1347
SCHOOL OF
CIVIL ENGINEERING

INDIANA

DEPARTMENT OF HIGHWAYS

JOINT HIGHWAY RESEARCH PROJECT

FHWA/IN/JHRP-82/13

EFFECTS OF SOIL PASTEURIZATION,
FUNGICIDE APPLICATION AND
TEMPERATURE ON MYCORRHIZAL
DEVELOPMENT AND PLANT GROWTH

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Interim Report

EFFECTS OF SOIL PASTEURIZATION, FUNGICIDE APPLICATION AND TEMPERATURE
ON MYCORRHIZAL DEVELOPMENT AND PLANT GROWTH

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

September 9, 1982

FROM: David F. Hamilton
Horticulture

Project: C-36-48H

File: 9-5-8

Attached is an Interim Report on the HPR Part II Study titled "Techniques to Increase Survival of New Highway Plantings". The title of the Report is as noted above and is the third one concerned with the mycorrhizal portion of the Study.

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

Sincerely,

David F. Hamilton/ms

David F. Hamilton
Horticulture

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INTERIM REPORT

EFFECTS OF SOIL PASTEURIZATION, FUNGICIDE APPLICATION
AND TEMPERATURE ON MYCORRHIZAL DEVELOPMENT AND PLANT GROWTH

by

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and
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Joint Highway Research Project
Project No. C-36-48H
File No. 9-5-8

Prepared as Part of an Investigation

Conducted by

Joint Highway Research Project
Engineering Experiment Station
Purdue University

in cooperation with the

Indiana Department of Highways

and the

U.S. Department of Transportation
Federal Highway Administration

The contents of this report reflect the views of the author who is 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.

Purdue University
West Lafayette, Indiana
September 9, 1982

1. Report No. FHWA/IN/JHRP-82/13	2. Government Accession No.	3. Recipient's Catalog No.	
4. Title and Subtitle EFFECTS OF SOIL PASTEURIZATION, FUNGICIDE APPLICATION AND TEMPERATURE ON MYCORRHIZAL DEVELOPMENT AND PLANT GROWTH		5. Report Date September 9, 1982	
		6. Performing Organization Code	
7. Author(s) Stephen D. Verkade and David F. Hamilton		8. Performing Organization Report No. JHRP-82-13	
9. Performing Organization Name and Address Joint Highway Research Project Civil Engineering Building Purdue University West Lafayette, Indiana 47907		10. Work Unit No.	
		11. Contract or Grant No. HPR-1(20) Part II	
12. Sponsoring Agency Name and Address* Indiana Department of Highways State Office Building 100 North Senate Avenue Indianapolis, Indiana 46204		13. Type of Report and Period Covered Interim Report Mycorrhizal Portion	
		14. Sponsoring Agency Code	
15. Supplementary Notes Prepared in cooperation with the U.S. Department of Transportation, Federal Highway Administration. Portion of Study titled "Techniques to Increase Survival of New Highway Plantings".			
16. Abstract <p>Highway construction generally results in a plant environment which creates permanent temperature extremes plus moisture and nutritional stress to plants used for revegetating the highway sites and stabilizing the slopes. Additionally, beneficial soil microorganisms are eliminated, preventing reestablishment of the natural vegetation to the site.</p> <p>The use of mycorrhizal plants or mycorrhizal inoculation of highway plantings may correct the microbial imbalance and reduce the physical stress of the environment through increased moisture and nutrient uptake contributed by fungal hyphae. Although the planting or seeding of mycorrhizal plants during revegetation could improve plant growth and establishment on the harsh highway sites, many cultural techniques currently used in plant production may be reducing or preventing normal mycorrhizal development.</p> <p>Soil pasteurization negates the beneficial effects of mycorrhizal fungi and should be done only before inoculation. Certain fungicides routinely used for pathogen control also reduce mycorrhizal formation. Benlate in particular should not be used following mycorrhizal inoculation. Finally, the high temperatures common in some plant growth environments may promote the activity of pathogens to the extent that they are not balanced by the beneficial effects of mycorrhizae unless the compatibility of the plant and mycorrhizal fungi is very high. The use of mycorrhizal plants for highway site revegetation may result in greater transplanting or seeding success, but production of mycorrhizal plants may require special cultural considerations.</p>			
17. Key Words Highway Revegetation, Fertility, Mycorrhizae, Tulip Poplar		18. Distribution Statement No restrictions. This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.	
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 22	22. Price

TABLE OF CONTENTS

	Page No.
INTRODUCTION	
EXPERIMENT I	
Materials and Methods	6
Results and Discussion	7
EXPERIMENT II	
Materials and Methods	9
Results and Discussion	10
EXPERIMENT III	
Materials and Methods	11
Results and Discussion	12
CONCLUSIONS	15
LITERATURE CITED	16

LIST OF TABLES

Table Number		Page No.
1.	Percent of cortical cells infected with mycorrhizae in roots of inoculated and noninoculated <u>Lolium perenne</u> grown at 3 temperature levels.	7
2.	Effects of temperature and inoculation with <u>Glomus fasciculatus</u> on foliar nutrient concentration (% N, P, and K) of <u>Lolium perenne</u> .	8
3.	Effects of pasteurized <u>Glomus fasciculatus</u> on nutrient concentration (% N, P, and K) in roots and shoots of <u>Liriodendron tulipifera</u> .	11
4.	Percent of cortical cells infected with mycorrhizae in roots of inoculated and noninoculated <u>Liriodendron tulipifera</u> grown with and without Benlate fungicide.	13
5.	Effects of Benlate and <u>Glomus fasciculatus</u> on nutrient concentrations (% N, P, and K) in roots and shoots of <u>Liriodendron tulipifera</u> .	14

LIST OF FIGURES

Figure Number		Page No.
1.	Effects of temperature and inoculation with <u>Glomus fasciculatus</u> on dry weight of shoots (g) of <u>Lolium perenne</u> .	18
2.	Effects of pasteurized <u>Glomus fasciculatus</u> on growth of <u>Liriodendron tulipifera</u> .	19
3.	Inoculation with <u>Glomus fasciculatus</u> resulted in the most total shoot growth of <u>Liriodendron tulipifera</u> when fungicide was not applied.	20
4.	Effects of Benlate and inoculation with <u>Glomus fasciculatus</u> on growth of <u>Liriodendron tulipifera</u> .	21
5.	Root systems of <u>Liriodendron tulipifera</u> with mycorrhizal development tended to be more extensive than root systems treated with Benlate and not inoculated with <u>Glomus fasciculatus</u> .	22

Highlight Summary

Three cultural factors were examined for effects on growth and development of mycorrhizal plants. Cultural factors tested included pasteurization of growing media and inoculum, fungicide application, and temperature.

The effects of pasteurization of endomycorrhizal inoculum on plant growth were determined. Seedlings of Liriodendron tulipifera were grown in 0.725 liter pots under greenhouse conditions. Half were inoculated with Glomus fasciculatus inoculum steam pasteurized at 225°C for 3 hours. The other half were inoculated with inoculum which had not been steam pasteurized. Inoculation of growing media with pasteurized G. fasciculatus showed no mycorrhizal development and did not enhance plant growth. Steam pasteurization apparently renders G. fasciculatus nonviable.

Effects of temperature and inoculation with Glomus fasciculatus on growth of Lolium perenne were also 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 seedlings were inoculated with G. fasciculatus inoculum (containing spores, hyphae, small amounts of soil, and fragments of roots from the previous culture), and half the seedlings were not inoculated. Plants from each group were grown at 40/35°C, 30/25°C, or 20/15°C day/night temperatures.

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

Effects of fungicide applications on growth of mycorrhizal and nonmycorrhizal plants were also determined. Seedlings of Liriodendron tulipifera were planted in containers and half were inoculated with the endomycorrhizae Glomus fasciculatus. The other half were not inoculated. 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:

Plant growth can be significantly improved by symbiotic association with mycorrhizal fungi. Enhanced plant growth results from increased nutrient and moisture uptake by the fungal hyphae. In addition to greater total growth, mycorrhizal plants have been shown to have improved success at survival and establishment in the landscape. This aspect is critical for revegetation of plants in the harsh environment of highway sites.

Endomycorrhizae are believed to be the most important type of mycorrhizae for herbaceous and woody landscape plants under natural conditions. Endomycorrhizal spores germinate and penetrate the smaller plant roots, resulting in symbiotic mycorrhizal association. Once inside the root, the hyphae grow into and around the cortical cells near the outer edges of the root. Structures called vesicles and arbuscles form within these cells. Vesicles are lipid droplets which have a storage function or can act as resting spores. Arbuscles are clusters of fine hyphae which periodically form and dissolve, and may be the mode of exchange between the plant and the fungi. Outside of the root, an extensive network of hyphae develops and creates a larger surface area for greater uptake of moisture and nutrients than in nonmycorrhizal plants. Thus, mycorrhizal plants may become more tolerant of nutrient deficiencies and drought.

Disruption of the natural soil environment, such as that occurring during highway construction, severely reduces the soil microbe population. Disrupted sites may require over one hundred years for natural levels of soil microbes to reestablish. Therefore, to obtain plants having normal or sufficient mycorrhizae, either the highway soil must be inoculated or plants must have mycorrhizal development prior to planting in the highway landscape. Inoculation during culture and production of plants in the nursery or during

seeding operations would be most economical since it inoculates a large number of plants in a smaller volume of soil or seed mix and would insure the earliest possible mycorrhizal formation. Although mycorrhizal inoculation during production may be critical for plant establishment and survival on landscape sites, many cultural techniques routinely used during production in the nursery can have detrimental effects on mycorrhizal development.

Often during plant propagation and production, the growing medium or soil is pasteurized using steam heat. The purpose of such soil pasteurization is to reduce the viability of pathogenic microorganisms in the growing medium. However, during this process beneficial microorganisms such as mycorrhizal fungi may also be adversely affected, reducing the endogenous fungal population. Soil pasteurization or treatment with other soil sterilants, such as methyl bromide plus chloropicrin, vapam, and vorlex are important in any plant production program designed to produce superior mycorrhizal plants.

Another cultural practice which may have a considerable impact on the development of mycorrhizal roots is the use of fungicides as preventative or curative pesticides for fungal pathogens. A fungicide routinely used during herbaceous and woody landscape plant production is Benlate or benomyl. Fungicides are also routinely used for coating of seeds before planting to control pathogenic organisms after germination. However, they may also negate benefits of beneficial organisms. Fungicides also do not effect all microbes uniformly or even all species within a genus, including mycorrhizal fungi.

Benlate has reduced the percentage root infection and number of spores of Glomus mosseae in wheat (Triticum aestivum L.) (Jalal and Domesch, 1975). Formation of mycorrhizae with clover (Trifolium spp.) was prevented by drench treatments of Benlate, and phosphate uptake by mycorrhizal onion (Allium cepa L.) and strawberry (Fragaria vesica L.) was reduced by similar

treatments (Boatman et al., 1978).

Application of Benlate also reduced mycorrhizal development on soybean (Glycine max L.) (Bailey and Safir, 1977), bean (Phaseolus sp. L.) (Sutton and Sheppard, 1976), and sour orange (Citrus aurantium L.) (Nemec, 1980). Benlate 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 (Zea mays L.), Benlate resulted in fewer spores in the soil, less root length infected, less cortex infected, and fewer entry points for fungal penetration. There were also fewer vesicles in mycorrhizal roots of maize after treatment with Benlate.

Benlate is a fungicide consisting of 50% benomyl (1-(N-butylcarbamol)-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). Benlate 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, Benlate has been shown to have negative effects on the growth of some mycorrhizal plants (Nemec, 1980).

Application of Benlate 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). Benlate 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.

Benlate is a very important fungicide in the nursery industry and is used to control many fungal diseases affecting landscape plants. However, its

effects on mycorrhizal development of more plant species should be determined before it is used in the cultural programs producing mycorrhizal plants. Many of the detrimental effects of Benlate reported have not been with plants used for highway revegetation or on species of fungi colonizing roots of these plants.

A final consideration important both to plant growth and the growth of mycorrhizal fungi is temperature. 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 soil 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). However, effects of temperature may vary considerably between ecto- and endomycorrhizal fungi.

Because of the difficulty of growing endomycorrhizae in pure culture, little research has been conducted on effects of temperature 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 Glomus mosseae occurred at 20°C (Schenck et al., 1975). Inoculum of Glomus fasciculatus (consisting of fragments of roots, small amounts of soil, 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 rapid root growth of mycorrhizal soybeans. Both root and arbuscular development in soybeans was slowed as temperatures decreased, but the decrease in arbuscular development was 90% compared to 60% reduction in root growth. This may indicate that mycorrhizal fungi are developmentally less active at low temperatures than are roots. The number of Endogone spores in the soil was greatest between 27.5 and 35°C. At temperatures above 35°C, arbuscles, 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 also depended upon the total length of the roots available for infection.

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.

Soil temperature may vary greatly, especially in container plant production and in the extreme exposure of soils at highway sites. The optimum temperature for growth of mycorrhizal plants may vary from that of nonmycorrhizal plants. Control of soil temperature could be achieved through the use of mulches,

shading or proper planting and seeding mixtures along highway sites.

The objectives of these experiments were to determine the effects of soil temperature (experiment I), pasteurization of growing medium and inoculum (experiment II), and fungicide application (experiment III), on mycorrhizal development and plant growth.

EXPERIMENT I

Materials and Methods

Seeds of Lolium perenne L. (perennial ryegrass) 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). This seeding rate would give complete cover on a larger scale such as a highway site, assuming most seeds germinate and grow. Plants were fertilized with 'Osmocote' 19N-6P-12K controlled-release fertilizer at 2 g/liter Nitrogen (N). Half the plants were treated with Glomus fasciculatus (Thaxter) Gerdemann and Trappe inoculum (containing spores, hyphae, small amounts of soil, and fragments of roots from the previous culture) at a rate of 44,400 spores/m² of soil surface. The other half of the plants were not inoculated. Ten containers of 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. Soil and air temperatures were the same. After 8 weeks plants were harvested and dry weights of shoots (g), foliar nitrogen (N), phosphorus (P), and potassium (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, 1979). 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 with a model 9200 Unicam flame spectrophotometer. For each temperature regime, there were ten replicates in a completely randomized design.

Results and Discussion

Inoculation of L. perenne with G. fasciculatus resulted in slight infection. Less than 2% of the cortical cells were infected. Noninoculated plants did not develop mycorrhizae (Table 1). Furthermore, inoculation did not increase growth under temperature regimes used (Fig. 1). In fact, growth of perennial ryegrass was reduced when plants were inoculated and grown at 40/35°C. Nitrogen, and K concentrations were not significantly reduced in inoculated plants as the growing temperature increased while foliar P concentration increased in plants grown at 40/35°C (Table 2). This indicates only 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), even though inoculation did not further stimulate growth. This suggests that additional species of endomycorrhizal fungi should be tested for possible synergistic benefits with perennial ryegrass.

Table 1. Percent of cortical cells infected with mycorrhizae in roots of inoculated and noninoculated Lolium 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 2. Effects of temperature and inoculation with Glomus fasciculatus on foliar nutrient concentration (% N, P, and K) of Lolium perenne (% per g dry weight).^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.

The reduction in growth and K concentration of inoculated plants grown at 40/35°C may be due to the presence of pathogenic organisms 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 fragments of roots, spores, hyphae, soil and associated microbes of the previous host (tomato) (Lycopersicon esculentum Mill.). If a more compatible fungal species could be found for the inoculum, the negative effect of high temperature on inoculation might be 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 mycorrhizal fungi.

The results indicate that impure inoculum should not be used for plants growing at excessively high temperatures in a pasteurized medium particularly if plant-fungi compatibility is low. However, the use of impure inoculum for highly compatible symbionts may still result in growth promotions at high temperatures. Although inoculum does contain impurities, current methods of inoculation are the most routinely used and readily available.

EXPERIMENT II

Materials and Methods

Seeds of Liriodendron tulipifera L. (tulip poplar) were pregerminated in a medium of perlite:vermiculite (1:1, v/v) in a growth chamber ($26^{\circ} \pm 2^{\circ}\text{C}$, with a 13-hour photoperiod). Twenty uniform seedlings were planted in steam-pasteurized perlite:sphagnum peat moss:soil (2:2:1, v/v/v) in 0.725 liter containers (one 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/l N of 'Osmocote' 19N-6P-12K controlled-release fertilizer incorporated into the top 2 to 3 cm. Ten seedlings were inoculated with inoculum containing fragments of roots, Glomus fasciculatus (Thaxter) spores, hyphae, and growing medium (perlite:sphagnum peat moss:soil) (2:2:1, v/v/v) from the previous culture. The inoculum was steam pasteurized at 225°C for three hours. Plants were grown under greenhouse conditions ($24 \pm 3^{\circ}\text{C}$, with a 16-hour photoperiod) and watered as needed with tap water. Plants were completely randomized, with 10 replicates per treatment.

After 14 weeks, plants were harvested and roots and shoots separated. Measurements included shoot length, dry weight of roots and shoots, root length (Tennant, 1975; Graca, et al., 1981), and nutrient concentration of roots and shoots. Nitrogen concentration 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 with a model 9200 Unicam flame spectrophotometer. 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). Data were analyzed by analysis of variance, and the Newman-Keuls' test of significance used to separate means.

Results and Discussion

Incorporation of steam-pasteurized inoculum into the growing medium did not result in mycorrhizal development. Neither treatment developed mycorrhizal roots. Inoculation of the rhizosphere with pasteurized inoculum also had no effect on plant growth (Fig. 2, Table 3). There were no significant differences in shoot length, dry weight of roots and shoots, root length, and nutrient concentrations in roots or in shoots of the two treatments.

These results indicate that steam pasteurization renders endomycorrhizal inoculum nonviable and incapable of any subsequent synergistic effects of plant growth in container production. Therefore, since steam pasteurization is routinely used for most soil media in container production, incorporation of mycorrhizal spores into growing media should be done after pasteurization of the medium. Endomycorrhizal inoculum must be viable and spores capable of germination to be useful for promoting growth of woody plants during container production.

Table 3. Effects of pasteurized Glomus fasciculatus on nutrient concentration (% N, P, and K) in roots and shoots of Liriodendron tulipifera (% per g dry weight).^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.

EXPERIMENT III

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/1 NO₃⁻, 0.8 mg/1 P, 2.58 mg/1 K, and a pH of 5.8. Plants were fertilized with 'Osmocote' 19N-6P-12K controlled-release fertilizer at a rate of 2 g/1 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, root fragments and small amounts of 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 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 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; Graca, et al., 1981), 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 with 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 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 4). Noninoculated plants had no mycorrhizal development regardless of fungicide treatment.

Inoculation resulted in the most growth when fungicide was not used (Fig. 3). Inoculated plants with no Benlate applied were tallest, and had the heaviest shoots and roots (Figs. 3, 4, and 5). 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 (Fig. 5).

Inoculation significantly reduced concentrations of foliar K and root N, and slightly reduced concentrations of root K (Table 5). The concentration of P in roots was increased by mycorrhizal development.

These results indicate that while inoculation of plants in containers with endomycorrhizal fungi can significantly enhance growth, the use of some fungicides 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 4. 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 (No Benlate)	10-30	0

Table 5. Effects of Benlate and Glomus fasciculatus on nutrient concentrations (% N, P, and K) in roots and shoots of Liriodendron tulipifera (% per g dry weight).^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	1.42b	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.

Conclusions

1. Development of highly mycorrhizal plants may require special considerations in plant culture and production.
2. 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.
3. Benlate fungicide is also used commonly in plant production and prevents mycorrhizal formation by some species. For sensitive species, alternative fungicides should be used.
4. There is some specificity between plant and fungal species. Low 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.

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Figure 1. Effects of temperature and inoculation with *Glomus fasciculatus* on dry weight of shoots (g) of *Lolium perenne*.^z

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

Figure 2. Effects of pasteurized *Glomus fasciculatus* on growth of *Liriodendron tulipifera*.^z

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

Fig. 3 Inoculation with Glomus fasciculatus resulted in the most total height growth of Liriodendron tulipifera when fungicide was not applied.

Figure 4. Effects of Benlate and inoculation with Glomus fasciculatus on growth of Liriodendron tulipifera.

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

Fig. 5 Root systems of *Liriodendron tulipifera* with mycorrhizal development tended to be more extensive than root systems treated with Benlate and not inoculated, *Glomus fasciculatus*.
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