can be recognized by the fungal sheath formed around the exterior of the root, creating a fuzzy appearance. From this sheath, hyphae extend into the soil, and also inward around the cortical cells of the root. The inward extension is termed a Hartig Net and provides intimate contact for exchange between the two symbionts.

The second type of mycorrhiza, the endomycorrhizal group, does not have any outwardly visible distinction, as there is no fungal sheath. Inside the root, however, the fungus does penetrate the individual epidermal and cortical cells. Upon infection, the fungi produce structures called arbuscles, which are fine clusters of hyphae within the cortical cells of the root. At some point these structures are digested and the contents absorbed by the plant cell. The formation and dissolution of these structures may serve as the prime mode of exchange between the two symbionts. Endomycorrhizae also contain structures called vesicles, which are ovate to spherical formations containing oil droplets. These may remain thin-walled and serve as storage, or become thick-walled and serve as resting spores. Endomycorrhizae seem to be the most common type, and are perhaps the most important type for growth of landscape plants commonly used for the revegetation of disturbed highway soils. The objective of this project was to examine the effects of endomycorrhizal

inoculation on growth of selected landscape plants used for revegetation of highway sites.

CHAPTER I
REVIEW OF LITERATURE

CHAPTER I

REVIEW OF LITERATURE

Occurrence of Endomycorrhizae in the Plant Kingdom

The existence of mycorrhizal interactions has been known for over 100 years (Hayman, 1978), yet the factors maintaining mycorrhizal associations symbiotic rather than parasitic are not clearly understood (Meyer, 1974). Natural endomycorrhizal symbioses are extensive in the plant kingdom and are believed to play important roles in the growth of plants (Gerdemann, 1968, 1975; Hayman, 1978; Meyer, 1974). Mycorrhizae occur in all climates of the world but are generally thought to be lacking in aquatic habitats (Hayman, 1978). However, recent studies have isolated water tolerant endomycorrhizal species (Sondergaard and Laegaard, 1977; Keeley, 1980).

In addition to wide geographic distribution, mycorrhizae occur in more than 80% of plant taxa (Meyer, 1974). Endomycorrhizae are common in Bryophytes, Pteridophytes, Gymnosperms, and Angiosperms (Gerdemann, 1975; Hayman, 1978). There are many reports of mycorrhizal growth enhancement of woody and herbaceous landscape plants (Trappe, 1962). Seedlings of Liquidambar styraciflua L. and Liriodendron tulipifera L. inoculated with Glomus fasciculatus had heavier shoots with more phosphorus than did noninoculated seedlings (Gerdemann, 1965; Gray and Gerdemann, 1967). Similar increases in height and dry weight of L. styraciflua occurred following inoculation with Glomus mosseae (Kormanik et al., 1977) or with a combination of G. mosseae and G. etunicatus (Schultz et al., 1979). Growth of Malus speciosa Mill. (Benson and Covey, 1976), Magnolia grandiflora L., and Juniperus horizontalis Moench (Maronek et al., 1980) have also been stimulated following endomycorrhizal inoculation.

The effects of mycorrhizal inoculation on grass species have not been consistent. Growth of Lolium perenne L. was promoted by endomycorrhizal inoculation with Gigaspora margarita and Glomus tenuis (Powell, 1977, 1979). However, growth of other grass species was found to be depressed by association with Glomus tenuis and Glomus mosseae (Sparling and Tinker, 1978). Growth of L. perenne is also promoted by association with endomycorrhizal Rhizophagus tenuis (Crush, 1973). Inoculation of L. perenne with G. mosseae resulted in slightly higher lipid content of roots (Cooper and Losel, 1978).

Species with fibrous root systems, such as grass species, seem to be less dependent on mycorrhizal formation than do plants without root hairs, such as L. styraciflua and L. tulipifera. Lack of consistent results following inoculation with endomycorrhizae may be caused by variability of experimental conditions. The beneficial effects of endomycorrhizae on growth of both herbaceous and woody plants are influenced by numerous environmental factors, including soil fertility and temperature (Slankis, 1974).

Effects of Mycorrhizae on Soil Nutrient Use

Inoculation with endomycorrhizal fungi result in more efficient use of soil nutrients by many landscape plants, and increasing plant size (Hayman, 1978). Mycorrhizal plants have an advantage under conditions of low fertility, due to the absorptive surface added to the root system by the fungal hyphae and perhaps to more efficient absorption (Harley, 1971). Mycorrhizal development is even reduced under conditions of high fertility, with some plant species (Menge et al., 1978; Maronek et al., 1980; Mosse, 1973; Johnson et al., 1980; Powell, 1980). However, there are also reports of increased growth of mycorrhizal plants at high fertility levels (Meyer, 1974; Wright, 1971).

Mycorrhizae promote growth of woody and herbaceous plants over a wide range of nutrient levels. Inoculation of L. styraciflua with a mixture of G. mosseae and G. etunicatus enhanced plant growth from 140 to 1120 kg/ha of 10N-10P-10K (Schultz et al., 1979; Kormanik et al., 1977). Glomus fasciculatus increased growth of several citrus cultivars over a wide range of fertility (Menge et al., 1978). Glomus fasciculatus also promoted growth of avocado plants (Persea americana Mill.) over a wide range of fertility, except when zinc was withheld and phosphorus was present in large amounts (Menge et al., 1980).

Height of Magnolia grandiflora was promoted by inoculation with G. fasciculatus at 1.1 kg/m³ and 4.5 kg/m³ (Maronek et al., 1980). Fresh weights of roots and shoots of Podocarpus macrophyllus Thunb., and Rhododendron simsii Planch. inoculated with a mixture of G. mosseae and G. fasciculatus were increased when fertilized with 250 to 1250 mg/l N and K with 50 to 250 mg/l Mg (Johnson et al., 1980).

Uptake of several nutrients is reported to be improved by endomycorrhizal formation (Gerdemann, 1975; Mosse, 1973), but these reports are inconsistent. Nitrogen uptake has been enhanced by mycorrhizae in soybeans (Glycine max L.) (Ross and Harper, 1970; Ross, 1971), and ericaceous plants (Read and Stribley, 1973; Stribley and Read, 1980). Stribley and Read (1980) also report that mycorrhizae utilize nitrogen more effectively by using simple organic compounds not used by nonmycorrhizal plants. Mycorrhizal roots may also have increased nitrate reducing capacity (Ho and Trappe, 1975).

Uptake of sulfur by mycorrhizal onion (Rhodes and Gerdemann, 1978), red clover (Trifolium pratense L.), and maize (Zea mays L.) is also higher than by nonmycorrhizal plants. In addition, uptake of zinc was greater by mycorrhizal, than by nonmycorrhizal Malus speciosa Mill. (Benson and Covey, 1976). There are also some reports of increased uptake of K, Ca, Mg, Fe, Cu, Mn, Na, and B by mycorrhizal plants (Gerdemann, 1975).

Reports of increased phosphorus uptake by mycorrhizal plants have been more numerous than for any other nutrient. In fact, enhancement of plant growth by mycorrhizae occurs particularly under conditions of low soil phosphorus (Hayman, 1978; Mosse, 1973; Stribley, et al., 1980). Increases in P concentration, rather than total P content in the plant, have been reported for tomato (Lycopersicon esculentum Mill.) (Cress et al., 1979), subterranean clover (Trifolium subterraneum L.), cassave (Manihot esculenta Mill.), soybean, and maize (Stribley et al., 1980). However, Schultz et al. found decreases in concentrations of N, P, K, and Mg in mycorrhizal L. styraciflua.

As mycorrhizal plants increase in size, their total P content rises proportionately, but the P concentration of shoots or roots may remain the same as that of nonmycorrhizal plants. Species with greater total P content include mycorrhizal tulip poplar (Gray and Gerdemann, 1967), perennial ryegrass (Powell, 1977), onion (Allium cepa L.) (Gray and Gerdemann, 1967; Hattingh et al., 1973), and alfalfa (Medicago sativa L.) (Barea et al., 1980). Other reports have not been consistent and mycorrhizal plants either have higher, equal, or lower P concentrations than nonmycorrhizal plants. This most likely depends on the availability of the nutrients and rate of plant growth.

One explanation for increased nutrient uptake is that the mycorrhizal hyphae act as fine roots or root hairs (Baylis, 1972), and simply serve as an additional, well distributed surface for absorbing nutrients (Mosse, 1973; Hattingh et al., 1973; Sanders and Tinker, 1973). Other authors suggest the possibility that not only are mycorrhizal root systems more extensive, but they are also more effective for nutrient uptake. Cress et al. (1979) reported that increased P uptake may be due to a greater affinity of mycorrhizal absorbing sites for H_2PO_4^- . It is a widespread view that mycorrhizae can solubilize unavailable phosphate, but Mosse (1973) reported that mycorrhizal plants used

the same P source as nonmycorrhizal plants. Furthermore, some nonmycorrhizal plants may have a minimum threshold concentration of soil P, below which they cannot absorb phosphate. According to this theory, mycorrhizal plants have a lower threshold or no threshold at all.

The varied growth response of mycorrhizal plants to phosphorus may be controlled by two opposing factors (Harley, 1969; Stribely et al., 1980). There is a stimulating effect due to enhanced P uptake, and a detrimental effect due to the fungal drain on host photosynthate. Therefore, if the plant is adequately supplied with P, the plant can actually suffer a yield loss in the presence of mycorrhizae (Crush, 1975; Stribely et al., 1980). According to Gerdemann (1975), if the nutrient is limiting plant growth, it may occur in higher concentrations in the mycorrhizal plant. If a mycorrhizal plant does not reach its full growth potential relative to P availability, due to a lack of other nutrients or a fixed carbon loss, the plant will have a greater percent P and a lower dry weight.

Effect of Temperature on Growth of Mycorrhizal Herbaceous Plants

In addition to the direct effects of temperature on plant growth, temperature also influences mycorrhizal fungi in the rhizosphere. Optimum temperatures for growth of ectomycorrhizal mycelium in culture vary with the medium used. Temperatures tested in vitro have not been useful for predicting the effect of temperature in the rhizosphere because optimum temperatures for infection and subsequent growth of roots may differ greatly from those for best mycelial growth of ectomycorrhizae in culture (Slankis, 1974). Ectomycorrhizae tolerate temperatures from 3 to 60°C, but optimum temperatures for ectomycorrhizal development are as high as 34°C (Slankis, 1974).

Because of the difficulty of growing endomycorrhizae in pure culture,

little research has been conducted on temperature effects on spore germination and hyphal development. In agar culture, spore germination of endomycorrhizal Gigaspora coralloidea and G. heterogama was best at 34°C while best spore germination of G. mosseae occurred at 20°C (Schenck et al., 1975). Inoculum of G. fasciculatus (consisting of roots, spores, and hyphae) was rendered ineffective when exposed to 52.5°C for 10 minutes, but not when exposed to 51.5°C (Menge et al., 1979). Germination of Glomus epigeous in soil was best at 18 to 25°C (Daniels and Trappe, 1980).

Other important studies involve the effects of temperature on endomycorrhizal fungi in symbiosis with plant partners. Maximum mycelial growth of Endogone on roots of soybean was between 28 and 34°C (Schenck and Schroder, 1974). Arbuscular development was maximum near 30°C, the same temperature for best root growth of mycorrhizal soybeans. Both root and arbuscular development decreased with decreasing temperature, but the decrease was greater for arbuscules (90%) than for roots (60%). This may indicate that the mycorrhizal fungi are more influenced by low temperature than are roots. The number of Endogone spores in the soil was greatest at 27.5 and 35°C. At temperatures above 35°C, arbuscules, hyphae, and root development decreased and at 41°C plant growth ceased.

Smith and Bowen (1979) reported effects of temperature on endomycorrhizae during the preinfection phase of mycorrhizal development on Medicago truncatula and Trifolium subterraneum. Increased temperature up to 25°C promoted the formation of entry points for fungal penetration of the root. However, the total number of entry points on the root system would also depend on the total length of roots available for infection (Smith, 1979).

Unfavorable temperature and extremes of light intensity influenced the appearance and effectiveness of mycorrhizal infection in onion more than they

influenced the percentage of the root system infected with mycorrhizae (Hayman, 1974). In addition, plant growth was much more affected by temperature than was mycorrhizal infection. At 41°C with low light intensity, mycorrhizal development resulted in no growth stimulation in onion. Increases in dry weight of mycorrhizal onion was greatest from 14 to 23°C.

In summary, temperature is an important factor in the growth of all plants. Mycorrhizal roots are generally more affected by temperature than are shoots of mycorrhizal plants, and in some cases plant growth may be more influenced than mycorrhizal infection. Mycorrhizal development could therefore be beneficial to plant growth under extremes of temperature for some plant species. Different species of mycorrhizal fungi have specific temperatures for optimum development. This should be considered when selecting fungal symbionts for plants growing in extreme temperatures.

Effect of Benlate on Mycorrhizal Plants

Benlate is a fungicide consisting of 50% benomyl (1-(N-buylcarbamol)-2-(methoxycarboximide)-benzimidazole) and is manufactured by E. I. duPont de Nemours and Co., Wilmington, Delaware. It is a systemic fungicide which may persist in plant tissue and in the soil (deBertoldi et al., 1977). Benomyl is used to control pathogenic fungi on crops and to reduce spoilage of fruits and vegetables in storage and in transit. Some systemic fungicides including captan (Nemec, 1980), prothiocarb, and pyroxychlor (Smith, 1978) have little effect on mycorrhizal endophytes of some plant species. However, benomyl consistently has negative effects on the growth of mycorrhizal plants.

Application of benomyl to endomycorrhizal onions resulted in a 22 to 25% reduction in diameter, and a 31 to 34% reduction in dry weight, but had little effect on fungi in the rhizosphere (deBertoldi et al., 1977). Benomyl does not affect other phycomycetes as greatly as it does the Endogone, and the effects

may be mediated by the plant since it is systemic.

Benomyl has reduced infection and number of spores of G. mosseae in association with wheat (Triticum aestivum L.) (Jalai and Domesch, 1975). Formation of mycorrhizae in association with clover (Trifolium spp.) was prevented by drench treatments of benomyl, and phosphate uptake by mycorrhizal onion and strawberry (Fragaria vesica L.) was reduced by these treatments (Boatman et al., 1978).

Application of benomyl also reduced mycorrhizal development on soybean (Bailey and Safir, 1977), bean (Sutton and Sheppard, 1976), and sour orange (Citrus aurantium L.) (Nemec, 1980). Benomyl applied to potato (Solanum tuberosum L.) reduced spore populations in the soil (Ocampo and Hyman, 1980). When applied to barley (Hordeum vulgare L.) and maize, benomyl resulted in fewer spores in the soil, less root length infected, less cortex infected, and fewer entry points for fungal penetration. There also were fewer vesicles in mycorrhizal roots of maize.

In summary, benomyl formulations are commonly used in the production of woody landscape plants as preventative or curative pesticide for fungal pathogens. However, mycorrhizal development is also hindered by benomyl, possibly resulting in reduced plant growth and depressed nutrient status. These factors should be considered when selecting a fungicide for use on plants which are dependent on mycorrhizal fungi.

Effects of Mycorrhizal Inoculum on Rooting of Cuttings

Propagation by cuttings is common for production of woody ornamentals. However, some plant species root poorly, and less convenient methods, such as grafting, must be sought. The presence of mycorrhizal inoculum in a rooting medium might promote root initiation or root development through chemical or

biological interaction of the mycorrhizal fungi with the cutting.

Linderman and Call (1977) found that when ectomycorrhizal inoculum of Thelephora terrestris Ehrh. ex. Fr. was incorporated into the rooting medium of bearberry (Arctostaphylos uva-ursi L. Spreng.) and huckleberry (Vaccinium ovatum Parsh.), rooting percentage and root volume increased. The increased rooting percentage reflects the effect of ectomycorrhizae on root initiation, which may be due to the root promoting effect of growth substances excreted into the soil by the ectomycorrhizal fungi. Evidence supporting this theory is the growth promoting effect of mycorrhizal filtrates. In contrast, the increased root volume simply reflects the usual promotions of root growth after mycorrhizal formation. There are presently no additional reports confirming the effect of ectomycorrhizae on root initiation, and there are no studies indicating that endomycorrhizae promote root initiation. However, there are some reports on effects of mycorrhizae on root development of cuttings.

Holden (1978) confirmed the beneficial effect of unidentified mycorrhizae on the root development and growth of bearberry. Unidentified endomycorrhizae did not promote the growth of mycorrhizal heather (Calluna vulgaris L.) cuttings under high nutrient regimes. However, under low nutrient regimes, dry weights of roots and shoots were greater for cuttings with mycorrhizal roots (Bannister and Norton, 1974). Incorporation of Conocybe tenera into the rooting medium also promoted the root weights of poplar cuttings (Veldeman, 1980).

There is only limited evidence for the promoting effect of mycorrhizal fungi on root initiation, the beneficial effect of mycorrhizae on root development is a more accepted concept. Mycorrhizae cannot be expected to increase the rooting percentage of many plant species, but inoculation in the propagation stage does insure the earliest possible mycorrhizal development and

subsequent promotion of growth.

Techniques for Extracting Spores of Mycorrhizal Fungi from Soil

Members of the fungal family Endogonaceae are major endomycorrhizal symbionts (Gerdemann, 1955; Gerdemann and Trappe, 1974; Mosse and Bowen, 1968). With the Endogonaceae, as with nearly all fungi, spores have a prominent role in the propagation of the organism (Gerdemann and Trappe, 1974; Mosse and Bowen, 1968). In mycorrhizal research, the estimation of spore content of a volume of soil is important in many phases of research, particularly in the inoculation stage. To scientifically inoculate a plant specimen with the fungal symbiont, it is critical to know the approximate amount of spore material being added to the soil. Without this information, it would be impossible to standardize inoculation among treatments, or to evaluate the success of an inoculation attempt.

The diversity of spore types produced by fungi creates a major difficulty in determining a single technique which would be best for all situations. In the Endogonaceae, several types of spores have been reported. Spore size, shape, and color vary greatly (Gerdemann, 1955; Gerdemann and Trappe, 1974). Gerdemann recorded spore diameters ranging from 22 microns to greater than 500 microns. Shapes ranged from spherical to irregular, and the color also varied from light yellow to dark brown.

This diversity of physical characteristics reflects the nature of the problems which might be represented when isolating and counting spores of mycorrhizal fungi. Despite the problem of isolating spores of such diverse characteristics, there are several techniques commonly in practice for spore extraction as used in the estimation of total soil content.

All techniques reviewed rely on direct microscopic observation of an

extract from a dilute soil suspension, and manual spore counting for use in estimating the soil spore count (Ohms, 1957; Jenkins, 1964; Smith and Skipper, 1979). Most techniques included modifications intended to remove non-spore material, such as large organic matter and mineral particles, from the sample.

Many include methods such as soil sieving, which relies on the differences in physical size of the various materials in the soil preparation (Jenkins, 1964; Smith and Skipper, 1979). In addition, other modifications, such as floatation and centrifugation, depend on the concept of mass differentiation. Each variation differs both in the simplicity of the procedure, and in the precision of the results.

The most simple and direct method for spore extraction from a soil sample is the New Plate Method, as described by Smith and Skipper (1979). In this very easy procedure, one gram of moist soil is added to 9.0 ml of distilled water in a test tube. The test tube is then capped with a rubber stopper and shaken vigorously. One ml is immediately pipeted in parallel lines onto a 9 cm filter paper disc in a petri dish. This can then be scanned, either wet or dry, under a dissecting microscope (7 to 30X) to facilitate spore counting. From this count, the number of spores in the sample can be calculated and the total number of spores in the soil volume projected.

The advantage of this method lies in the simplicity of the technique. It is rapid and requires a minimum of steps. Since there is no requirement for sieving, centrifugation, or floatation, few spores are lost in processing the sample. It has been found that this method recovers more than two times as many spores as techniques employing modification (Smith and Skipper, 1979).

This technique, however, is not the best for all situations. In cases where there is a lot of organic matter in the soil medium obstructing vision, this method can become a very tedious one. In addition, for species of fungi

having extremely small spores it can be difficult to distinguish the spores under the low powers of a dissecting microscope. For occasions when a spore count is very low, this method would also not be adequate because it only samples 0.1 gram of soil and risks missing the spores completely (Smith and Skipper, 1979). Thus, although this seems to be a very accurate and simple procedure, researchers are often forced to take extra steps to remove unwanted materials and to isolate the spores.

To achieve this goal, two types of modifications can be introduced. The first is to wet sieve the sample through a series of U.S. Standard sieves, removing larger particles and catching the spores on a very fine sieve (Smith and Skipper, 1979). The second type of modification uses the characteristic mass of the spores to isolate them either by floatation and sedimentation techniques, or by centrifugation (Jenkins, 1964; Ohms, 1957; Smith and Skipper, 1979). These procedures can be used alone, or in combination to achieve spore isolation.

In such a case where further steps must be taken to isolate the spores, wet sieving is the modification most often employed (Gerdemann and Nicolson, 1963; Gerdemann and Trappe, 1974; Hwang and Ko, 1976). The first one or two sieves, having larger openings (250 to 500 μm), are used to remove large and undesirable particles. The final sieve has smaller openings (44 μm), and is used to collect the spores. This material can either be examined under a microscope, or undergo further treatment to extract the spores.

This method has the advantage of isolating spores from bulky soil material, and can greatly facilitate spore counting. It has the disadvantage of requiring more time and introducing spore losses from the system (Smith and Skipper, 1979). Spores not passing through to the collection sieve, or not successfully removed from the collection sieve for examination would introduce

bias into the system. This bias would tend to lower all spore counts.

Wet sieving, despite its drawbacks, is an important tool for the isolation of the spores of mycorrhizal fungi. If the degree of isolation provided at this point is still not sufficient, further steps can be added to achieve this purpose. In many cases, wet sieving is used in conjunction with the techniques of floatation and centrifugation (Furlan and Fortin, 1975; Jenkins, 1964; Ohms, 1957; Smith and Skipper, 1979; Sutton and Barron, 1972).

Several methods of floatation have been developed and are adaptable for the isolation of fungal spores (Furlan and Fortin, 1975; Ohms, 1957; Smith and Skipper, 1979; Sutton and Barron, 1972). In the floatation method, a 10.0 gram moist soil sample is placed into a 50 ml graduated cylinder (Smith and Skipper, 1979). This is filled with approximately 63 ml of water, stoppered, and shaken vigorously for 10 to 15 seconds. After settling for two minutes, the suspension is decanted into a 150 ml separatory funnel. This process is repeated twice. The suspension in the separatory funnel is allowed to stand for two to three minutes. The water is then drained from the separatory funnel at a rate of 75 to 100 ml/minutes into a second separatory funnel, which is then drained in the same way as the first. The material on the walls of the separatory funnels include the spores, which are rinsed off onto a filter paper for viewing under the microscope.

The technique further purifies the spore extract, but again introduces the possibility for some error and spore loss. As reported by Smith and Skipper (1979), the number of spores recovered by this method is significantly less than the number recovered by the plate method. It is however, effective for removing debris and unwanted material in an effort to isolate the fungal spores.

An alternative to simple floatation is the sucrose centrifugation method

(Jenkins, 1974; Smith and Skipper, 1979). The principle of this technique is again floatation, but with two modifications. In this case, a 100 to 500 ml aliquot is taken from a blended sample and passed through a 500 um sieve. This mixture is then passed through a 44 um sieve and collected. This procedure is repeated and the collected residue transferred into two 50 ml centrifuge tubes. The two tubes are then centrifuged at 1750 rpm for four to five minutes.

The supernatant is poured off, and a sugar solution is added (1 lb. cane sugar/1 liter H₂O). This solution is mixed thoroughly and centrifuged at 1750 rpm for 0.5 to 1 minute. The supernatant containing the spores is poured onto a 44 um sieve and placed on filter paper for counting under the microscope.

According to Smith and Skipper (1979), this method produces results which are not significantly different from those of the basic floatation method. It again has the advantage of isolating spores with little debris, and has the disadvantages of being labor intensive and having low spore recovery relative to the plate method.

Several conclusions can be drawn about the techniques reviewed. When using recovery of spores as the criteria of a good extraction technique, the simple plate method is most effective (Smith and Skipper, 1979). This method also has the advantage of being quick and easy to perform. In cases where a sample contains a lot of organic matter, small spores or very few spores, this method may not prove to be effective. The procedures used to further isolate spores, such as wet sieving, floatation and centrifugation, tend to significantly lower the number of spores recovered from a volume of soil (Jenkins, 1964; Ohms, 1957; Smith and Skipper, 1979). These modifications are, nonetheless, very important techniques in cases where further isolation is required.

Identification of Endogonaceae Spores

Identification of the Endogone is dependent on morphological criteria including size, shape and wall structure of spores, as well as the appearance of the hyphae from which they arise (Mosse and Bowen, 1968; Talbot, 1971). Of these, spore characteristics are most often utilized for positive identification of the fungus. Classical work was done by Thaxter (1922) in characterizing the spores of the Endogone. More recently, other workers, including Mosse and Bowen (1968), as well as Gerdemann and Trappe (1974), have further added to and modified this scheme.

Several diagnostic characteristics of the spores can be considered (Gerdemann and Trappe, 1974; Mosse and Bowen, 1968). First, after wet sieving and extraction, the spores usually remain attached to a short portion of the hyphae from which they arose. The spores are generally larger than the vegetative spores of most other fungi. In addition, their thick yellow-brown spore walls distinguish them from the large sexual spores of other phycomycetes.

More spores of the Endogone are borne terminally on hyphae which are either simple, bulbous, or swollen in character (Mosse and Bowen, 1968). The spores having simple hyphal connections have either a small opening between the spore and the hyphae, or a thin membrane between the two. This type of spore stains readily. The spores having swollen hyphal connections either have a wide lone hyphae with an opening to the spore, or a shorter and more one-sided hyphae, which with time, forms a separation layer between the spore and the hyphae. These spores do not stain well. Spores with bulbous hyphae have small pores at the tips of the bulbs, which are not continuous at maturity.

The structure of the spore wall can also be used as a diagnostic characteristic for the Endogone (Mosse and Bowen, 1968). Mature spores have

very resistant walls, often consisting of two or more separate layers with different staining properties. The thick, often colored outer wall, fractures when crushed, leaving straight-sided pieces with wide angles. The inner wall is tough and membranous and folds rather than fracturing. This inner wall stains easily. The relative position of these two walls can be a very characteristic feature of the spore for many species.

Spore content may also be observed (Mosse and Bowen, 1968). The appearance of spore content varies with age, but there are two distinct patterns. In the first category, the cytoplasmic reticulatum consisting of a series of very fine beaded strands, resulting in a crystalline appearance. In the second group, the cytoplasm is vacuolate consisting of a continuous homogeneous phase with many lipid droplets.

Spore color and shape are also obvious diagnostic characteristics, although these are not standard enough for definitive identification (Mosse and Bowen, 1968). Most spores are yellow or brown, becoming black as the contents of the spore degenerates. Spores also may be colorless.

In size, spores can differ significantly (Mosse and Bowen, 1968). For a given soil, they may vary only 15% from the mean size, although spores from different soils may have different mean spore sizes. The three main shapes that occur are spherical, usually with simple hyphae; round to pear-shaped, usually with swollen hyphae; and ovoid shaped, usually with bulbous hyphae. While spore size, shape, and color are obvious, aspects of the spores, other criteria, such as the spore wall structure and spore content, are considered more diagnostically sound.

Although these characteristics are very useful in identifying spores of the Endogone, there is some confusion in the nomenclature of this genus (Hayman, 1978). Regardless of the problems in nomenclature, mycorrhizae formed with

members of the Endogone encompass a wide range of plant species, families, and orders. Mycorrhizae are very common in nature and are very important to the growth of plants under stress. Mycorrhizal inoculation may also be useful in crop production systems for promoting growth and reducing requirements for expensive fertilizers.

Uses for Mycorrhizae in Revegetation of Disturbed Sites

A plant environment which has been severely disturbed is one in which all vegetation existing prior to construction has been removed and destroyed. Often the topsoil layer has undergone extended storage, or has been destroyed by mixing it with other soil layers, resulting in a soil poorly suited for plant growth. The topsoil surviving may have an abnormally high pH, poor fertility and is often devoid of the normal beneficial microflora contributing to plant growth. Even in cases where the topsoil has been retained, the amount of viable mycorrhizal inoculum in undisturbed soil can be as much as ten times that of topsoil stored for three years (Rives et al., 1980). Furthermore, the soil to be revegetated is directly exposed to the heat of the sun, which creates temperatures detrimental to plant growth (Masiunas, 1981). Soil on exposed slopes of a highway reached daytime temperatures as high as 51°C, well above the optimum plant growth temperatures. The soil may also be exposed to erosion by wind and water, further complicating the problem of revegetation.

The presence of mycorrhizae on difficult-to-revegetate sites is important because of their ability to increase plant uptake of water and nutrients. This factor can be critical in the hot, dry and unfertile conditions frequently found on highway sites being revegetated. Mycorrhizae could improve the survival of the plants used and increase the success of highway revegetation efforts. In addition, by promoting plant growth, a complete plant cover is

achieved earlier and could reduce the problems associated with soil erosion.

Mycorrhizae can recolonize a site naturally, however this may take years and would not be present during the initial period critical to the stabilization of highway slopes. Allen and Allen (1980) report that reclaimed stripmines, with conditions similar to those occurring on highway sites, mycorrhizal infection and spore counts increased to up to 50% of the levels in undisturbed soils after two to three years. The natural spread of mycorrhizal fungi through the soil into nonmycorrhizal seedlings occurs at a rate of 0.6 to 1.5 meters per year (Powell, 1979). If mycorrhizae are not present on a site, the plants successfully recolonizing the location are limited to plants capable of surviving without mycorrhizae (Allen and Allen, 1980).

The use of mycorrhizal plants or inoculation of the highway sites could hasten the reestablishment of normal microbe populations which assist in the growth and survival of plants normally used to revegetate highway construction sites. The result could be more rapid and successful revegetation of highway sites and stabilization of highway slopes.

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CHAPTER II

INFLUENCE OF MYCORRHIZAL INOCULATION AND
FERTILITY LEVELS ON GROWTH OF SELECTED LANDSCAPE PLANTS

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Abstract

Effects of endomycorrhizal inoculation on growth of selected landscape plants fertilized at different nutrient levels were determined. Liriodendron tulipifera, Forsythia x intermedia, Acer platanoides, and Lolium perenne were grown in 3.28 liter containers under greenhouse conditions. Thirty-nine plants of each species were inoculated either with Glomus fasciculatus or Glomus mosseae. For each plant species, inoculated and noninoculated plants were treated with 0, 2, or 4 g/l Nitrogen of 19N-6P-12K controlled release fertilizer.

The optimum fertility level for most plant species studied was 2 g/l N. Mycorrhizal development increased growth of L. tulipifera infected with G. fasciculatus at both 2 and 4 g/l N. When no supplemental fertilizer was applied, there was no growth increase of mycorrhizal plants. Growth of A. platanoides and L. perenne was not improved by mycorrhizal association with G. fasciculatus, and growth of F. x intermedia was not improved by association with G. mosseae. Other fungal symbionts may increase growth of these plants.

Introduction

In nature and under cultivation, many horticultural plants are capable of forming symbiotic mycorrhizal associations (Gerdemann, 1965; Gray and Gerdemann, 1967; Johnson et al., 1980; Kormanik et al., 1976, 1977; Maronek et al., 1980). Mycorrhizae benefit plant growth mainly through increased nutrient and water uptake (Hayman, 1978). When soil microbes are not present, as in sterile media or soils of highly disturbed landscape sites, plant growth may be slowed.

Soils at highly disturbed sites, such as reclaimed highway slopes and strip-mines, have very low levels of beneficial soil microbes and are difficult to revegetate (Ponder, 1979). Reclamation costs, particularly for fertilizers and plant material, are rising rapidly as inflation increases.

The introduction of mycorrhizal fungi to the rhizosphere of plants grown in containers may make it possible to use soil nutrients more efficiently and reduce production costs. Mycorrhizal roots also may increase growth and survival of these plants once they are transplanted to the landscape, particularly on harsh sites.

Although many plants are known to have mycorrhizal symbionts which increase height and plant weight (Clark, 1963, 1964; Gerdemann, 1965; Gray and Gerdemann, 1967; Hayman, 1978; Powell, 1977, 1979), effects of mycorrhizal development on plant growth at a range of fertility levels are unknown. The objective of this study was to determine the effects of mycorrhizal inoculation and soil fertility on the growth of selected landscape plants used for the revegetation of highway slopes.

Materials and Methods

Seeds of tulip poplar (Liriodendron tulipifera L.) and Norway maple (Acer platanoides L.) were planted in perlite and sand (1:1, v/v) and grown for 4 weeks in a growth chamber at $26 \pm 2^{\circ}\text{C}$ under a 13 hour photoperiod. Terminal cuttings of forsythia (Forsythia x intermedia Zab.) (20 cm long with 8 leaves) were rooted in perlite and vermiculite (1:1, v/v) under intermittent mist (12 sec./10 min.) in a greenhouse with approximately 25% shade. Indolebutyric acid (IBA) was used at 0.1% (w/v) to enhance rooting. Rooted cuttings of forsythia, seedlings of tulip poplar (4 cm tall), and Norway maple (4 to 8 cm tall) were transplanted into 3.28 liter pots (one gallon trade designation) containing

steam pasteurized medium of perlite, sphagnum peat moss, and soil (2:2:1, v/v/v). Seeds of perennial ryegrass (Lolium perenne L.) were seeded directly into 3.28 liter pots at a rate of 12.33 g/m², and covered with 0.5 cm of pasteurized medium. Before addition of fertilizer, the medium for all plants contained 9 mg/l N, 1 mg/l P, 4 mg/l K.

Half the plants of each species were inoculated either with Glomus fasciculatus (Thaxter) Gerd. and Trappe or Glomus mosseae Nicol. & Gerd. at a rate of 44,000 spores/m² of soil surface area (Table II-1). The inoculum contained tomato roots (Lycopersicon esculentum Mill.), fungal spores, hyphae, and the growing medium (perlite:sphagnum peat moss:soil (2:2:1, v/v/v)). The inoculum was inserted into three vertical cores around the plant to a depth of 10 cm in the pot.

Table II-1. Combinations of plant and fungal species used, and subsequent compatibility.

=====		
<u>Plant Species</u>	<u>Mycorrhizal Fungi</u>	<u>Growth Increase</u>
<u>Acer platanoides</u>	<u>Glomus fasciculatus</u>	None
<u>Forsythia x intermedia</u>	<u>Glomus mosseae</u>	None
<u>Liriodendron tulipifera</u>	<u>Glomus fasciculatus</u>	Positive
<u>Lolium perenne</u>	<u>Glomus fasciculatus</u>	None

Inoculated and noninoculated plants were grown with fertilizer additions of 0, 2, or 4 g/l N of medium, supplied by controlled release 'Osmocote' fertilizer (Sierra Chemical Company, Milpitas, California) (19N-6P-12K) in a 3 to 4 month release period. As N was increased, P and K were increased proportionally. Fertilizer was incorporated to a depth of 5 cm. Plants were grown in a greenhouse (24 ± 3°C under a 16 hour photoperiod) and watered as needed with tap water.

After three months the plants were harvested and the roots and shoots were separated. Measurements included shoot length, dry weights of roots and shoots, total root length (Tennant, 1975), and the percentages of N, P, and K in roots and shoots. The percentage of N was determined by Nesslerization, P by the ammonium-phospho-molybdate method with 1,2,4-amino naphthol sulphonic acid as the reducing agent (Jackson, 1958), and K by flame spectrophotometry with a model 9200 Unicam flame spectrophotometer. Nutrient concentration of roots and root length were determined for all species except perennial ryegrass. Shoot length was measured for all species except forsythia and perennial ryegrass. The amount of mycorrhizal infection was determined by visual estimation using root staining and microscopy (Gray and Gerdemann, 1967; Phillips and Hayman, 1970; Giovanetti and Mosse, 1979). The treatments were arranged in a randomized complete block design with 13 replicated for height measurements and 6 replicates for all other measurements. Data were analyzed by analysis of variance, with the Newman-Keuls' test of significance used to separate means.

Results

1. Liriodendron tulipifera. Inoculation of L. tulipifera with viable G. fasciculatus resulted in mycorrhizal development (Table II-2, appendix A). Roots of inoculated plants grown at 2 or 4 g/l N had high mycorrhizal development (more than 50% of cortical cells infected with hyphae). Inoculated plants grown without supplemental fertilizer (0 g/l N) had very little mycorrhizal development (less than 5% of cortical cells infected with hyphae). Noninoculated plants had no mycorrhizal infection.

Table II-2. Percent of cortical cells infected with mycorrhizae in roots of inoculated and noninoculated L. tulipifera, A. platanoides, F. x intermedia, and L. perenne grown at three fertility levels.

Plant	Fertility (g/l N)	Inoculated	Noninoculated
<u>L. tulipifera</u>	0	0-2	0
	2	60-80	0
	4	60-80	0
<u>A. platanoides</u>	0	0-2	0
	2	0-2	0
	4	0-2	0
<u>F. x intermedia</u>	0	0-2	0
	2	0-2	0
	4	0-2	0
<u>L. perenne</u>	0	0-2	0
	2	0-2	0
	4	0-2	0

Inoculation with G. fasciculatus increased plant growth of fertilized plants (Figure II-1). Without supplemental fertility, there was no significance increase in the heights of plants with or without mycorrhizal inoculation. At 2 or 4 g/l N, inoculated plants were taller than noninoculated plants, but there was no significant difference between the two fertility levels.

Mycorrhizal inoculation also increased the dry weights of shoots from fertilized plants. Without fertilization, there was no difference between the dry weight of shoots from inoculated and noninoculated plants. At 2 and 4 g/l N, shoots of inoculated plants were significantly heavier than shoots of noninoculated plants. Dry weights of shoots from inoculated plants fertilized

with 4 g/l N were also greater than shoots from inoculated plants fertilized with 2 g/l N. Dry weights of noninoculated plants did not differ significantly regardless of fertility level.

Inoculation also increased the dry weights of roots from fertilized plants. There was no difference between the dry weights of roots from inoculated and noninoculated plants grown without fertilizer. At 2 and 4 g/l N, dry weights of roots from inoculated plants were heavier than those from noninoculated plants. However, there was no difference between the dry weights of roots from plants grown at the two fertility levels.

Mycorrhizae also increased root lengths of fertilized plants. Inoculated plants grown at 2 or 4 g/l N had longer roots than those from other treatments, but were not significantly different from each other. Root length of inoculated plants grown without fertilizer and noninoculated plants grown at all nutrient levels did not differ.

Finally, there were differences in the nutrient concentrations of inoculated and noninoculated plants at the three fertility levels (Table II-3). Inoculation did not affect foliar N concentrations, although foliar N concentration was increased by additions of fertilizer. Inoculation slightly increased the percentage of N in roots of plants grown at 2 or 4 g/l N. Nitrogen in roots also was less in both inoculated and noninoculated plants grown without fertilizer.

Foliar P concentration was the same for all treatments except noninoculated plants grown without supplemental fertilizer, which had less phosphorus than the other treatments. Therefore, mycorrhizal development increased the P concentration in shoots of inoculated plants grown without supplemental fertilizer. Phosphorus concentrations were greatest in noninoculated roots from plants grown at 2 g/l N.

Figure II-1. Effects of fertility and inoculation with mycorrhizal *Glomus fasciculatus* on growth of *Liriodendron tulipifera*.²

² Separation of means by the Newman-Keuls test of significance, 5% level. Mean of six values.

Table II-3. Effects of fertility and inoculation with Glomus fasciculatus on nutrient concentration (% N, P, and K) in roots and shoots of Liriodendron tulipifera.^z

Nutrient (%)	Fertility Level (g N/l)	Shoot		Roots	
		Inoculated	Noninoculated	Inoculated	Noninoculated
N	0	1.069b	0.706b	0.418s	0.323d
	2	2.037a	2.584a	1.467bc	1.183c
	4	2.329a	3.108a	2.025a	1.867ab
P	0	0.205a	0.062b	0.193b	0.198b
	2	0.157a	0.138a	0.413b	0.978a
	4	0.161a	0.228a	0.378b	0.201b
K	0	1.075c	0.578c	1.833b	1.870b
	2	1.438b	1.662b	1.973a	2.970a
	4	1.112bc	2.275a	3.063a	3.150a

^z Separation of means by the Newman-Keuls' test of significance, 5% level. Mean of 6 values. Differing letters represent significance in rows and columns for each nutrient in roots and shoots.

Inoculation increased foliar K concentration of unfertilized plants. However, the highest foliar K concentration was in noninoculated plants grown at 4 g/l N. Mycorrhizal development did not affect the concentration of K in roots. The percentage of K in roots was significantly less in plants grown without fertilizer.

2. Acer platanoides. Inoculation of A. platanoides with G. fasciculatus resulted in minimal mycorrhizal development (Table II-2). The least mycorrhizal development on inoculated plants occurred when no fertilizer was added. High fertility also suppressed mycorrhizal development, since infection was greatest at 2 g/l N. However, even at 2 g/l N mycorrhizal development was

not great. Noninoculated plants had no mycorrhizal development.

Although inoculation resulted in some mycorrhizal development, it did not enhance plant growth at any fertility level studied (Table II-4). Addition of fertilizer did increase growth. Height, dry weights of shoots and roots, and root length were the same for all treatments except plants grown without supplemental fertilizer, which had less growth than other treatments. Increasing fertility from 2 to 4 g/l N did not further increase growth.

Table II-4. Effects of fertility and inoculation with Glomus fasciculatus on growth of Acer platanoides.^z

Plant	Fertility (g/l N)	Inoculated	Noninoculated
=====			
Height Increase (cm) ^y	0	0.64b	0.34b
	2	55.35a	43.41a
	4	49.32a	49.13a
Dry Weight of Shoots (g) ^x	0	0.343b	0.517b
	2	1.382a	1.270a
	4	1.890a	1.471a
Dry Weight of Roots (g) ^x	0	0.311b	0.309b
	2	7.283a	7.117a
	4	7.8967a	6.817a
Root Length (cm) ^x	0	1297.0b	1290.6b
	2	5848.2a	8229.7a
	4	8724.9a	8227.7a

^z Separation of means by the Newman-Keuls' test of significance, 5% level. Differing letters represent significance in rows and columns for each plant response.

^y Mean of 13 values.

^x Mean of 6 values.

While inoculation did not promote overall growth, there were some effects on plant nutrient concentrations (Table II-5). Inoculation and increasing the fertilizer application from 2 to 4 g/l N did not significantly affect the N concentration in roots and shoots of inoculated plants, there was a trend of increased N concentration in shoots and a trend of decreased N concentration in roots of inoculated plants.

Foliar P concentration of plants grown at 2 or 4 g/l N decreased following inoculation. However, inoculation increased the P concentration in roots of plants at these fertility levels.

Mycorrhizal inoculation also did not affect K concentration in roots or shoots of Norway maple. Potassium concentration was least when no fertilizer was applied (0 g/l N), but raising the fertility level from 2 to 4 g/l N did not affect percentage of potassium in roots or shoots.

3. Forsythia x intermedia. Mycorrhizal inoculation of forsythia with G. mosseae did not result in infection, except for a small amount with inoculated plants grown without fertilizer (Table II-2). Plants grown at 4 g/l N were heaviest with unfertilized plants weighing the least (Table II-6). Inoculation with G. mosseae did not significantly increase shoot weight, although inoculated plants tended to be heavier.

Dry weights of roots were not significantly influenced by inoculation, but were increased by fertilization. Fertilization with 2 g/l N increased root dry weight, but additional fertilizer application did not further increase growth.

Inoculation did not influence foliar N and P concentrations of forsythia, but did slightly decrease foliar K concentration (Table II-7). Fertilization increased foliar N concentration, but increasing the fertilizer rate from 2 to 4 g/l N did not further increase N concentration.

Table II-5. Effects of fertility and inoculation with Glomus fasciculatus on nutrient concentration (% N, P, and K) in root and shoots of Acer platanoides.^z

=====					
Fertility					
Nutrient (%)	Level (g N/l)	Shoots		Roots	
		Inoculated	Noninoculated	Inoculated	Noninoculated
N	0	0.49b	0.81b	0.37c	0.44c
	2	2.43a	2.41a	1.64b	1.77ab
	4	2.72a	2.60a	1.69b	2.03a
P	0	0.09b	0.14b	0.11c	0.14bc
	2	0.18b	0.29a	0.24a	0.17b
	4	0.21b	0.34a	0.25a	0.18b
K	0	0.65b	0.94b	0.94b	1.30b
	2	1.75a	1.64a	1.93a	2.06a
	4	1.52a	1.65a	2.00a	1.95a
=====					

^a Separation of means by the Newman-Keuls' test of significance, 5% level. Mean of 6 values. Differing letters represent significance in rows and columns for each nutrient in roots and shoots.

Table II-6. Effects of fertility and inoculation with Glomus mosseae on growth of Forsythia x intermedia.^z

Plant Response	Fertility (g/l N)	Inoculated	Noninoculated
<hr/>			
Dry Weight of			
Shoots (g)	0	0.750c	0.941c
	2	10.250b	9.123b
	4	14.328a	12.913a
Dry Weight of			
Roots (g)	0	1.099b	0.688b
	2	2.975a	3.140a
	4	3.874a	3.241a
Root Length (cm)	0	418.2a	615.2a
	2	4088.4a	3465.2a
	4	2425.5a	1579.3a
<hr/>			

^z Separation of means by the Newman-Keuls' test of significance, 5% level. Mean of 6 values. Letters represent significance in rows and columns for each plant response.

Table II-7. Effects of fertility and inoculation with *Glomus mosseae* on nutrient concentration (% N, P, and K) in roots and shoots of *Forsythia x intermedia*.^z

Nutrient (%)	Fertility Level (g N/l)	Shoots		Roots	
		Inoculated	Noninoculated	Inoculated	Noninoculated
N	0	0.458b	0.727b	0.858b	0.613b
	2	3.354a	3.342a	1.729ac	0.577b
	4	3.317a	3.185a	1.953a	0.557b
P	0	0.088b	0.087b	0.078c	0.032d
	2	0.250a	0.240a	0.203a	0.135b
	4	0.218a	0.243a	0.165b	0.146b
K	0	0.745d	0.792d	1.351c	1.388c
	2	2.184bc	2.318ab	2.580b	3.198a
	4	2.048c	2.473a	2.563b	3.273a

^z Separation of means by the Newman-Keuls' test of significance, 5% level. Mean of 6 values. Differing letters represent significance in rows and columns for each nutrient in roots and shoots.

Inoculation increased the N concentration in roots of fertilized plants (2 and 4 g/l N), but fertility had no effect on noninoculated plants. Inoculation also increased P concentration of roots at 0 or 2 g/l N. Phosphorus concentration was greatest in inoculated plants grown at 2 g/l N. Potassium concentration of roots was increased in fertilized plants, but was not further increased by additional fertilizer. Inoculation decreased K concentration in roots.

Root length was not significantly influenced by fertility or inoculation, although inoculation tended to increase root length of fertilized plants. Plants grown at 2 g/l N tended to have the greatest total root length, while plants grown at 0 g/l N tended to have the shortest total root length.

4. Lolium perenne. Mycorrhizal development of L. perenne with G. fasciculatus was not extensive (Table II-2). There was slight infection (less than 5% of cortical cells infected) on roots of all inoculated plants. Mycorrhizal infection was greatest at 2 g/l N and was less at higher or lower nutrient levels.

Dry weight of shoots was not influenced by inoculation (Table II-8). Fertilization increased shoot growth, but raising fertilizer applications from 2 to 4 g/l N did not further improve growth. Foliar N was also unaffected by inoculation, although it was influenced by fertility. Nitrogen concentration was highest in plants grown at 2 g/l N. Foliar P concentration was increased by inoculation at 2 g/l N, and was lowest in unfertilized plants. Potassium concentration was increased by fertilization, but not affected by inoculation. Increasing fertility from 2 to 4 g/l N did not enhance foliar K concentration.

Discussion

With the species tested, mycorrhizal inoculation promoted plant growth in tulip poplar infected with G. fasciculatus. Inoculation of tulip poplar was effective for increasing plant growth only under fertilized conditions. Plants grew taller with longer and heavier roots at both 2 to 4 g/l N, but shoots were heaviest at 4 g/l N. Without supplement fertilization at the growing medium, mycorrhizal inoculation was not beneficial to the host plant.

The data indicated that inoculated plants at 4 g/l N had greater shoot:root ratios than those of plants grown at 2 g/l N. This is indicated by the significant increase in dry weights of shoots of inoculated plants at 4 g/l N relative to those at 2 g/l N, but no similar increase for dry weights of roots. The altered shoot:root ratio may be caused by the increased nitrogen supply at the 4 g/l N level. An excessively high shoot:root ratio could prohibit establishment and survival of the plants in the landscape.

Mycorrhizae have a potential role in the production and landscape use of tulip poplar because of the increases in growth following inoculation. Establishment of an inoculation program can enable more efficient use of nutrients by plants.

Table II-8. Effects of fertility and inoculation with Glomus fasciculatus on shoot dry weight (g) and nutrient concentration (% N, P, and K) of Lolium perenne.^z

Plant Response	Fertility (g/l N)	Inoculated	Noninoculated
Shoot Dry Weight			
(g)	0	0.032b	0.035b
	2	2.977a	3.082a
	4	2.754a	3.012a
Foliar N (%)	0	1.353c	1.218c
	2	3.367a	3.533a
	4	3.112b	3.000b
Foliar P (%)	0	0.365c	0.344c
	2	0.575a	0.452b
	4	0.528a	0.547a
Foliar K (%)	0	1.953b	1.991b
	2	3.388a	3.532a
	4	3.185a	3.255a

^z Separation of means by the Newman-Keuls' test of significance, 5% level. Mean of 6 values. Differing letters represent significance in rows and columns for each plant response.

However, for other plant species studied inoculation had no beneficial effect on plant growth at the fertility levels tested, even though slight mycorrhizal development was observed. Plants with roots which are better adapted for nutrient uptake may be less dependent on mycorrhizal formation, and therefore may have greater specificity for a particular fungal symbiont before increased growth occurs. Perhaps such plant-fungal associations would result in growth increases at fertility levels more marginal for plant growth than those studied (less than 2 g/l N but higher than 0 g/l N).

For all plant species, foliar and root nutrient concentrations were influenced by inoculation, indicating definite interactions even when plant growth was not influenced. If growth is limited by any factor, it is feasible that mycorrhizae may still influence uptake of some nutrients, resulting in higher concentrations of those nutrients in the plant. However, if mycorrhizal development acts to reduce the effect of the limiting factor even slightly, then the concentration of some nutrients may be reduced as the nutrients are spread over a larger volume of plant tissue.

The optimum nutrient level appears to be 2 g/l N for the plants studied regardless of interactions with mycorrhizal fungi, with the exception of inoculated tulip poplar, which had heavier shoots when fertilized with 4 g/l N. Addition of fertilizer greater than 2 g/l N would not result in any growth increases for some plant species.

In plants which did not receive a growth stimulation from mycorrhizal formation, there was no detrimental effect from mycorrhizal inoculation. This suggests that when several plant species are being inoculated, an inoculum source could contain numerous species of mycorrhizal fungi to accommodate all plants present. Plants forming mycorrhizal roots without growth increases showed no inhibition of growth following inoculation under extremes of

fertility conditions. However, in such cases where the plant and fungal species are not highly compatible, other environmental conditions, such as extreme temperature, may result in inhibition of growth of inoculated plants. Inoculum intended for various plant species should have at least one highly compatible fungal species for each plant species present, to insure increased growth.

In summary, it appears that minimum nutrient requirements must be met before inoculation with mycorrhizal fungi will result in increased growth. Inoculation of tulip poplar was beneficial to plant growth even under fertile conditions (4 g/l N of 19N-6P-12K), but the optimum fertilizer rate for most species studied was 2 g/l N. Establishment of an inoculation program can enable more efficient use of nutrients by plants. For growth increases to occur, the plant and fungal symbiots must be compatible, and nutrients must be available. The economic feasibility of maintaining an inoculum source will determine the commercial success of an inoculation program.

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CHAPTER III

EFFECTS OF TEMPERATURE AND INOCULATION WITH
GLOMUS FASCICULATUS ON GROWTH OF LOLIUM PERENNE

CHAPTER III

EFFECTS OF TEMPERATURE AND INOCULATION WITH

GLOMUS FASCICULATUS ON GROWTH OF LOLIUM PERENNE

Abstract

Effects of temperature and inoculation with Glomus fasciculatus on growth of Lolium perenne were determined. Seeds of L. perenne were sown in 0.725 liter pots containing pasteurized medium amended with 2 g/liter N of 19N-6P-12K controlled release fertilizer. Half the plants were inoculated with G. fasciculatus inoculum (containing spores, hyphae, soil, and roots from the previous culture). Plants from each group were grown at 40.35°C, 30.25°C or 20/15°C.

Mycorrhizal inoculation resulted in slight infection (less than 2% of cortical cells infected) and did not increase growth. Plant growth and foliar K concentration were reduced when inoculated plants were grown at 40/35°C, but P concentration increased at 40/35°C. The negative effect on plant growth by inoculation at high temperatures is attributed to the presence of pathogens in impure inoculum, and their activity at high temperatures.

Introduction

Perennial ryegrass (Lolium perenne L.) is a major component of grass seed mixtures used to revegetate disturbed sites after construction. Soil conditions on disturbed sites, including fertility and moisture, are generally not optimal for plant growth (Carpenter et al., 1976). Plant growth is further limited by high temperature, particularly on south and west facing slopes without established vegetation. Temperatures as high as 51°C have been recorded for exposed slopes (Masiunas, 1981). However, best shoot growth of perennial ryegrass occurs below 25°C (Sullivan and Sprague, 1949; Beevers and

Cooper, 1978). Root growth of perennial ryegrass is best below 26.6 to 32.3°C (Sullivan and Sprague, 1949).

Development of mycorrhizae is also temperature dependent. Optimum temperatures for spore germination depends on the species of fungi, but may be as high as 34°C (Schenck et al., 1975). Hyphal development may be greatest at temperatures also optimum for root growth (Schenck and Schroder, 1974). Mycorrhizal development in onion (Allium cepa L.) did not increase plant growth at high temperatures (41°C), but did promote growth at lower temperatures (14 to 23°C). Furlan and Fortin (1973) found that the rate of mycorrhizal infection was greatest at 21°C day/26°C night.

Glomus fasciculatus (Thaxter) Gerdemann and Trappe did infect perennial ryegrass, but did not increase growth under the various fertility levels tested (Chapter I). It is not known if infection of perennial ryegrass by G. fasciculatus can promote growth of plants at extreme temperatures. The objective of this experiment was to examine the effect of temperature and inoculation with G. fasciculatus on growth of perennial ryegrass.

Materials and Methods

Seeds of Lolium perenne L. were sown at a rate of 12.33 g/m² and covered with 3 to 5 cm of vermiculite in 0.725 liter pots containing steam pasteurized perlite:sphagnum peat moss:soil (2:2:1, v/v/v). Plants were fertilized with 'Osmocote' 19N-6P-12K controlled-release fertilizer at 2 g/liter Nitrogen. Half the plants were treated with Glomus fasciculatus (Thaxter) Gerdemann and Trappe inoculum (containing spores, hyphae, soil, and roots from the previous culture) at a rate of 44,400 spores/m² of soil surface. Ten plants of each group were grown in growth chambers at 40°C day/35°C night, 30°C day/25°C night, or 20°C day/15°C night. After 5 to 8 weeks plants were harvested and

dry weights of shoots (g), foliar N, P, and K concentration, and degree of mycorrhizal infection were determined. The degree of mycorrhizal infection was estimated using root staining and microscopy (Phillips and Hayman, 1970; Gray and Gerdemann, 1967; Giovanetti and Mosse, 1970). The N concentration was determined by the Nesslerization method, P by the ammonium-phospho-molybdate method with 1,2,4-amino naphthol sulphonic acid as the reducing agent (Jackson, 1958), and K by flame spectrophotometry with a model 9200 Unicam flame spectrophotometer. For each temperature level, there were ten replicates arranged in a completely randomized design.

Results and Discussion

Inoculation of L. perenne with G. fasciculatus resulted in slight infection (less than 2% of cortical cells infected). Noninoculated plants did not develop mycorrhizae (Table III-1). Furthermore, inoculation did not increase growth at any temperature used (Figure III-1). In fact, growth of perennial ryegrass was reduced when plants were grown at 40/35°C or 30/25°C, but foliar K concentration was reduced by inoculation at 40/35°C (Table III-2). However, inoculation increased foliar P concentration of plants grown at 40/35°C, indicating a limited beneficial effect of inoculation at high temperatures. Growth of this cool-season grass was best at the lowest temperature tested (20/15°C).

Table III-1. Percent of cortical cells infected with mycorrhizae in roots of inoculated and noninoculated L. perenne grown at 3 temperature levels.

Temperature (day/night)	Inoculated	Noninoculated
20/15°C	0-2	0
30/25°C	0-2	0
40/35°C	0-2	0

Table III-2. Effects of temperature and inoculation with Glomus fasciculatus on foliar nutrient concentration (% N, P, and K) of Lolium perenne.^z

Temperature (day/night)	Nutrient	Inoculated	Noninoculated
20/15°C	N	2.440a	2.508a
	P	0.360a	0.379a
	K	3.991a	4.013a
30/25°C	N	1.918a	2.063a
	P	0.202a	0.224a
	K	2.215a	2.248a
40/35°C	N	4.342a	4.789a
	P	0.945a	0.648b
	K	2.704b	3.317a

^z Separation of means by the Newman-Keuls' test of significance, 5% level. Mean of 10 values. Differing letters within rows indicate significance.

Figure III-1. effects of temperature and inoculation with *Glomus fasciculatus* on dry weight of shoots (g) of *Lolium perenne*.²

² Separation of means by the Newman-Keuls test of significance, 5% level. Mean of 10 values.

The reduction in growth and K concentration of inoculated plants grown at 40/35°C are probably due to the presence of pathogens in the inoculum, which are most active and exerted a detrimental effect only at high temperatures. Pathogenic microorganisms could be present in the inoculum since it consists of the roots, spores, hyphae, soil and associated microbes of the previous host (Lycopersicon esculentum Mill.). If a more compatible fungal species was used as the inoculum, the negative effect of the inoculum at the high temperature might have been compensated for, resulting in a promotion of growth by inoculation. Impure inoculum may be rendered more effective through the use of pesticides which can reduce the activity of the pathogens present, but not reduce the activity of mycorrhizae.

These results indicate that impure inoculum should not be used for plants growing in pasteurized medium at excessively high temperatures, particularly if plant-fungi compatibility is low. However, the use of impure inoculum for highly compatible symtients may still result in growth promotion at high temperatures.

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CHAPTER IV
EFFECTS OF ENDOMYCORRHIZAL INOCULATION ON ROOT
INITIATION AND GROWTH OF CUTTINGS OF
LIGUSTRUM OBTUSIFOLIUM VAR. REGELIANUM

CHAPTER IV

EFFECTS OF ENDOMYCORRHIZAL INOCULATION ON ROOT INITIATION AND
GROWTH OF CUTTINGS OF LIGUSTRUM OBTUSIFOLIUM VAR. REGELIANUMAbstract

Effects of mycorrhizal inoculation on root initiation and growth were determined. Terminal cuttings of Ligustrum obtusifolium Var. regelianum were rooted under intermittent mist either in an unamended medium of vermiculite: perlite (1:1, v/v), medium amended with Glomus mosseae inoculum, or in medium amended with pasteurized inoculum. Amendment with inoculum resulted in mycorrhizal development and enhanced root growth, but did not increase root initiation. Amendment of a rooting medium with inoculum results in the earliest possible establishment of growth enhancing mycorrhizal roots.

Introduction

Vegetative propagation by rooting of cuttings is currently the most economically important propagation technique in the production of woody landscape plants used to revegetate highway construction sites. However, many plants are difficult to root or require long periods for development of adequate root systems, adding considerably to production time and cost. Amendment of a rooting medium with mycorrhizal inoculum might result in improved rooting, root development, and establishment of a mycorrhizal root system important for the subsequent establishment of the plants on highway soils.

Ectomycorrhizal inoculation was reported to increase rooting percentage of bearberry (Arctostaphylos uva-ursi L. Spreng.) and huckleberry (Vaccinium ovatum Parsh.) (Lindermann and Call, 1977). There are more frequent reports that mycorrhizal inoculation promotes root development following rooting

(Holden, 1978; Veldeman, 1981; Bannister and Norton, 1974; Crews et al., 1978). The objective of this experiment was to determine the effect of endomycorrhizal inoculation on root initiation and subsequent growth of regel's privet cuttings (Ligustrum obtusifolium Var. regelianum (Koehne) Rehd.).

Materials and Methods

Terminal cuttings of L. obtusifolium Var. regelianum (16.25 cm long with 8 leaves) were treated with 0.1% (w/v) IBA (indolebutyric acid) and rooted either in an unamended rooting medium of vermiculite:perlite (1:1, v/v), in inoculum:rooting medium (1:3, v/v), or in pasteurized inoculum:rooting medium (1:3, v/v). The mycorrhizal inoculum consisted of roots of tomato (Lycopersicum esculentum Mill.), spores and hyphae of Glomus mosseae Nicol. and Gerd., and soil mix (2 perlite:2 sphagnum peat moss: 1 soil, v/v/v). Inoculum and pasteurized inoculum treatments contained 40,800 spores (2.19 spores/cm³).

Cuttings were inserted into the medium in flats (35 x 42.5 x 12.5 cm) and placed under intermittent mist (12 seconds/8 minutes) in a greenhouse with approximately 25% shade. Eight cuttings were harvested from each treatment on the third and sixth weeks after sticking cuttings. Measurements included fresh weight of roots and number of root initials visibly penetrating the stems of the cuttings. Subsample error was used to test significance.

Results and Discussion

On the third week after sticking cuttings, there were no differences between treatments in number or fresh weight of roots (Figure IV-1). During the sixth week, there was no difference in the number of roots formed, but there was a difference in the fresh weight of roots (Figure IV-2). The fresh weight of roots from cuttings rooted in medium amended with inoculum were

significantly greater than those from cuttings rooted in the rooting medium with no amendment. The fresh weight of roots from cuttings rooted in medium amended with pasteurized inoculum were not significantly different from either of the other treatments.

Amendment of the rooting medium resulted in mycorrhizal development following rooting. By the sixth week after removal from the stock plant, the roots of cuttings rooted in medium amended with inoculum were mycorrhizal (5 to 10% of cortical cells infected), roots of cuttings rooted in medium amended with pasteurized inoculum had slight mycorrhizal development (0 to 2% of cortical cells infected), and roots of cuttings rooted in unamended medium had no mycorrhizal development.

No evidence was found linking inoculation with endomycorrhizal fungi to increased root initiation. Amendment of the rooting medium with inoculum did lead to early development of mycorrhizae and increased fresh weight of roots. Fresh weight of roots from cuttings rooted in medium amended with pasteurized inoculum were intermediate to other treatments probably because of two factors. First, the physical effect of the amendment on moisture and aeration may result in an improved rooting medium. Secondly, some mycorrhizal development was detected on roots of cuttings rooted in medium amended with pasteurized inoculum. This indicates that a portion of the inoculum remained viable after steam treatment and may have increased root development.

Inoculation of woody plants in the propagation stage of production may prove to be the most efficient time, since it results in the earliest development of mycorrhizal roots and subsequent enhancement of growth and establishment of the plants on the highway site. Since early mycorrhizal development is achieved, subsequent growth promotions could reduce the cost of producing these plants. In addition, since mycorrhizal plants are produced, it may be unnecessary to further inoculate the highway soil.

Figure IV-1. Effects of *G. mosseae* and pasteurized *G. mosseae* on root number and root fresh weight (g) of cuttings of *L. obtusifolium* Var. *regelianum* after three weeks.^z

^z Separation of means by the Newman-Keuls test of significance 5% level. Mean of 8 values.

Figure IV-2. Effects of *G. mosseae* and pasteurized *G. mosseae* on root number and root fresh weight (g) of cuttings of *L. obtusifolium* Var. *regelianum* after six weeks.²

² Separation of means by the Newman-Keuls test of significance, 5% level. Mean of 8 values.

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CHAPTER V

EFFECTS OF FUNGICIDE ON MYCORRHIZAL
DEVELOPMENT BY GLOMUS FASCICULATUS

CHAPTER V

EFFECTS OF FUNGICIDE ON MYCORRHIZAL DEVELOPMENT BY GLOMUS FASCICULATUSAbstract

The effects of fungicide applications on growth of mycorrhizal and nonmycorrhizal plants were determined. Seedlings of Liriodendron tulipifera were planted in containers and half were inoculated with the endomycorrhizae Glomus fasciculatus. Every two weeks plants from each group were treated with a Benlate drench.

Inoculated plants without fungicide application were tallest and heaviest. Fungicide application increased growth of nonmycorrhizal plants, but reduced growth of mycorrhizal plants. To gain the benefit of mycorrhizal development, Benlate should not be used on inoculated plants.

Introduction

Mycorrhizal inoculation may have important uses for increasing growth of landscape plants used for highway revegetation. Often woody landscape plants are cultured in containers and nurserymen have used chemicals to enhance growth by reducing the activity of diseases and pathogens. Fungicides are routinely used, particularly during propagation, to prevent the occurrence of fungal diseases associated with moist or humid conditions. However, fungicides may have a detrimental effect on mycorrhizal development, and the subsequent enhancement of plant growth associated with mycorrhizae.

Benlate was reported to have a negative effect on growth of mycorrhizal plants, while Captan was less inhibitory (deBertoldi et al., 1977; Jalai and Domesch, 1975; Boatman et al., 1978; Nemec, 1980; Bailey and Safair, 1977; Sutton and Sheppard, 1976). Benlate has an active ingredient of 50% benomyl (1(N-buylcarbamoyl)-2-(methoxycarboximidazole) and is used to control fungal

pathogens responsible for diseases including many root rots, powdery mildew and botrytis. The objective of this experiment was to determine the effects of Benlate application on growth of Liriodendron tulipifera L. inoculated with mycorrhizal fungi.

Materials and Methods

Stratified seeds of Liriodendron tulipifera L. were sown in a medium of perlite:vermiculite (1:1, v/v) and grown in a growth chamber for five weeks (23.5°C day/22.5°C night $\pm$ 1°C, with a 16 hour photoperiod). Twenty uniform seedlings were transplanted into 0.725 liter pots (one quart trade designation) containing a medium of perlite:sphagnum peat moss:soil (2:2:1, v/v/v), with less than 10 mg/l NO₃, 0.8 mg/l P, 2.58 mg/l K, and a pH of 5.8. Plants were fertilized with 'Osmocote' 19N-6P-12K controlled-release fertilizer at a rate of 2 g/l Nitrogen. Half the seedlings were inoculated with Glomus fasciculatus (Thaxter) Gerdemann & Trappe at a rate of 44,400 spores/m² of container surface area. The inoculum contained spores, hyphae, roots and soil from the previous culture and was inserted in three cores to a depth of 6 cm in the container. Every two weeks, 78 ml of Benlate (50% Benomyl) was applied to 5 plants of each treatment as a drench at 4.5 g/3.8 l. Control plants were drenched with 78 ml of tap water.

After seven weeks, plants were harvested and shoots separated from roots. Measurements included seedling height (cm), dry weight of roots and shoots (g), estimated total root length (m) (Tennant, 1975), and nutrient analysis (% N, P, and K) of roots and shoots. Nitrogen was determined by the Nesslerization method, P by the ammonium-phospho-molybdate method with 1,2,4-amino naphthol sulphonic acid as the reducing agent (Jackson, 1958), and K by flame spectrophotometry using a model 9200 Unicam flame spectrophotometer.

Degree of mycorrhizal infection was evaluated by visual estimation using root staining and microscopy (Gray and Gerdemann, 1967; Phillips and Hayman, 1970; Giovanetti and Mosse, 1979). Plants were arranged in a completely randomized design with 5 replications per treatment combination. Data were analyzed using analysis of variance, with the Newman-Keuls' test of significance to separate means.

Results and Discussion

Inoculation resulted in mycorrhizal development and significant enhancement of plant growth. However, mycorrhizal development and growth enhancement was prevented by fungicide application. Inoculated plants not treated with Benlate had extensive endomycorrhizal development, but inoculated plants treated with Benlate had limited endomycorrhizal development (Table V-1). Noninoculated plants had no mycorrhizal development regardless of fungicide treatment.

Inoculation resulted in the most growth when fungicide was not used. Inoculated plants with no Benlate applied were tallest, and had the heaviest shoots and roots (Figure V-1). Fungicide application enhanced growth of nonmycorrhizal plants, but reduced growth of mycorrhizal plants. Root systems of mycorrhizal plants tended to be longest and fungicide application tended to reduce total root length.

Table V-1. Percent of cortical cells infected with mycorrhizae in roots of inoculated and noninoculated Liriodendron tulipifera grown with and without Benlate fungicide.

Fungicide Treatment	Inoculated	Noninoculated
Benlate	0-2	0
Control	10-30	0

Figure V-1. Effects of Benlate and inoculation with *Glomus fasciculatus* on growth of *Liriodendron tulipifera*.^z

^z Separation of means by the Newman-Keuls test of significance, 5% level.

Inoculation significantly reduced concentrations of foliar K and root N, and slightly reduced concentrations of root K (Table V-2). The concentration of P in roots was increased by mycorrhizal development.

These results indicate that while inoculation with endomycorrhizal fungi can significantly enhance growth and produce mycorrhizal plants suitable for highway planting, the use of some fungicides during culture may prevent the beneficial effect of inoculation. Therefore, if mycorrhizal inoculation is used commercially, cultural aspects must be considered and certain fungicides avoided to benefit from mycorrhizal development.

Table V-2. Effects of Benlate and Glomus fasciculatus on nutrient concentrations (% N, P, and K) in roots and shoots of Liriodendron tulipifera.^z

Nutrient (%)	Fungicide Treatment	Shoots		Roots	
		Inoculated	Noninoculated	Inoculated	Noninoculated
N	Benlate	2.495a	2.340a	2.556a	3.273a
	Control	1.375a	2.275a	1.420b	2.953a
P	Benlate	0.189a	0.173a	0.265b	0.201b
	Control	0.206a	0.176a	0.663a	0.156b
K	Benlate	1.986a	2.044a	3.558a	2.990ab
	Control	0.850b	1.498a	2.518b	3.838a

^z Separation of means by the Newman-Keuls' test of significance, 5% level. Mean of 6 values. Differing letters represent significance in rows and columns for each nutrient in roots and shoots.

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CHAPTER VI
NUMBER OF MYCORRHIZAL SPORES
IN HIGHLY DISTURBED HIGHWAY SOILS

CHAPTER VI

NUMBER OF MYCORRHIZAL SPORES IN HIGHLY DISTURBED HIGHWAY SOILS

Abstract

Following construction of a new highway, the soil is unavoidably disturbed, providing an unfavorable environment for the reestablishment of plants on these sites. These locations are generally characterized by high air and soil temperatures, low moisture, poor fertility, and lack of beneficial mycorrhizal fungi.

Three highway soils were tested for presence of spores from mycorrhizal fungi, to examine the role of mycorrhizal fungi in the revegetation of disturbed highway soils. A newly disturbed soil had virtually no mycorrhizal spores present, while a soil successfully revegetated with herbaceous species and one revegetated with herbaceous and woody species had very high numbers of mycorrhizal spores. Initially there are no mycorrhizal spores present in highly disturbed soils, but the reoccurrence of mycorrhizal spores coincides with the successful reestablishment of landscape plants on harsh highway sites.

Introduction

After the construction of a new highway, the soils remaining are unavoidably disturbed and provide harsh environments for the reestablishment of vegetation on these sites. Unfavorable aspects of newly established highway locations often include high air and soil temperatures, low or excess moisture, poor fertility, and lack of beneficial soil microorganisms (Carpenter et al., 1976). The soils under consideration are often disturbed subsoils with microbe populations generally less than normal. Ultimate establishment and growth of the plant may depend on higher than normal populations of beneficial soil microorganisms. Perhaps the most useful of the beneficial soil microbes are symbiotic mycorrhizal fungi.

Conditions on very disturbed highway sites are similar to conditions on newly regraded strip mine sites. Conditions on strip mine sites include limited fertility, poor water relations, high temperatures, and low microbe populations (Miller et al., 1979). Prior to surface mining, the topsoil is piled and stored for replacement over recontoured soil during reclamation (Rives et al., 1980). The physical disruption of soil during strip mining and grading results in a heterogeneous mix of rock, clay, coal, topsoil, and subsoil (Ponder, 1979). The resulting mixture is varied, complex, and provides a poor environment for revegetation. Mycorrhizal fungi may play an important role in reestablishment of plant material in the harsh environments of strip mine sites (Allen and Allen, 1980; Khan, 1978).

On highly disturbed mine sites, endomycorrhizae are generally not present after regrading (Ponder, 1979) and it can take several years to develop high microbial populations (Miller et al., 1979). Often the top soil is stored for several years before regrading. Rives et al. (1980) found that storage of topsoil for three years greatly reduced the viability of the natural mycorrhizal inoculum present in the soil. In regrading, the concentration of remaining viable inoculum can be diluted through mixture with subsoil or rock, or may be buried too deeply to interact with plants colonizing the area. Moorman and Reeves (1979) confirm that disturbance of the land reduces the population of viable mycorrhizal fungi.

Reeves et al. (1979) found that plant cover in an undisturbed ecosystem on an arid site in Colorado was nearly 99% mycorrhizal, while the existent vegetation on an adjacent, highly disturbed site was only 1% mycorrhizal. They suggest that the lack of mycorrhizal fungi on the disturbed site results in the establishment of nonmycorrhizal plants that are effective colonizers during initial revegetation.

This means that plant population on the disturbed site has a greater percentage of plant species which can survive under harsh conditions without mycorrhizae, than does the original undisturbed site. This condition may be undesirable if the goal of revegetation is to restore the disturbed site to the original condition and plant community. Mycorrhizal fungi spread relatively slowly through the soil (up to 1.5 m/year) and altered population in the plant community could persist for some time (Powell, 1979).

Even after 2 to 3 years after reclamation, strip mine sites have up to 50% fewer mycorrhizal plants and have up to 50% fewer mycorrhizal spores present (Allen and Allen, 1980). However, Khan (1978) reports very variable, but high populations of mycorrhizal plants on reclaimed mine sites. The length of time of reclamation was not reported.

Available evidence suggests that populations of viable mycorrhizal fungi in highly disturbed soils are greatly reduced, resulting in initial colonization by nonmycorrhizal plants. Many years may pass before normal populations of microbes are present, permitting the return of the original floral population to the site. Inoculation with mycorrhizal fungi during reclamation of harsh sites may hasten revegetation and result in quicker reestablishment of the natural plant cover. The objective of this study was to determine the number of spores present before and after revegetation, as a measure of the importance of mycorrhizae in the reestablishment of plants on highly disturbed highway sites.

Materials and Methods

The number of spores were estimated in a newly disturbed soil, a highway soil revegetated with herbaceous plants, and a highway soil revegetated with herbaceous and woody plants. On the newly disturbed site, no vegetation was

present. The second site had been successfully revegetated for approximately eight years with herbaceous plants including Lolium perenne L., Poa pratensis L., and Coronilla varia L. The third site had also been successfully revegetated for approximately eight years, but in addition to the herbaceous species, it also had the woody plants Viburnum dentatum L., Lonicera morrowii A., and Fraxinus pennsylvanica Marsh. All three sites were located in West Lafayette, Indiana.

Ten soil samples were taken randomly from the top 6 cm of soil. For each site, the soil samples were mixed to form a composite sample, before subsampling for the spore counts. From each composite soil sample, 3 one-gram subsamples were examined for content of spores from mycorrhizal fungi. Spore isolation was done using the sucrose centrifugation method of Jenkins (1964), and quantification was done visually with the aid of a dissecting microscope. Analysis of variance was conducted using the Newman-Keuls' test of statistical significance.

Results and Discussion

Mycorrhizae do play a role in the revegetation of harsh highway sites. The newly disturbed soil had virtually no spores present. However, the soils that were successfully revegetated had much greater numbers of spores from mycorrhizal fungi (Table IV-1). There was no difference between the number of spores found in soil revegetated with herbaceous species, and those found in soil revegetated with herbaceous and woody species. This indicates that initially there were no mycorrhizal spores present in highly disturbed soils, but mycorrhizal development coincides with the reestablishment of landscape plants on harsh highway sites. Both herbaceous and woody plant species appear to rely on the development of mycorrhizae under the suboptimal conditions

present during revegetation. With increasing costs of plant material, fertilizers, energy, and labor, more efficient use of resources will become increasingly important in the future. Successful development of mycorrhizal inoculation programs for plants used in highway plantings could result in savings of limited financial resources spent on plant material and fertilizers.

Table VI-1. Numbers of mycorrhizal spores present in a newly disturbed soil, a highway soil revegetated with herbaceous plants, and one revegetated with woody and herbaceous plants.^z

Soil condition	Number of Mycorrhizal spores/g of soil
Newly Disturbed	0.1a
Revegetated with Herbaceous plants	24.3b
Revegetated with Herbaceous and Woody plants	32.6b

^z Separation of means by the Newman-Keuls' test of significance, 5% level. Mean of eight values used.

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CHAPTER VII
EFFECTS OF PASTEURIZED ENDOMYCORRHIZAL
INOCULUM ON PLANT GROWTH

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Abstract

The effects of pasteurized endomycorrhizal inoculum on plant growth were determined. Seedlings of Liriodendron tulipifera were grown in 0.725 liter pots under greenhouse conditions, and half were inoculated with steam pasteurized (225°C for 3 hours) Glomus fasciculatus inoculum. Inoculation with pasteurized G. fasciculatus resulted in no mycorrhizal development and did not influence growth. Steam pasteurization can render G. fasciculatus nonviable and incapable of increasing plant growth.

Introduction

It is known that inoculation with endomycorrhizae is an effective means for enhancing plant growth during production, yielding mycorrhizal plants better adapted to survival and establishment on harsh sites (Maronek et al., 1980; Johnson et al., 1980). However, soil media is often pasteurized to reduce disease problems and it is also important to know the effect of steam heat pasteurization on the viability of mycorrhizal inoculum, and the ability of pasteurized inoculum to form mycorrhizae and increase plant growth. The objective of this experiment was to examine the effects of pasteurized endomycorrhizal inoculum on plant growth.

Materials and Methods

Seeds of Liriodendron tulipifera L. were germinated in a medium of perlite:vermiculite (1:1, v/v) in a growth chamber (26 ± 2°C, with a 13 hour photoperiod). Twenty uniform seedlings were planted in pasteurized medium of perlite:sphagnum peat moss:soil (2:2:1, v/v/v) in 0.725 liter containers (1

quart trade designation). The unfertilized medium had 1.2 mg/l N, 0.4 mg/l P, 3.9 mg/l K, and had a pH of 6.2. Plants were treated with 2 g/liter N of 'Osmocote' (19N-6P-12K) controlled release fertilizer worked into the top 2 to 3 cm. Ten seedlings were inoculated with steam pasteurized (225°C for 3 hours) inoculum containing roots, Glomus fasciculatus (Thaxter) spores, hyphae, and growing medium (perlite:sphagnum peat moss:soil (2:2:1, v/v/v)) from the previous culture. Plants were grown under greenhouse conditions ($24 \pm 3^{\circ}\text{C}$, with a 16 hour photoperiod) and watered as needed with tap water. The plants were arranged in a completely randomized design, with 10 replicates per treatment.

After three months of growth, the plants were harvested and roots separated from shoots. Measurements included height increase, dry weights of roots and shoots, estimated root length (Tennant, 1975), and nutrient concentration in roots and shoots. Nitrogen concentration was determined by Nesslerization, P by the ammonium-molybdate method with 1,2,4-amino naphthol sulphonic acid as the reducing agent (Jackson, 1958), and K by flame spectrophotometry using a model 9200 Unicam flame spectrophotometer. The amount of mycorrhizal infection was evaluated by visual estimation using root staining and microscopy (Gray and Gerdemann, 1967; Phillips and Hayman, 1970; Giovanetti and Mosse, 1979). Data were analyzed by analysis of variance, with the Newman-Keuls' test of significance to separate means.

Results and Discussion

Inoculation with pasteurized inoculum resulted in no mycorrhizal development. Neither treatment developed mycorrhizal roots. Inoculation with pasteurized inoculum also had no effect on plant growth (Figure A-1, Table A-1). There were no differences between the heights, dry weights of roots and

shoots, root lengths, and nutrient concentrations in roots or in shoots of the two treatments.

These results indicate that steam pasteurization renders endomycorrhizal inoculum inviable and incapable of enhancing plant growth in container production. Therefore, since steam pasteurization is commonly used for soil media in container production, inoculation should occur after pasteurization of the medium. Endomycorrhizal inoculum must be viable to be useful for promoting growth of woody plants in container production and in the field.

Table A-1. Effects of pasteurized Glomus fasciculatus on nutrient concentration (% N, P, K) in roots and shoots of Liriodendron tulipifera.^z

Nutrient	Shoots		Roots	
	Control	Pasteurized Inoculum	Control	Pasteurized Inoculum
N	2.290a	2.492a	1.324a	1.440a
P	0.169a	0.192a	0.174a	0.176a
K	1.662a	1.830a	2.298a	2.382a

^z Separation of means by the Newman-Keuls' test of significance, 5% level. Mean of 10 values. Differing letters indicate significance in rows for each nutrient in roots and in shoots.

Figure A-1. Effects of pasteurized *Glomus fasciculatus* on growth of *Liriodendron tulipifera*.²

² Separation of means by the Newman-Keuls test of significance, 5% level.

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CONCLUSIONS

General conclusions of these and other studies:

- 1) Construction of a highway renders an environment which presents formidable problems for revegetation. The soils are often steeply sloped, and rocky. Soil temperature is often abnormally high and inhibitive to plant growth. Nutrients and moisture may also be limiting to plant growth. The soil may also be biologically void, having no microbes present to aid in weathering the soil or mycorrhizal fungi to aid in plant growth. Due to these problems, the selection of plant material capable of surviving on highly disturbed highway sites is presently severely limited.
- 2) Newly disturbed highway soils studied had virtually no mycorrhizal spores present, while successful revegetation of highway sites corresponds to the reestablishment of high spore numbers in the soil. Therefore, it is likely that mycorrhizae do play a role in the revegetation of harshly disturbed sites. Development of mycorrhizae has been found to enable the plant to take up moisture more readily and to utilize nutrients more efficiently.
- 3) Although storage of topsoil reduces the amount of viable mycorrhizae in soil, it still should be returned to the site when possible, since it does contain numerous mycorrhizal spores and provides a better growing environment than the exposed sub-soils (Powell, 1979).
- 4) The experiments conducted establish that endomycorrhizal inoculation does promote growth of some landscape plants used in highway plantings. Of particular importance is the ability of mycorrhizal plants to make more efficient use of scarce nutrients and limited water. However,

additional research is essential to establish the optimum combinations of more plant and fungal species used in highway plantings; to determine the most effective inoculation technique; and to further study the special environmental strain placed on plants growing on the sites, particularly the lack of topsoil and organic matter and the moisture stress related to deicing salt operations (Rives, et. al., 1980).

Conclusions primarily of these studies:

- 5) In harsh highway sites with no mycorrhizae present, non-mycotrophic early successional plants have an advantage over plants highly dependent on mycorrhizae. Exogenous introduction of mycorrhizae could shorten the time required for revegetation, and increase the number of plants suitable for highway use.
- 6) Natural movement of mycorrhizae in the soil from undisturbed to disturbed areas is very slow and can not be relied upon to adequately reestablish the microbe balance in disturbed highway sites.
- 7) Development of highly mycorrhizal plants may require special considerations in plant culture and production.
- 8) Soil sterilization or pasteurization often conducted in plant production eliminates endogenous mycorrhizal populations and increases the need for inoculation. If used, soil sterilants should be applied prior to the introduction of mycorrhizal inoculum.
- 9) Benlate fungicide is also used commonly in plant production and prevents mycorrhizal formation by some species. For sensitive species, alternative fungicides should be used.
- 10) Low plant and fungal compatibility can be detrimental to plants grown at high temperatures, due to the presence of pathogens in impure

inoculum cultures. Compatibility should be known prior to inoculation or selected fungicides should be used to reduce the activity of pathogens present.

- 11) Minimum nutrient requirements must be met before inoculation with mycorrhizal fungi will result in increased growth. In other words, increases in growth of plants attributable to mycorrhizal development are greatest with some supplemental nutrient source.
- 12) Mycorrhizal development can occur under conditions of very low to very high fertility during production but plant responses may be limited (i.e. increased growth). Inoculation during production is possible even with high rates of supplemental fertilizer. Such successful mycorrhizal inoculation during production will yield plants pre-adapted to transplanting and will be beneficial especially on difficult-to-revegetate sites.
- 13) This study indicates that the growth of all species of plants are not promoted by the same mycorrhizal fungi. For growth increases to occur, the plant and fungal symbionts must be compatible. It is possible to get limited mycorrhizal infection with no positive growth responses. This emphasizes further that growth responses due to mycorrhizal development may occur with only very specific fungi, even though infection can occur with several species of fungi. Therefore, mycorrhizal inoculation used for highway sites should include several fungal species, to accomodate the various plants important in highway revegetation.

APPENDIX A

HIGHWAY EXPERIMENTS ESTABLISHED 1980

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HIGHWAY EXPERIMENTS
ESTABLISHED 1980

An experiment to determine survivability of spores, growth of plants and compatibility of plants and spores was set up at the Intersection of State Highway 43 and Interstate 65 in spring 1980. A completely randomized experiment was set up using Forsythia intermedia (Forsythia) with various fertilizer-mycorrhizal fungus combinations. Plants were watered and mulched and other procedures suggested by the highway department followed.

Growth was monitored and spore viability determined. Measurement taken in late summer 1980 showed no viable spores at this site and less than 30% plant survival.

This experiment alone showed the importance of subsequent greenhouse and field studies.

A similar experiment was set up at the Maxwell Tract Research Farm with soil excavated from a construction site. The experiment was with Acer rubrum (red maple), and results also showed the necessity for further greenhouse studies before adequate field research could be conducted.

A third field experiment designed for selected sites in Montgomery and Parke Counties was unsuccessful.

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