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FHWA/IN/JHRP-85/12

Final Report

EFFECTS OF ENDOMYCORRHIZAL INOCULATION
ON GROWTH OF SELECTED LANDSCAPE PLANTS
USED IN HIGHWAY REVEGETATION

Stephen D. Verkade
Philip L. Carpenter

PURDUE UNIVERSITY

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SELECTED LANDSCAPE PLANTS USED IN HIGHWAY REVEGETATION

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

July 2, 1985
Revised: January 1986

FROM: Philip L. Carpenter, Research Associate
Department of Horticulture

Project: C-36-48J
File: 9-5-10

Attached is the Final Report on the HPR Part II Study entitled, "Mycorrhizae and Their Role in Maintenance of Highway Plantings". The research was conducted by Stephen D. Verkade, Graduate Assistant in Research in the Department of Horticulture under the direction of Professors D. F. Hamilton and Philip L. Carpenter, also of the Department of Horticulture.

The report documents the research conducted and the results found. A major finding was that little immediate value of mycorrhizal inoculation occurred at the time of planting on highway sites.

The research was completed early because of these findings, thus resulting in a substantial lesser cost than that budgeted and approved.

The report after review by IDOH and FHWA and revisions required is available for publication by JHRP in the usual manner.

Respectfully submitted,

Philip L. Carpenter 2/2/86

Philip L. Carpenter
Research Associate

PLC/tb

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by

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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 of policies of the Federal Highway Administration. This report does not constitute a standard, specification or regulation.

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16. Abstract <p>Verkade and Hamilton (1980) found that extensive mycorrhizal development occurred on roots of regal privet (<i>Ligustrum obtusifolium</i> var. <i>regelianum</i> Koehne Rehd.) after six weeks of rooting in media containing inoculum of <i>Glomus mosseae</i> (Thaxter) Gerdemann and Trappe. This coincided with substantial increases in root development. It, hence, was reasonable to conclude that inoculation in the propagation state of production would insure the earliest possible mycorrhizal development and subsequent promotion of growth. The objective of this experiment was to evaluate the effectiveness of mycorrhizal inoculation during clonal propagation by cuttings, and to determine the effect of mycorrhizae on root initiation and growth of <i>Viburnum</i>.</p> <p>Mycorrhizal inoculation with <i>G. fasciculatum</i> significantly increased root growth and root initiation of arrowwood <i>Viburnum</i> (<i>V. dentatum</i>). Roots of inoculated cuttings were highly mycorrhizal (30-50% cortical cells infected), while roots of non-inoculated cuttings were virtually non-mycorrhizal (0-2% cortical cells infected). Inoculation significantly increased the number of root initials penetrating the stems of cuttings after five weeks of rooting, with smaller increases during subsequent weeks. Increased root initiation, however, occurred only after other roots had begun to form.</p> <p>Other environmental factors, however, were found to have a greater influence on plant establishment on the roadside than the presence of mycorrhizal. If the conditions are too adverse for good plant establishment, (i.e., moisture and temperature stresses) plant failure results even if they are inoculated with mycorrhizal fungi.</p>			
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Highlight Summary

Cuttings of arrowwood viburnum (Viburnum dentatum L.) were inoculated with spores of Glomus fasciculatum (Thaxter) Gerdemann and Trappe. On the 4th through 9th weeks, fresh weight, number, and mycorrhizal condition of roots were determined. Inoculation resulted in uniform mycorrhizal infection, increased root development, and an interaction between mycorrhizal development and root initiation.

Seedlings of tulip-tree (Liriodendron tulipifera L.) were inoculated with G. fasciculatum (Thaxter) Gerdemann and Trappe or Glomus mosseae (Thaxter) Gerdemann and Trappe. Plants were grown in highly fertile medium and were watered as needed with nutrient solution. Both fungal species successfully promoted plant growth under highly fertile conditions, but G. mosseae tended to be more effective in promoting growth.

The mycorrhizal dependency of Norway maple (Acer platanoides L.), redstem dogwood (Cornus sericea L.) and Russian olive (Elaeagnus angustifolia L.) inoculated with Glomus epigaeum Daniels and Trappe, Glomus etunicatum Becker and Gerd., G. fasciculatum, Glomus macrocarpum Tul. and Tul., G. mosseae, or Gigaspora margarita Becker and Hall. Analyses included height, dry weights of shoots and roots, nutrient concentrations in leaves and roots, and carbohydrate concentration of stems and roots. Inoculation of Norway maple (A. platanoides), redstem dogwood (C. sericea), and Russian olive (E. angustifolia) with each fungal species resulted in mycorrhizal development. Plant growth response to inoculation varied depending upon fungal species. Growth of Norway maple (A. platanoides) and Russian olive (E. angustifolia) were maximized by inoculation with G. etunicatum, while growth of redstem dogwood (C. sericea) was maximized by inoculation with G. mosseae.

Spore isolation by wet sieving and centrifugation was improved by the use of colloidal silica solution as centrifugation media in place of sucrose solution. Spores of G. mosseae were isolated using either sucrose or Ludox solutions. The use of Ludox resulted in the recovery of more spores than sucrose and was most effective at specific gravities from 1.16 to 1.20.

Beneficial effects of inoculation were not present on plants grown on highway sites. Other environmental conditions probably were more limiting. The presence of spores on highway sites may be a limiting factor if other factors for plant growth are satisfactory.

Introduction

Mycorrhizal associations have been documented extensively for nearly all plants existing in their natural habitats. In mycorrhizal symbiosis, plants benefit through increased nutrient uptake, increased moisture uptake, and possibly through hormonal action and prevention of pathogen invasion. The fungi benefit by using the plant as a source of needed carbohydrate.

While there are several types of mycorrhizae, woody landscape plants on highway slopes are most commonly found to be associated with the endomycorrhizal type. Within an endomycorrhizal root, the fungal hyphae surround and penetrate cell walls in the cortical region. Vesicles and arbuscles are characteristic structures within invaginations in the cortical cells of endomycorrhizal roots. Vesicles contain lipid and serve a function of food storage when thin-walled, and act as resting spores when thick-walled. Arbuscles are fine concentrations of hyphae whose formation and dissolution may serve as a means of exchange between the two symbionts.

To date, most research has concentrated on the importance of mycorrhizal symbiosis when nutrient availability is extremely low, such as revegetation of strip-mines or other marginal sites. Mycorrhizal development by plants used to revegetate highway sites may increase the ability of the plant to become established and grow under harsh conditions. Aspects under consideration in this study include improvement of a simple spore isolation technique, mycorrhizal inoculation during plant propagation, mycorrhizal plant growth under high fertility found in the production of plants used in highway revegetation, and fungal compatibility of selected landscape plant species commonly used to revegetate highway sites.

Chapter I

Effect of Endomycorrhizal Inoculum on Root Initiation and Development of Arrowwood Viburnum (Viburnum dentatum) Cuttings

Introduction and Literature Review

Mycorrhizal development has been shown to increase growth of many plant species during production (Verkade and Hamilton, 1983; Maronek et al, 1980; Johnson et al, 1980; Menge et al, 1979; Menge et al, 1980; Wick and Moore, 1983). However, efficient and economical inoculation techniques must be developed before the use of mycorrhizae can become widespread for plant production. Mycorrhizal plants would grow more rapidly during production, reducing the costs of the plants, and may also improve initial growth and establishment of the plants in the highway landscape.

Most inoculation techniques are currently employed at transplanting or after propagation when all plants must be treated individually. It would be more efficient to inoculate during propagation when large numbers of plants could be treated with mycorrhizal inoculum in one operation.

Propagation by cuttings is common for production of woody landscape plants used in highway revegetation. However, some plant species are difficult to root, and costly methods such as grafting, must be used. 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. The mycorrhizal plant may also be more competitive in the highway landscape.

Linderman and Call (1977) found that ectomycorrhizal inoculum of Thelephora terrestris Ehrh. ex. Fr. in the rooting medium of cuttings of bearberry (Arctostaphylos uvaursi L. Spreng.) and huckleberry (Vaccinium ovatum

Parsh.), increased rooting percentage and root volume. This was possibly due to root-promoting growth substances excreted into the soil by the ectomycorrhizal fungi. There are no additional reports confirming the effects of ectomycorrhizae on root initiation, and there are no reports indicating that endomycorrhizae promote root initiation. However, there are reports on the promotional effects of mycorrhizae on root development of cuttings, reflecting the promotion of root growth after mycorrhizal formation (Holden, 1978; Bannister and Norton, 1984; Veldeman, 1980; Verkade and Hamilton, 1980).

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

Verkade and Hamilton (1980) found that extensive mycorrhizal development occurred on roots of regal privet (Ligustrum obtusifolium var. regelianum (Koehne) Rehd.) after six weeks of rooting in media containing inoculum of Glomus mosseae (Thaxter) Gerdemann and Trappe. This coincided with substantial increases in root development. No effect on root initiation was found for this species combination.

Although the role of mycorrhizae in root initiation prior to infection is not conclusive from the evidence presented above, mycorrhizae clearly promote root development following infection (Linderman and Call, 1977; Bannister and Norton, 1974; Verkade and Hamilton, 1980; Veldeman, 1980; Holden, 1978). It is reasonable to conclude that inoculation in the propagation stage of production

insures the earliest possible mycorrhizal development and subsequent promotion of growth.

The objective of this experiment was to evaluate the effectiveness of mycorrhizal inoculation during clonal propagation by cuttings, and to determine the effect of mycorrhizae on root initiation and growth of viburnum (Viburnum spp. L.).

Materials and Methods

Terminal cuttings of arrowwood viburnum (Viburnum dentatum L.), 20 cm long with six leaves, were taken in July, 1981. The cuttings were treated with 0.1% W/W Indolebutyric Acid and rooted in a medium of perlite:vermiculite (1:1, v/v), amended either with potting soil [perlite:sphagnum peat moss:topsoil (2:2:1, v/v)] at a rate of 7 volumes of the rooting medium to 1 volume of potting soil, or with mycorrhizal inoculum at the same rate. The mycorrhizal inoculum consisted of roots of tomato (Lycopersicon esculentum L.), spores and hyphae of Glomus fasciculatum (Thaxter) Gerdemann & Trappe, and potting soil [perlite:sphagnum peat moss:topsoil (2:2:1, v/v)]. Spore isolation was not included in this experiment to insure development of an inoculation technique practical for general nursery use. The cuttings were stuck in flats and placed under intermittent mist (12 seconds/8 minutes) in a greenhouse with approximately 25% shade. A total of 54,600 spores were added to the medium of each treated flat (5 spores/cm³).

Each treatment was replicated four times, with three subsamples within each replicate. Roots were sampled weekly for five weeks beginning with the fourth week following initiation of the experiment. Measurements included fresh weight of roots and number of root initials penetrating stems of the cuttings. Mycorrhizal development was determined by root staining and visual

observation (Phillips and Hayman, 1970; Gray and Gerdemann, 1973; Giovanetti and Mosse, 1979). Data were analyzed by analysis of variance, using Student-Newman-Keuls test of significance (5% level) to separate means.

Results and Discussion

Propagation is the earliest possible time during production of plants needed to revegetate highway sites for efficient and uniform inoculation. Infection by G. fasciculatum significantly increased root growth and root initiation of arrowwood viburnum (V. dentatum). Roots of inoculated cuttings were highly mycorrhizal (30-50% cortical cells infected), while roots of non-inoculated cuttings were virtually non-mycorrhizal (0-2% cortical cells infected).

Inoculation significantly increased the number of root initials penetrating the stems of cuttings after five weeks of rooting, with smaller increases during subsequent weeks (Table I-1). Increased root initiation occurred only after other roots had begun to form. Therefore, it is likely that this effect occurs only after infection and is mediated through an effect of the fungi on plant metabolism.

Mycorrhizal infection increased root growth after 7 weeks of rooting, as measured by root fresh weight (Table I-2). Several weeks of rooting are necessary for significant differences in root growth to occur on cuttings.

Mycorrhizal inoculation of woody plants during propagation is a convenient and cost effective time in the production cycle to efficiently inoculate large numbers of plants at the earliest possible time. There is no other time in the production cycle during which plant density is as high, resulting in maximum efficiency of inoculation. Plants inoculated at this time are already mycorrhizal at the time of planting in the highway site, requiring no further special planting techniques.

Table I-1. Effect of Glomus fasciculatum on number of root initials formed by cuttings of arrowwood viburnum (Viburnum dentatum) harvested after 4, 5, 6, 7, or 8 weeks of rooting.

Week	<u>Number of Root Initials</u> Inoculated	Control
4	5.25 c ^z	2.25 c
5	33.75 ab	8.25 c
6	33.50 ab	25.00 b
7	49.75 a	34.25 ab
8	42.75 ab	27.50 ab

^zSeparations of means by the Student-Newman-Keuls test of significance, 5% level. Differing letters represent significances in rows and columns for each treatment combination.

Table I-2. Effect of Glomus fasciculatum on root fresh weight (g) of arrowwood viburnum (Viburnum dentatum) cuttings after 4, 5, 6, 7, and 8 weeks of rooting.

Week	<u>Root Fresh Weight (g)</u>	
	Inoculated	Control
4	0.00 d ^z	0.00 d
5	0.00 d	0.00 d
6	0.00 d	0.00 d
7	0.50 b	0.27 c
8	0.77 a	0.26 c

^zSeparation of means by the Student-Newman-Keuls test of significance, 5% level. Differing letters represent significance in rows and columns for each treatment combination.

Literature Cited

- Bannister, P. and W.M. Norton. 1974. The response of mycorrhizal and non-mycorrhizal rooted cuttings of heather (Calluna vulgaris (L.) (Hull) to variations in nutrient and water regimes. *New Phytol.* 73:81-89.
- Giovanetti, M. and B. Mosse. 1979. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84:489-500.
- Gray, L.E. and J.W. Gerdemann. 1973. Uptake of sulfur-35 by vesicular-arbuscular mycorrhizae. *Plant Soil* 39:687-689.
- Holden, V.L. 1978. The use of mycorrhizae in the propagation of Arctostaphylos uva-ursi. *Int. Plant Prop. Soc. Comb. Proc.* 28:132-133.
- Johnson, C.R., J.N. Joiner, and C.E. Crews. 1980. Effects of N, K, and Mg on growth and leaf nutrient composition of three container grown woody ornamentals inoculated with mycorrhizae. *J. Amer. Soc. Hort. Sci.* 105:286-288.
- Linderman, R.G. and C.A. Call. 1977. Enhanced rooting of woody plant cuttings by mycorrhizal fungi. *J. Amer. Soc. Hort. Sci.* 102(5):206-208.
- Maronek, D.M., J.W. Hendrix, and J. Kiernan. 1980. Differential growth response to the mycorrhizal fungus Glomus fasciculatus of southern magnolia and bar harbor junipers grown in containers in composted hardwood bark-shale. *J. Amer. Soc. Hort. Sci.* 105:206-208.
- Menge, J.A., E.L.V. Johnson, and R.G. Platt. 1978. Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. *New Phytol.* 81:553-559.
- Menge, J.A., J. LaRue, C.K. Labananskas, and E.L.V. Johnson. 1980. The effect of two mycorrhizal fungi upon growth and nutrition of avocado seedlings grown with six fertilizer treatments. *J. Amer. Soc. Hort. Sci.* 105(3):400-404.
- Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55(1):158-161.
- Veldemann, R. 1981. Influence of mycorrhizae on the rooting of poplar cuttings. *Mededelingen Van De Faculteit Landbouwwetenschappen Rijksuniversiteit Gent.* 45(2):169-173 (Abstract).
- Verkade, S.D. and D.F. Hamilton. 1980. Mycorrhizae and their uses in the nursery. *The International Plant Propagators' Combined Proceedings* 30:353-363.

- Verkade, S.D. and D.F. Hamilton. 1983. Effects of soil fertility on growth, nutrient concentration and mycorrhizal development of Liriodendron tulipifera seedlings inoculated with the vesicular-arbuscular fungus, Glomus fasciculatus. *Scientia Horticulturae*. 21:243-252.
- Wick, R.L. and L.D. Moore. 1983. Japanese holly: A host for endomycorrhizal fungi. *Journal of Environmental Horticulture* 1(1):12-14.

Chapter II

Effect of *Glomus fasciculatum* and *Glomus mosseae* on Growth of tulip-tree (*Liriodendron tulipifera* L.) Under High Fertility

Introduction and Literature Review

Mycorrhizal fungi symbiotically contribute to plant growth by expanding the absorptive surface available to the plant for uptake of soil nutrients and moisture in general, and possibly by enhancing absorption of phosphorus. Fertilizer is generally supplied to plants both during production and in the highway landscape at times when mycorrhizal inoculation might take place.

Commercial production of woody landscape plants in containers generally employs sterile and/or soil-less media which contain no mycorrhizal fungi. Under these conditions, mycorrhizal development can be expected to be considerably delayed, reduced, or absent. Mycorrhizal inoculation may therefore result in plants with accelerated growth and increased potential for establishment in the highway landscape.

Several reports have indicated the importance of mycorrhizal development in plant production (Graham and Timmer, 1984; Hayman, 1980; Holevas, 1966; Maronek et al., 1981; Mosse and Hayman, 1971; Ruehle, 1980). These reports generally have stressed that mycorrhizal inoculation is well suited for container production with media initially devoid of mycorrhizal fungi, and also suitable for field production when soil phosphorus is abnormally low or when natural mycorrhizal levels have been eliminated by steam sterilization or fumigation. Although container production using sterile media seems ideal for mycorrhizal inoculation, conventional plant production practices include use of moderate-to-high fertility levels, whereas most mycorrhizal research has been conducted under conditions of low fertility. Fertilizers are routinely supplied to plants at the time of planting on highway slopes.

Gray and Gerdemann (1967) found that Glomus fasciculatum significantly increased phosphorus uptake by tulip-tree (Liriodendron tulipifera), which indicates that mycorrhizal plants may be more competitive scavengers of phosphorus when this nutrient is limiting. Growth promotion due to mycorrhizal development occurs readily under conditions of low fertility because plants have increased reliance on the fungi to absorb nutrients from the soil. However, when soil nutrients are in abundant supply, the plant can achieve adequate nutrient uptake and normal growth without assistance from the fungus. Furthermore, mycorrhizal infection has been reported to be decreased by high phosphorus levels (Maronek et al., 1981).

Information regarding growth of mycorrhizal plants under low fertility is very useful for situations such as strip mines and extreme conditions on highway construction sites, but provides no indication of the practicality of mycorrhizal inoculation earlier in production when inoculation would be more efficient or for use of woody plants when soil fertility is increased by the use of fertilizer in the highway landscape.

There is evidence that minimum nutrient levels must be met before mycorrhizal promotion of growth can occur (Verkade and Hamilton, 1983a). This research indicated no promotion of growth of tulip-tree (Liriodendron tulipifera) occurred following mycorrhizal inoculation of a low fertility soil without fertilizer. However, when plants were fertilized with 2 or 4 g/l nitrogen as 19-6-12 slow-release fertilizer, there were significant growth increases due to mycorrhizal development by Glomus fasciculatum. Fertilization of endomycorrhizal magnolia with slow-release fertilizer at the manufacturer's recommended rate did not inhibit growth response (Maronek et al., 1980). Johnson et al. (1980) also found significant growth increases of podocarpus (Podocarpus spp.), pittosporum (Pittosporum spp.), and rhododendron

(Rhododendron spp.) from mycorrhizae at high levels of fertilization. This information suggests that mycorrhizae may promote plant growth of certain plant species even with relatively high soil fertility. The objective of this experiment was to determine the effect of mycorrhizal inoculation on growth of tulip-tree (Liriodendron tulipifera L.) grown at high levels of soil fertility.

Materials and Methods

Seeds of tulip-tree (Liriodendron tulipifera L.) were stratified for 90 days at 4.4°C. Stratified seeds were soaked in 10% (v/v) HCl for 10 minutes and were sown in a medium of vermiculite:perlite (1:1, v/v) in a growth chamber (24°C day/22°C night, with a 16-hour photoperiod). Seedlings 4-cm tall and 30-days old were transplanted into one-gallon (3.28 l) containers of medium which had been previously steam pasteurized at 121°C for two hours. The growing medium consisted of perlite, sphagnum peat moss, and loam soil (2:2:1, v/v/v) and had a nutrient analysis of 164 mg/l soluble salts, 120 mg/l NO₃, 53 mg/l P, 130 mg/l K, 12 mg/l NH₄, and a pH of 6.2.

Plants either were inoculated with Glomus fasciculatum (Thaxter) Gerdemann and Trappe, inoculated with Glomus mosseae (Thaxter) Gerdemann and Trappe, or not inoculated as a control treatment. The inoculum contained chlamydospores, hyphae, fragmented roots, and growing-medium from the previous culture of the fungus with tomato (Lycopersicon esculentum Mill.).

At transplanting, inoculum containing 400 spores (44,400 spores m⁻² of container surface area) was inserted into the containers to a depth of 6 cm, in cores equidistant from the plant and positioned just outside the root mass of the seedling to prevent root damage from the making of the cores. Inoculum was placed into cores to concentrate chlamydospores adjacent to initial root growth, permitting maximum infection.

Plants were transferred to a greenhouse ($24^{\circ}\text{C} + 3^{\circ}\text{C}$, with a 16 hour photoperiod) and were irrigated as needed with nutrient solution containing 516.6 mg/l KNO_3 , 367.6 mg/l NH_4NO_3 , and 0.124 ml/l 75% H_3PO_4 . After 16 weeks of growth, height and mycorrhizal condition of roots were determined. Degree of mycorrhizal infection was evaluated by visually estimating % cortical cells infected in stained roots (Phillips and Hayman, 1970; Gray and Gerdemann, 1973; Giovanetti and Mosse, 1979). Plants were arranged in a completely randomized design, and data were analyzed by analysis of variance with the Student-Newman-Keuls test of significance to determine differences between means.

Results and Discussion

Inoculation with Glomus fasciculatum or Glomus mosseae resulted in moderate mycorrhizal infection of 30-60% of root cells. Inoculation with either fungal species significantly increased the height of tulip-tree (L. tulipifera) grown under highly fertile conditions (Figure II-1). These results are consistent with previous work by Verkade and Hamilton (1983a) in which mycorrhizal development increased growth of tulip-tree (Liriodendron) fertilized at moderate levels of N and K. However, in the present study, phosphorus also was raised to levels normally maintained during intensive container production, and significant promotion of growth by mycorrhizae still occurred.

Plants inoculated with G. mosseae tended to be taller than those inoculated with G. fasciculatum (Figure II-1). These results confirm those of Pope et al. (1983), who observed a similar trend with tulip-tree (Liriodendron) and also found that inoculation with Glomus macrocarpum resulted in an even greater growth promotion than either G. mosseae or G. fasciculatum. Lower fertility levels (Pope et al., 1983) may amplify the growth-promoting

differential between G. mosseae and G. fasciculatum since the statistically-significant difference between the effects of these two fungal species found by Pope et al. (1983) were not observed in our study. There are several reasons for variable fungal compatibility or mycorrhizal dependency of plants (Pope et al., 1983), including genotype (Kormanik et al., 1977a; Menge et al., 1978), soil type (Daft and Hacskeylo, 1977), soil phosphorus (Gray and Gerdemann, 1967; Menge et al., 1978; Pope, 1980; Verkade and Hamilton, 1983a), and mycorrhizal species (Daft and Hacskeylo, 1977; Kormanik et al., 1977b).

These results indicate that mycorrhizal inoculation of some species intended for highway use can be inoculated effectively during production without an antagonistic effect of high fertility. Inoculation early in production requires less labor and inoculum than field inoculation, resulting in substantial monetary savings. Since mycorrhizal fungi require a considerable time period to become established in plant roots, plants arriving at a highway planting site with mycorrhizal roots may be more competitive in the initially critical period of establishment than plants inoculated at the site. It is also clear that growth and establishment of many highway landscape plants may be optimized through the use of both fertilizers and mycorrhizal inoculation.

Figure II-1. Effect of endomycorrhizal inoculation on height of tulip-tree (*Liriodendron tulipifera*) grown under high fertility. Separation of means by the Newman-Keuls' test of significance, 5% level. Means of 12 values.

Literature Cited

- Daft, M.J. and E. Hacskeylo. 1977. Growth of endomycorrhizal red maple seedlings in sand and anthracite spoil. *Forest Science* 23:207-216.
- Giovanetti, M. and B. Mosse. 1979. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84:489-500.
- Graham, J.H. and L.W. Timmer. 1984. Vesicular-arbuscular mycorrhizal development and growth response of rough lemon in soil and soilless media: Effect of phosphorus source. *J. Amer. Soc. Hort. Sci.* 109(1):118-121.
- Gray, L.E. and J.W. Gerdemann. 1973. Uptake of sulfur-35 by vesicular-arbuscular mycorrhizae. *Plant Soil* 39:687-689.
- Gray, L.E. and J.W. Gerdemann. 1967. Influence of vesicular-arbuscular mycorrhizae on the uptake of phosphorus-32 by Liriodendron tulipifera and Liquidambar styraciflua. *Nature* 213:106-107.
- Hayman, D.S. 1980. Mycorrhiza and crop production. *Nature* 287:487-489.
- Holevas, C.D. 1966. The effect of a vesicular-arbuscular mycorrhiza on the uptake of soil phosphorus by strawberry (Fragaria sp. var. Cambridge favorite). *J. Hort. Sci.* 41:57-64.
- Johnson, C.R., J.N. Joiner and C.E. Crews. 1980. Effects of N, K, and Mg on growth and leaf nutrient composition of three container-grown woody ornamentals inoculated with mycorrhizae. *J. Amer. Soc. Hort. Sci.* 105:286-288.
- Kleinschmidt, G.D. and J.W. Gerdemann. 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhizae. *Phytopat.* 62:1447-1453.
- Kormanik, P.P., W.C. Bryan and R.C. Schultz. 1977a. Influence of endomycorrhizae on growth of sweetgum seedlings from eight mother trees. *Forest Science* 23:500-506.
- Kormanik, P.P., W.C. Bryan and R.C. Schultz. 1977b. Quality hardwood seedlings require early mycorrhizal development in nursery beds. Proceedings of the 14th Southern Forestry Tree Improvement Conference, June 1977, Gainesville, FL. pp 289-293.
- Maronek, D.M., J.W. Hendrix and J. Kiernan. 1981. Mycorrhizal fungi and their importance in horticultural crop production. In: *Horticultural Reviews*, Jules Janick, ed. AVI Publishing Co., Inc., Westport, CT. 13:172-213.
- Maronek, D.M., J.W. Hendrix and J. Kiernan. 1980. Differential growth responses to the mycorrhizal fungus Glomus fasciculatus of southern magnolia and Bar Harbor juniper grown in composted hardwood bark-shale. *J. Amer. Soc. Hort. Sci.* 105(2):206-208.

- Menge, J.A., L.V. Johnson, and R.G. Platt. 1978. Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. *New Phytol.* 81:553-559.
- Mosse, B. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. IV. In soil given additional phosphate. *New Phytol.* 72:127-136.
- Mosse, B. and D.S. Hayman. 1971. Plant growth responses to vesicular-arbuscular mycorrhiza. II. In unsterilized field soils. *New Phytol.* 70:29-34.
- Nemic, S. 1980. Effects of 11 fungicides on endomycorrhizal development in sour orange. *Can. J. of Bot.* 58:522-526.
- Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.* 55:158-161.
- Pope, P.E. 1980. Influence of Glomus fasciculatus mycorrhizae on some physical and chemical characteristics of Platanus occidentalis seedlings. *Can. J. of Bot.* 58:1601-1606.
- Pope, P.E., W.R. Chaney, J.D. Rhodes and S. Woodhead. 1983. The mycorrhizal dependency of four hardwood tree species. *Can. J. of Bot.* 61(2):412-417.
- Powell, C.L. 1980. Effect of phosphate fertilizers on the production of mycorrhizal inoculum in soil. *New Zealand J. of Agric. Res.* 23:219-223.
- Ruele, J.L. 1980. Ectomycorrhizal colonization of container-grown Northern red oaks as affected by fertility. *U.S.D.A. For. Ser. Res. Note SE-297:1-5.*
- Verkade, S.D. and D.F. Hamilton. 1983a. Effects of soil fertility on growth, nutrient concentration and mycorrhizal development of Liriodendron tulipifera seedlings inoculated with the vesicular-arbuscular fungus, Glomus fasciculatus. *Scientia Horticulturae* 21:243-252.
- Verkade, S.D. and D.F. Hamilton. 1983b. Effects of Benomyl on growth of Liriodendron tulipifera L. seedlings inoculated with the vesicular-arbuscular fungus Glomus fasciculatus. *Scientia Horticulturae* 21:253-260.

Chapter III

Effects of Fungal Species on Growth of Endomycorrhizal Highway Landscape Plants

Introduction and Literature Review

Overwhelming numbers of reports indicate that mycorrhizae promote growth and establishment of woody plants under conditions which limit nutrient availability, especially that of phosphorus (Harley and Smith, 1983; Maronek, 1981). Recent evidence indicates that mycorrhizal inoculation also will be possible during the production of landscape plants under relatively fertile conditions present in culture, eliminating the need for special inoculation steps during planting in the highway landscape (Verkade and Hamilton, 1983a; Maronek, Hendrix and Kiernan, 1980; Kormanik et al., 1977; Schultz et al., 1979; Johnson et al., 1983).

If mycorrhizal inoculation is to become a common practice with woody landscape plants on highway sites, it is imperative to maximize compatibility of the symbionts by providing the appropriate mycorrhizal fungi in the inoculum source for the plant species.

Several researchers have documented variable mycorrhizal dependency in plants (Daft and Nicholson, 1976; Geddeda et al., 1983; Mosse, 1975; Pope et al., 1983; and Schultz et al., 1981). Woody plant species which have been shown to exhibit mycorrhizal response dependent upon fungal species include common apple (Malus domestica Borkh.) (Geddeda et al., 1983), green ash (Fraxinus pennsylvanica Marsh.), sweet-gum (Liquidambar styraciflua L.), tulip-tree (Liriodendron tulipifera L.) and sycamore (Platanus occidentalis L.). Considerable research is needed to identify the mycorrhizal compatibility of woody landscape plants.

In order to thoroughly determine the fungal compatibility of a plant, all aspects of plant condition, health, and growth must be considered. In addition to growth measurements of height and weight, nutrient concentration and the concentration of food reserves should be considered. The levels of starch and sugars contribute to the present and future growth potential of the plant, while current nutrient status of a plant is both an indication of nutrient-dependent growth and current health of the plant.

Ectomycorrhizae and ericaceous endomycorrhizae affect the production of fungi-specific carbohydrates including trehalose, glycogen, and mannitol (Lewis and Harley, 1965abc; Stribley and Read, 1974; Wedding and Harley, 1976). Glycogen has been found in vesicular-arbuscular mycorrhizae (Bevenge et al., 1975), while trehalose and mannitol have not been found (Hepper and Mosse, 1973; Bevenge et al., 1975). Lipids associated with vesicles, hyphae, and chlamydospores may be increased by infection (Nagy et al., 1980; and Cooper and Losel, 1978). Distinct increases in reducing, non-reducing, or total sugars have not been demonstrated.

In ectomycorrhizal Monterey pine (Pinus radiata), cut-tail (Eucalyptus fastigiata), and European beech (Fagus sylvatica), starch concentrations were reduced (Foster and Marks, 1966; Ling-Lee et al., 1977; and Wedding and Haley, 1976). In endomycorrhizal soybean (King and Brown, 1981) and clover (Trifolium sp.) (Mosse, 1962), starch also has been found to decrease upon infection. Little work has been conducted with woody plants and no clear changes in the starch concentrations of woody plants have been found in response to mycorrhizal development (Douds, 1984).

Phosphorus concentration is consistently increased with plants infected with endomycorrhizae (Harley and Smith, 1983). The effects of endomycorrhizae on the concentrations of nitrogen and potassium are less clear, with the

occurrence of both increased and decreased concentrations (Verkade and Hamilton, 1983; Harley and Smith, 1983).

The objective of this experiment was to determine the mycorrhizal compatibility of three plant species with six common fungal species.

Materials and Methods

Media consisting of topsoil:peat:perlite (1:2:2, v/v/v) was steam pasteurized for 2 hours at 250°F, placed in 2500 cm³ pots (one gallon trade designation) and leached several times (9) to reduce accumulated ammonium and to lower soil nutrient content. Soil nutrient concentration (ppm) at planting as 0 NO₃, 9.0 NH₄, 0.75 P, 6.55 K, and soil pH was 7.25 (soil nutrient analysis conducted by Purdue Soils Laboratory). Seeds of Norway maple (Acer platanoides L.), redstem dogwood (Cornus sericea L.) and Russian olive (Elaeagnus angustifolia) were surface sterilized with 5% HCl solution and germinated in a growth chamber (27°C day/22°C night±1°C). Norway maple (Acer platanoides) seedlings 8-9 cm tall with 1 pair of expanded leaves, redstem dogwood (Cornus sericea) seedlings 5-6 cm tall with 2 pairs of expanded leaves, and Russian olive (Elaeagnus angustifolia) seedlings 9-10 cm tall with 3-4 pairs of expanded leaves were transplanted into the containers and placed into a greenhouse (25°C±3°C, under a 16 hour photoperiod using incandescent lights to supplement daylight).

Mycorrhizal spores were isolated by wet sieving and centrifugation, and were stored in Ringer's Solution under refrigeration (8-9°C) until use. Plants were inoculated either with Gigaspora margarita Becker and Hall, Glomus epigaeum Daniels and Trappe, Glomus etunicatum Becker and Gerdemann, Glomus fasciculatum (Thaxter) Gerdemann and Trappe, Glomus macrocarpum Tul. and Tul., Glomus mosseae Nicol. and Gerdemann, or not inoculated as a control treatment.

Thirty-five ml of distilled H₂O containing 700 spores were thoroughly mixed into 300 ml of pasteurized medium for use as inoculum for each treated pot. Control plants received 300 ml of pasteurized medium mixed with 35 ml of distilled H₂O lacking mycorrhizal spores.

The treatments were placed in three holes equally spaced around the seedlings to a depth of 10 cm. Plants were watered with Hoagland's Solution #2 with P after planting and each subsequent week with Hoagland's Solution lacking P. Plants were watered as needed with tap water through a Chapin watering system to prevent contamination by splashing water. Plants were arranged in a completely randomized design with six replications per treatment combination.

The Norway maple (A. platanoides) were harvested after 21 weeks of growth, redstem dogwood (C. sericea) after 23 weeks, and Russian olive (E. angustifolia) after 19 weeks. Leaves, stems, and roots were separated for analysis. Measurements included height; dry weights of leaves, stems and roots; content of N, P, and K in stems and roots; carbohydrate content of stems and roots; and the degree of mycorrhizal infection. The percentage of N was determined by Nesslerization, P by the ammonium-phospho-molybdate method with 2,3,4-amino naphthol sulphonic acid as the reducing agent (Jackson, 1958), and by flame spectrophotometry with a model 9200 Unicam flame spectrophotometer.

A 100 mg sample of stem or root tissue was lyophilized and amalgamized for use in estimating total sugars, reducing sugars and starch. Soluble sugars were extracted three times by shaking the sample in 10 ml of 80% ethanol for 20 minutes and centrifuged at 1650xg for 25 minutes. The pooled supernatant fractions were analyzed for total sugars by the phenol sulfuric acid method (Dubois et al., 1956). Reducing sugars were analyzed by Nelson's method as outlined by Cherry (1973). An estimate of non-reducing sugars was obtained by subtracting reducing sugars from total sugars.

The pellet remaining after extraction and centrifugation was lyophilized and analyzed for starch concentration. Starch was solubilized with DMSO (90.9%), 55°C) and hydrolyzed with a 2 mg·ml⁻¹ solution of amyloglucosidase (Sigma) in 0.05M citrate buffer (pH 4.8, 45°C) for one hour (Libby, 1970). After hydrolyzation, 0.5 ml was removed for glucose determination via the glucose oxidase, peroxidase, o-dianisidine method (Sigma Kit No. 510-A).

The degree 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, with the Newman-Keuls test of significance used to separate means.

Results

Inoculation of Norway maple (Acer platanoides), redstem dogwood (Cornus sericea), and Russian olive (Elaeagnus angustifolia) with each mycorrhizal species resulted in mycorrhizal development (Table III-1). Plant response to inoculation varied depending upon fungal species, although growth increases were achieved for all plant species. Growth of maple (Acer) and oleaster (Elaeagnus) were maximized by inoculation with G. etunicatum, while growth of dogwood (Cornus) was maximized by inoculation with G. mosseae. Phosphorus concentration was increased by mycorrhizal development in all cases, while nitrogen and potassium were increased in only certain cases. The effect of mycorrhizae on total sugar, reducing sugar, non-reducing sugar and starch was not consistent among plant and fungal species.

Maple (Acer)

Stem height, shoot dry weight, and root dry weight of Norway maple (Acer platanoides) was significantly increased by inoculation with G. etunicatum, but not by any other fungal species tested (Fig. III-1).

Mycorrhizal development had no effect on foliar concentration of nitrogen and potassium (Table III-2). However, all species of fungi increased foliar concentration of phosphorus. Glomus epigaeum was most effective at increasing foliar phosphorus. Glomus fasciculatus was the only fungal species which increased the concentration of nitrogen and potassium in maple (Acer) roots (Table III-3). All species of fungi increased the concentration of phosphorus in maple (Acer) roots, while G. epigaeum most effectively increased phosphorus in roots.

Table III-1. Degree of mycorrhizal development (percent of cortical cells infected) of all plant-fungal combinations tested, as determined by visual estimation.

Fungi	<u>Plant Species</u>		
	Norway maple (<u>Acer</u> <u>platanoides</u>)	Redstem dogwood (<u>Cornus</u> <u>sericea</u>)	Russian olive (<u>Elaeagnus</u> <u>angustifolia</u>)
Control	0-2%	0-2%	0-2%
<u>Glomus epigaeum</u>	30-60%	30-60%	30-60%
<u>Glomus etunicatum</u>	30-60%	30-60%	10-30%
<u>Glomus fasciculatum</u>	30-60%	10-30%	30-60%
<u>Glomus macrocarpum</u>	10-30%	10-30%	10-30%
<u>Glomus mosseae</u>	30-60%	30-60%	30-60%
<u>Gigaspora margarita</u>	30-60%	30-60%	10-30%

Figure III-1. Effect of fungal species on height, dry weight of shoots, and dry weight of roots of Norway maple (*Acer platanooides*). Separation of means by the Newman-Keuls test of significance, 5% level. Differing letters indicate significance.

Figure III-1. Effect of fungal species on height, dry weight of shoots, and dry weight of roots of Norway maple (*Acer platanoides*). Separation of means by the Newman-Keuls test of significance, 5% level. Differing letters indicate significance.

Table III-2. Effect of fungal species on foliar nutrient concentration ($\mu\text{g}\cdot\text{g}^{-1}$) of Norway maple (Acer platanoides). Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance within columns.

Mycorrhizal Treatment	N	P	K
	$\mu\text{g}\cdot\text{g}^{-1}$		
Control	5.842 ^{ab}	0.674 ^b	25.207 ^a
<u>Glomus epigaeum</u>	5.920 ^{ab}	1.218 ^a	25.816 ^a
<u>Glomus etunicatum</u>	6.327 ^a	0.973 ^a	25.191 ^a
<u>Glomus fasciculatum</u>	5.800 ^b	0.986 ^a	26.036 ^a
<u>Glomus macrocarpum</u>	5.837 ^{ab}	1.069 ^a	23.797 ^a
<u>Glomus mosseae</u>	5.813 ^b	0.900 ^a	24.475 ^a
<u>Gigaspora margarita</u>	5.783 ^b	0.927 ^a	23.335 ^a

Table III-3. Effect of fungal species on concentration ($\mu\text{g}\cdot\text{g}^{-1}$) of N, P, and K in Norway maple (Acer platanoides) roots. Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance within columns.

Mycorrhizal Treatment	N	P	K
	$\mu\text{g}\cdot\text{g}^{-1}$		
Control	3.250 ^c	0.418 ^c	23.111 ^b
<u>Glomus epigaeum</u>	3.540 ^{ab}	1.321 ^a	27.975 ^{ab}
<u>Glomus etunicatum</u>	3.558 ^{ab}	1.119 ^{ab}	27.853 ^{ab}
<u>Glomus fasciculatum</u>	3.982 ^a	1.023 ^b	30.514 ^a
<u>Glomus macrocarpum</u>	3.375 ^c	0.885 ^b	22.905 ^b
<u>Glomus mosseae</u>	3.695 ^{ab}	0.926 ^b	22.905 ^{ab}
<u>Gigaspora margarita</u>	3.513 ^{ab}	0.913 ^b	28.098 ^{ab}

Mycorrhizal development had no clear effect on total sugar concentration, and also had little effect on reducing sugar concentration in Acer stems except for a decrease in stems infected with G. etunicatum (Table III-4). Starch concentration in stems was significantly increased by infection of all fungal species. In maple (Acer) roots, mycorrhizal development had no effect on reducing sugar concentration (Table III-5). Glomus macrocarpum and G. epigaeum slightly increased total sugar concentration in maple (Acer) roots, while G. fasciculatum slightly reduced the total sugar concentration. Mycorrhizal development had little effect on concentration of starch in roots.

Dogwood (Cornus)

Dry weights of dogwood (Cornus) shoots were significantly increased by all mycorrhizal species, and stem heights were significantly increased by all mycorrhizal species except Gigaspora margaritum (Fig. III-2). Glomus fasciculatum, Glomus mosseae, Gigaspora margaritum, Glomus macrocarpum, and Glomus epigaeum all maximized dry weight of dogwood (Cornus) shoots. Glomus mosseae and Glomus epigaeum maximized height of dogwood (Cornus) shoots. No mycorrhizal treatment had a significant effect on the dry weight of dogwood (Cornus) roots.

The concentration of nitrogen in dogwood (Cornus) leaves was significantly increased by association with Glomus mosseae, Gigaspora margaritum, and Glomus epigaeum (Table III-6). Phosphorus concentration in leaves was significantly increased by association with all fungal species. There was no effect of mycorrhizae on potassium concentration in dogwood (Cornus) leaves. In roots, nitrogen concentration was significantly decreased by all fungal species (Table III-7). The concentration of phosphorus in dogwood (Cornus) roots was significantly increased by all fungal species, but this effect was most pronounced when Gigaspora margaritum and Glomus etunicatum were the fungal

Table III-4. Effect of fungal species on carbohydrate concentration ($\mu\text{g}\cdot\text{mg}^{-1}$) in Norway maple (Acer platanoides) shoots. Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance in columns.

Mycorrhizal Treatment	Total Sugars	Reducing Sugars	Non-reducing Sugars	Starch
$\mu\text{g}\cdot\text{mg}^{-1}$				
Control	212.09 ^a	18.64 ^a	193.43 ^b	19.67 ^b
<u>Glomus epigaeum</u>	218.19 ^a	16.99 ^{ab}	201.19 ^b	54.37 ^a
<u>Glomus etunicatum</u>	147.87 ^b	8.36 ^c	139.51 ^b	52.22 ^a
<u>Glomus fasciculatum</u>	197.72 ^{ab}	11.52 ^{bc}	186.21 ^b	59.70 ^a
<u>Glomus macrocarpum</u>	225.07 ^a	15.70 ^{ab}	209.37 ^a	50.22 ^a
<u>Glomus mosseae</u>	205.51 ^{ab}	12.83 ^{abc}	192.67 ^b	62.88 ^a
<u>Gigaspora margarita</u>	175.29 ^{ab}	10.84 ^b	164.46 ^b	67.50 ^a

Table III-5. Effect of fungal species on carbohydrate concentration ($\mu\text{g}\cdot\text{mg}^{-1}$) in Norway maple (Acer platanoides) roots. Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance in columns.

Mycorrhizal Treatment	Total Sugars	Reducing Sugars	Non-reducing Sugars	Starch
$\mu\text{g}\cdot\text{mg}^{-1}$				
Control	224.43 ^{ab}	12.60 ^a	211.83 ^{ab}	42.13 ^a
<u>Glomus epigaeum</u>	271.69 ^a	12.91 ^a	258.78 ^a	44.16 ^a
<u>Glomus etunicatum</u>	247.31 ^{ab}	12.34 ^a	234.97 ^{ab}	20.77 ^a
<u>Glomus fasciculatum</u>	196.92 ^b	10.36 ^a	186.56 ^b	50.29 ^a
<u>Glomus macrocarpum</u>	288.58 ^a	11.812 ^a	276.77 ^a	38.98 ^a
<u>Glomus mosseae</u>	239.71 ^{ab}	11.32 ^a	228.39 ^{ab}	54.38 ^a
<u>Gigaspora margarita</u>	221.18 ^{ab}	12.39 ^a	208.79 ^{ab}	49.04 ^a

Figure III-2. Effect of fungal species on height, dry weight of shoots, and dry weight of roots of redstem dogwood (*Cornus sericea*). Separation of means by the Newman-Keuls test of significance, 5% level. Differing letters indicate significance.

Table III-6. Effect of fungal species on concentration ($\mu\text{g} \cdot \text{g}^{-1}$) of N, P, and K in redstem dogwood (Cornus sericea) shoots. Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance within columns.

Mycorrhizal Treatment	N	P	K
	$\mu\text{g} \cdot \text{g}^{-1}$		
Control	4.754 ^b	0.520 ^b	20.680 ^a
<u>Glomus epigaeum</u>	5.300 ^a	0.789 ^a	22.543 ^a
<u>Glomus etunicatum</u>	5.104 ^{ab}	0.750 ^a	22.630 ^a
<u>Glomus fasciculatum</u>	4.904 ^{ab}	0.695 ^a	22.975 ^a
<u>Glomus macrocarpum</u>	4.908 ^a	0.729 ^a	23.156 ^a
<u>Glomus mosseae</u>	5.411 ^a	0.753 ^a	20.683 ^a
<u>Gigaspora margarita</u>	5.338 ^a	0.791 ^a	21.141 ^a

symbionts. Inoculation with all fungal species greatly increased the concentration of potassium in dogwood (Cornus) roots.

Glomus fasciculatum was the only mycorrhizal fungus which increased the concentration of total sugars in dogwood (Cornus) stems, while Glomus etunicatum, Glomus macrocarpum, and Glomus epigaeum decreased the concentration of total sugars in dogwood (Cornus) stems (Table III-8). Only Glomus epigaeum increased the concentration of reducing sugars in dogwood (Cornus) stems. With the exception of Glomus fasciculatum, inoculation with all fungal species resulted in a reduction of starch concentration in dogwood (Cornus) stems. In dogwood (Cornus) roots, there was no effect of mycorrhizal development on the concentration of total sugars (Table III-9). Inoculation had little effect on the concentration of reducing sugars in dogwood (Cornus) roots, except for a reduction when plants were inoculated with Glomus fasciculatum. There also was little effect of inoculation on the concentration of starch in dogwood (Cornus) roots, except for a reduction when plants were inoculated with Glomus etunicatum.

Table III-7. Effect of fungal species on concentration ($\mu\text{g} \cdot \text{g}^{-1}$) of N, P, and K in redstem dogwood (Cornus sericea) roots. Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance within columns.

Mycorrhizal Treatment	N	P	K
	$\mu\text{g} \cdot \text{g}^{-1}$		
Control	2.838 ^a	0.210 ^c	7.427 ^b
<u>Glomus epigaeum</u>	1.863 ^{bc}	0.504 ^{ab}	17.480 ^a
<u>Glomus etunicatum</u>	2.046 ^{bc}	0.556 ^a	16.654 ^a
<u>Glomus fasciculatum</u>	2.096 ^b	0.493 ^{ab}	17.461 ^a
<u>Glomus macrocarpum</u>	1.775 ^{bc}	0.423 ^{ab}	15.648 ^a
<u>Glomus mosseae</u>	1.717 ^c	0.413 ^b	15.633 ^a
<u>Gigaspora margarita</u>	1.808 ^{bc}	0.523 ^a	19.012 ^a

Table III-8. Effect of fungal species on carbohydrate concentration ($\mu\text{g}\cdot\text{mg}^{-1}$) of redstem dogwood (Cornus sericea) shoots. Separation of means by the Newman-Keuls test in significance, 10% level. Differing letters indicate significance in columns.

Mycorrhizal Treatment	Total Sugars	Reducing Sugars	Non-reducing Sugars	Starch
$\mu\text{g}\cdot\text{mg}^{-1}$				
Control	211.54 ^b	12.37 ^b	199.16 ^{ab}	14.81 ^a
<u>Glomus epigaeum</u>	156.80 ^c	31.74 ^a	125.47 ^c	2.071 ^b
<u>Glomus etunicatum</u>	53.99 ^d	11.59 ^b	42.39 ^{cd}	3.40 ^b
<u>Glomus fasciculatum</u>	290.89 ^a	12.82 ^b	278.82 ^a	6.53 ^{ab}
<u>Glomus macrocarpum</u>	17.61 ^d	9.64 ^b	7.97 ^d	2.41 ^b
<u>Glomus mosseae</u>	183.40 ^b	13.05 ^b	170.34 ^{bc}	1.90 ^b
<u>Gigaspora margarita</u>	252.24 ^{ab}	13.32 ^b	239.91 ^{ab}	1.65 ^b

Table III-9. Effect of fungal species on carbohydrate concentration ($\text{ug} \cdot \text{mg}^{-1}$) of redstem dogwood (Cornus sericea) roots. Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance in columns.

Mycorrhizal Treatment	Total Sugars	Reducing Sugars	Non-reducing Sugars	Starch
$\text{ug} \cdot \text{mg}^{-1}$				
Control	241.50 ^a	75.02 ^a	166.49 ^a	52.05 ^{ab}
<u>Glomus epigaeum</u>	237.75 ^a	66.33 ^a	171.42 ^a	61.65 ^{ab}
<u>Glomus etunicatum</u>	200.20 ^a	61.21 ^{ab}	138.99 ^a	29.34 ^c
<u>Glomus fasciculatum</u>	195.01 ^a	46.87 ^c	148.14 ^a	46.58 ^{abc}
<u>Glomus macrocarpum</u>	247.13 ^a	65.55 ^a	181.58 ^a	52.14 ^a
<u>Glomus mosseae</u>	183.56 ^a	55.73 ^b	127.69 ^a	39.66 ^{bc}
<u>Gigaspora margarita</u>	173.67 ^a	57.59 ^{ab}	116.08 ^a	40.12 ^{bc}

Oleaster (Elaeagnus)

Not all fungal species increased the height of Russian olive (Elaeagnus angustifolia), although there were significant increases in stem heights of plants inoculated with Glomus fasciculatum, Glomus etunicatum, Glomus macrocarpum, and Glomus epigaeum (Figure III-3). The dry weights of oleaster (Elaeagnus) shoots were increased by association with all fungal species, but root dry weights were increased only by Glomus mosseae and Glomus etunicatum.

Neither the concentration of nitrogen nor potassium in oleaster (Elaeagnus) leaves were affected by inoculation (Table III-10). However, the concentration of phosphorus in leaves was significantly increased following infection with Glomus macrocarpum. In roots, inoculation with all fungal species resulted in a reduction of nitrogen concentration (Table III-11). In contrast, all species of fungi increased the concentration of phosphorus in oleaster (Elaeagnus) roots. Inoculation had no significant effect on potassium concentration in roots, when compared to control plants.

Glomus mosseae was the only fungus which significantly increased the concentration of total sugars in stems of oleaster (Elaeagnus) (Table III-12). All other treatments were not significantly different from control plants. No treatments had concentrations of reducing sugars or starch significantly different from those in stems of control plants. In roots, no treatments had significantly different concentrations of total sugars from those in roots of control plants (Table III-13). Reducing sugar concentration in roots was significantly reduced by infection by Glomus margaritum, Glomus etunicatum, and Glomus macrocarpum. No mycorrhizal treatments had an influence on starch concentration in oleaster (Elaeagnus) roots.

Figure III-3. Effect of fungal species on height, dry weight of shoots, and dry weight of roots of Russian olive (*Elaeagnus angustifolia*). Separation of means by the Newman-Keuls test of significance, 5% level. Differing letters indicate significance.

Table III-10. Effects of fungal species on foliar concentration ($\mu\text{g}\cdot\text{g}^{-1}$) of N, P, and K in Russian olive (Elaeagnus angustifolia). Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance within columns.

Mycorrhizal Treatment	N	P	K
	$\mu\text{g}\cdot\text{g}^{-1}$		
Control	4.328 ^a	0.717 ^{bc}	81.816 ^{ab}
<u>Glomus epigaeum</u>	4.127 ^a	1.081 ^{ab}	77.598 ^b
<u>Glomus etunicatum</u>	3.961 ^a	0.878 ^{bc}	91.423 ^a
<u>Glomus fasciculatum</u>	4.143 ^a	1.104 ^{ab}	81.833 ^{ab}
<u>Glomus macrocarpum</u>	3.940 ^a	1.160 ^a	77.422 ^b
<u>Glomus mosseae</u>	3.776 ^a	0.914 ^{bc}	81.826 ^{ab}
<u>Gigaspora margarita</u>	4.213 ^a	0.866 ^c	71.313 ^b

Table III-11. Effects of fungal species on concentration ($\text{ug} \cdot \text{g}^{-1}$) of N, P, and K of Russian olive (Elaeagnus angustifolia) roots. Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance in columns.

Mycorrhizal Treatment	N	P	K
	$\text{ug} \cdot \text{g}^{-1}$		
Control	4.990 ^a	0.585 ^c	16.723 ^{ab}
<u>Glomus epigaeum</u>	3.308 ^b	0.909 ^{ab}	17.374 ^{ab}
<u>Glomus etunicatum</u>	3.575 ^b	0.999 ^a	16.480 ^{ab}
<u>Glomus fasciculatum</u>	3.863 ^b	1.081 ^a	17.297 ^{ab}
<u>Glomus macrocarpum</u>	3.458 ^b	0.970 ^{ab}	18.546 ^a
<u>Glomus mosseae</u>	3.920 ^b	0.799 ^b	17.814 ^{ab}
<u>Gigaspora margarita</u>	3.425 ^b	0.983 ^a	16.129 ^b

Table III-12. Effect of fungal species on carbohydrate concentration ($\mu\text{g}\cdot\text{mg}^{-1}$) of Russian olive (*Elaeagnus angustifolia*) shoots. Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance in columns.

Mycorrhizal Treatment	Total Sugars	Reducing Sugars	Non-reducing Sugars	Starch
$\mu\text{g}\cdot\text{mg}^{-1}$				
Control	202.19 ^{bc}	10.02 ^{abc}	192.16 ^b	18.64 ^{ab}
<i>Glomus epigaeum</i>	136.00 ^c	8.73 ^{bc}	127.26 ^c	11.36 ^b
<i>Glomus etunicatum</i>	267.84 ^{abc}	12.51 ^a	255.32 ^{abc}	14.98 ^{ab}
<i>Glomus fasciculatum</i>	148.06 ^c	12.65 ^a	135.41 ^c	12.81 ^b
<i>Glomus macrocarpum</i>	148.76 ^c	8.35 ^c	140.41 ^c	11.81 ^b
<i>Glomus mosseae</i>	340.10 ^a	12.08 ^{ab}	328.01 ^a	22.14 ^a
<i>Gigaspora margarita</i>	298.59 ^{ab}	8.10 ^c	290.50 ^{ab}	16.26 ^{ab}

Discussion

Growth of all plant species was significantly increased by mycorrhizal inoculation with one or more of the fungal species tested. For a given plant species, some fungi were more effective at enhancing plant growth than were others, indicating variable mycorrhizal dependency.

Some of the growth enhancements credited to mycorrhizal association are obvious in the landscape, such as height and weight, which are a reflection of the mass and density of growth. Both of these attributes are important in the establishment and performance of plants in the landscape. Less obvious, but still important to plant health, are nutrient status and carbohydrate concentrations.

In this research, phosphorus concentrations were clearly increased by association of plants with mycorrhizal fungi. Increases and decreases in the concentrations of nitrogen and potassium were not consistent among all plant and fungal combinations, and may relate to the nutrient requirements of the plants and plant growth rate. In cases where nutrients in mycorrhizal plants were in higher concentrations than in control plants, the nutrients were apparently taken up by mycorrhizal roots at a rate faster than their use, resulting in a net increase in their concentration. When a decrease in nutrient concentration occurred, it is likely that the nutrients were diluted over a larger plant mass. Nitrogen in dogwood (Cornus) was distributed heavily to shoots, probably due to a strong sink created by the rapid growth of the shoots.

It is generally agreed that any increases in the uptake of nitrogen and potassium by mycorrhizal plants is achieved through the use of the surface area added by the fungal symbiont. However, in the case of phosphorus uptake several theories exist proposing a more effective system of phosphorus uptake in mycorrhizal roots (Harley and Smith, 1983; Maronek et al., 1981).

Table III-13. Effect of fungal species on carbohydrate concentration ($\text{ug} \cdot \text{mg}^{-1}$) of Russian olive (Elaeagnus angustifolia) roots. Separation of means by the Newman-Keuls test of significance, 10% level. Differing letters indicate significance in columns.

Mycorrhizal Treatment	Total Sugars	Reducing Sugars	Non-reducing Sugars	Starch
$\text{ug} \cdot \text{mg}^{-1}$				
Control	262.09 ^{ab}	31.41 ^a	233.00 ^{abc}	23.68 ^a
<u>Glomus epigaeum</u>	291.19 ^{ab}	30.59 ^a	260.62 ^{abc}	22.07 ^a
<u>Glomus etunicatum</u>	176.04 ^b	23.79 ^b	152.25 ^c	20.15 ^a
<u>Glomus fasciculatum</u>	358.24 ^a	26.89 ^{ab}	331.35 ^a	19.35 ^a
<u>Glomus macrocarpum</u>	268.41 ^{ab}	21.91 ^{ab}	246.50 ^{abc}	19.95 ^a
<u>Glomus mosseae</u>	328.20 ^a	28.136 ^{ab}	300.06 ^{ab}	21.03 ^a
<u>Gigaspora margarita</u>	191.34 ^b	15.28 ^c	176.06 ^b	23.47 ^a

Although there have been proposals that mycorrhizal fungi have the ability to solubilize insoluble forms of soil P, making them available to the plant (Douds, 1984; Pearson and Read, 1975; Mitchell and Read, 1981; Bartlett and Lewis, 1973), it is generally believed that vesicular-arbuscular mycorrhizal roots use the same pool of soluble P as do non-mycorrhizal roots (Hayman and Mosse, 1972; Mosse et al., 1973; Powell, 1975). Alternatively, it has been suggested that mycorrhizal infection may lower the threshold concentration from which plants are able to absorb phosphate (Mosse et al., 1973; Cress et al., 1979; Jintakamon et al., 1979; Howeler et al., 1979).

Another theory of increased phosphorus uptake stems from the knowledge that mycorrhizal plants in general respire at greater rates than do non-mycorrhizal plants (Harley and Smith, 1983). Phosphorus uptake is an active process which requires ATP produced during respiration. Therefore, it may be logical that if the elevated respiration of mycorrhizal roots results in a higher rate of ATP production, the added energy available may contribute to the impressive increases in phosphorus uptake by mycorrhizal plants. At present, no studies have been conducted to confirm this hypothesis. Additional evidence implicating increased energy supplies in the improved nutrient status of mycorrhizal plants is the distribution of ATPases in mycorrhizal plants (Douds, 1984; Harley and Smith, 1983).

Phosphorus uptake may be more affected by any increased energy supply than potassium because it has a lower K_m (Michaelis constant), or an affinity of nutrient ions for carrier sites involved in uptake (Epstein, 1972). At a given nutrient concentration, more phosphorus ions would be present at the carrier site than would be the case for potassium, therefore imposing a greater energy drain on the root for uptake of nutrient ions present at carrier sites. No clear trends were observed on the effect of mycorrhizal inoculation on

carbohydrate concentration. This is in agreement with that of Douds (1984) who observed seasonal variations, but no mycorrhizal treatment effect. In cases where mycorrhizal fungi significantly increase plant weight, but have no impact on soluble or insoluble carbohydrate concentrations, mycorrhizal symbiosis may still have a positive effect on net carbon balance of the plants since, the plants are larger in size (Douds, 1984).

Results reported here, as well as in the work of others (Pope et al., 1983), highlight the importance of providing a fungal symbiont with the greatest potential for symbiotic association with the plant species. Fungal species is important not only for growth as indicated by plant height and weight, but also to the nutrient status and health of the plants. If a mycorrhizal plant intended for use in the highway landscape is in symbiotic association with the proper fungal symbiont, significant growth increases are possible.

Literature Cited

- Bartlett, E.M. and D.H. Lewis. 1973. Surface phosphatase activity of mycorrhizal roots of beech. *Soil Biol. Biochem.* 5:249-257.
- Beverge, D.I., G.D. Bowen, and M.F. Skinner. 1975. Comparative physiology of ecto- and endomycorrhizas. In: *Endomycorrhizas*. F.E. Sanders, B. Mosse, and P.B. Skinner, Eds. Academic Press, NY. p 149-174.
- Cherry, J.H. 1973. *Molecular biology of plants. A text-manual.* Columbia University Press, Y. p 204.
- Cooper, K.M. and D.M. Losel. 1978. Lipid physiology of vesicular-arbuscular mycorrhiza. I. Composition of lipids in roots of onion, clover, and ryegrass infected with Glomus moseae. *New Phytol.* 80:143-151.
- Cress, W.A., G.O. Throneberry, and D.L. Lindsey. 1979. Kinetics of phosphorus absorption by mycorrhizal and non-mycorrhizal tomato roots. *Pl. Physiol.* 64:484-487.
- Daft, M.J. and T.H. Nicolson. 1976. Effect of Endogone mycorrhiza on plant growth. *New Phytol.* 65:343-350.
- Douds, D.D., Jr. 1984. Seasonal patterns of host growth, fungal morphology, and root carbohydrate and phosphorus content in a green ash mycorrhiza. Ph.D. Thesis, Purdue University.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Ribers, and F. Smith. 1956. Colorimetric method for determining sugars and related substances. *Anal. Chem.* 28:350-356.
- Epstein, E. 1972. *Mineral nutrition of plants: Principles and perspectives.* Wiley and Sons, NY. pp 83-285.
- Foster, R.C. and G.C. Marks. 1966. The fine structure of the mycorrhizas of Pinus radiata, D. Don. *Aust. J. Biol. Sci.* 18:1027-1038.
- Geddeda, Y.I., J.M. Trappe, and R.L. Stebbins. 1983. Vesicular-arbuscular mycorrhizal fungi associated with apples grown in Oregon. *HortSci.* 18(6):929-930.
- Giovanetti, H. and B. Mosse. 1979. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84:489-500.
- Gray, L.E. and J.W. Gerdemann. 1967. Influence of vesicular-arbuscular mycorrhiza on the uptake of phosphorus-32 by Liriodendron tulipifera and Liquidambar styraciflua. *Nature* 213:106-107.
- Harley, J.L. and S.E. Smith. 1983. *Mycorrhizal Symbiosis.* Academic Press, NY. p 15-103.

- Hayman, D.S. and B. Mosse. 1972. Plant growth responses to vesicular-arbuscular mycorrhiza. III. Increase uptake of labile P from soil. *New Phytol.* 71:41-47.
- Hepper, C.M. and B. Mosse. 1973. Trehalose and mannitol in VA mycorrhiza. *Rep. Rothamsted Exp. Sta.* 1972. P 81.
- Howeler, R.H., D.G. Edwards, and C.J. Asher. 1979. The effect of soil sterilization and mycorrhizal inoculation on the growth, nutrient uptake and critical phosphorus concentration of Cassava. *Proc. 5th International Tropical Root and Tuber Crops Symposium, Manila, Philippines.*
- Jackson, M.L. 1958. *Soil Chemical Analysis.* Prentice-Hall, Englewood Cliffs, NJ. pp 200-210.
- Jintakamon, S., D.G. Edwards, and C.J. Asher. 1979. An anomalous high external phosphorus requirement for young Cassava plants in solution culture. *Proc. 5th International Tropical Root and Tuber Crops Symposium, Manila, Philippines.*
- Johnson, C.R., J.N. Joiner, and C.E. Crews. 1980. Effects of N, K, and Mg on growth and leaf nutrient composition of three container grown woody ornamentals inoculated with mycorrhizae. *J. Amer. Soc. Hort. Sci.* 105(2):286-288.
- King, E.J. and M.F. Brown. 1981. Electron microscopy of developing arbuscles of Glomus fasciculatus in soybean mycorrhizae. In: *Fifth N. Amer. Conf. on Mycorrhizae, Quebec, Can. Program and Abstracts.* p 10.
- Knox, G.W. 1984. Light intensity as it affects growth and branching of three woody plant species. *Ph.D. Thesis, Purdue University.* pp 21-22.
- Kormanik, P.P., W.C. Bryan, and R.C. Schultz. 1977. Endomycorrhizal inoculation during transplanting improves growth of vegetatively propagated yellow-poplar. *Plant Propagator.* 23:4-5.
- Lewis, D.H. and J.L. Harley. 1965a. Carbohydrate physiology of mycorrhizal roots of beech. I. Identity of endogenous sugars and utilization of exogenous sugars. *New Phytol.* 64:224-237.
- Lewis, D.H. and J.L. Harley. 1965b. Carbohydrate physiology of mycorrhizal roots of beech. II. Utilization of exogenous sugars by uninfected and mycorrhizal roots. *New Phytol.* 64:238-256.
- Lewis, D.H. and J.L. Harley. 1965c. Carbohydrate physiology of mycorrhizal roots of beech. III. Movement of sugars between host and fungus. *New Phytol.* 64:256-269.
- Libby, R.A. 1970. Direct starch analysis using DMSO solubilization and glucoamylase. *Cereal Chem.* 70:273-281.
- Ling-Lee, M., A.E. Ashford, and G.A. Chilvers. 1977. A histochemical study of polysaccharide distribution in eucalypt mycorrhizas. *New Phytol.* 78:329-335.

- Maronek, D.M., J.W. Hendrix, and J. Kiernan. 1981. Mycorrhizal fungi and their importance in horticultural crop production. In: Horticultural Reviews, Vol. 3. J. Janick, Ed. AVI Publishing Company, Inc., Westport, CT. pp 172-213.
- Maronek, D.M., J.W. Hendrix, and J. Kiernan. 1980. Differential growth responses to the mycorrhizal fungus Glomus fasciculatus of southern magnolia and bar harbor juniper grown in containers in hardwood bark-shale. J. Amer. Soc. Hort. Sci. 105(2):206-208.
- Mitchell, D.T. and D.J. Read. 1981. Utilization of inorganic and organic phosphates by the mycorrhizal endophytes of Vaccinium macrocarpum and Rhododendron ponticum. Trans. Brit. Mycol. Soc. 76:255-260.
- Mosse, B. 1962. The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. J. Gen. Microbiol. 27:509-520.
- Mosse, B. 1975. Specificity of VA mycorrhizas. In: Endomycorrhizas. F.E. Sanders, B. Mosse, and P.B. Tinker, Eds. Academic Press, NY. pp 469-484.
- Mosse, B., D.S. Hayman, and D.J. Arnold. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labelled with ^{32}P . New Phytol. 72:809-815.
- Nagy, S., H.E. Nordby, and S. Nemic. 1980. Composition of lipids in roots in six citrus cultivars infected with the vesicular-arbuscular mycorrhizal fungus Glomus mosseae. New Phytol. 85:377-384.
- Pearson, V. and D.J. Read. 1975. The physiology of the mycorrhizal endophyte of Calluna vulgaris. Trans. Br. Mycol. Soc. 64:1-7.
- Philips, V. and D.S. Hayman. 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55:158-161.
- Pope, P.E., W.R. Chaney, J.D. Rhodes, and S.H. Woodhead. 1983. The mycorrhizal dependency of four hardwood tree species. Can. J. Bot. 61(2):412-417.
- Powell, C.C. 1975. Plant growth responses to vesicular-arbuscular mycorrhiza. VIII. Uptake of P by onion and clover infected with different Endogone spore types in ^{32}P -labelled soils. New Phytol. 75:563-566.
- Schultz, R.C., P.P. Kormanik, W.C. Bryan, and G.H. Brister. 1979. Vesicular-arbuscular mycorrhiza influence growth but not mineral concentrations in seedlings of eight sweetgum families. Can. J. For. Res. 9:218-223.
- Schultz, R.C., P.P. Kormanik, and W.C. Bryan. 1981. Effects of fertilization and vesicular-arbuscular inoculation on growth of hardwood seedlings. Soil Sci. Soc. Am. J. 45:961-965.

- Stribley, D.P. and D.J. Read. 1974. The biology of mycorrhiza in the Ericaceae. III. Movement of carbon-14 from the host to the fungus. New Phytol. 73:731-741.
- Verkade, S.D. and D.F. Hamilton. 1983a. Effects of soil fertility on growth, nutrient concentration and mycorrhizal development of Liriodendron tulipifera seedlings inoculated with the vesicular-arbuscular fungus, Glomus fasciculatus. Scientia Horticulturae 21:243-252.
- Verkade, S.D. and D.F. Hamilton. 1983b. Effects of Benomyl on growth of Liriodendron tulipifera L. seedlings inoculated with vesicular-arbuscular fungus, Glomus fasciculatus. Scientia Horticulturae 21:253-260.
- Wedding, R.T. and J.L. Harley. 1976. Fungal polyol metabolites in the control of carbohydrate metabolism of mycorrhizal roots of beech. New Phytol. 77:675-688.

Chapter IV

Use of Colloidal Silica Solution in the Isolation of Endomycorrhizal Spores

Introduction and Literature Review

Several techniques exist for isolating spores of mycorrhizal fungi from soil (Daniels and Skipper, 1982). The most simple technique is the new plate method (Smith and Skipper, 1979), which is useful for soils without a large amount of organic matter. In this procedure, one gram of soil is suspended in water and shaken. An aliquot is then placed on lined filter paper in a Petri dish and spores are counted. If organic matter content is high, additional steps are needed to eliminate debris from the isolation. It is desirable to keep the complexity of the procedure to a minimum since spores are easily lost or damaged by using sieves, containers, or devices. Procedures used to further purify spore solutions, such as wet sieving, flotation, and centrifugation tend to significantly lower the number of spores recovered from a given volume of soil (Jenkins, 1964; Ohms, 1957; Smith and Skipper, 1979). Nonetheless, these modifications are very important in cases where high organic matter content necessitates further isolation.

For general industrial use, it also is desirable to employ an uncomplicated technique which requires little investment in equipment. One effective procedure which has become popular is wet sieving and centrifugation.

This procedure was adapted from a technique used in the isolation of nematodes from soil (Jenkins, 1964). The major drawback of this procedure is that spores are suspended in sucrose solution in the final step of centrifugation. The sucrose is of high enough concentration (454 g/l) to apply osmotic stress upon delicate mycorrhizal spores.

Furlan et al. (1980) solved this problem by substituting various radiopaque media or Percoll for sucrose in the centrifugation step. These centrifugation media were effective, but could be cost prohibitive for large scale use.

The objective of this research was to refine the technique of wet sieving and sucrose centrifugation by substituting a low-cost centrifugation solution which will not impose osmotic stress on the spores. For this purpose, Ludox TM (manufactured by E.I. Dupont) was tested. Ludox is an inert solution of colloidal silica which has been used in the isolation of mitochondria.

Materials and Methods

Sporocarps from a culture of Glomus mosseae were isolated by the wet-sieving and centrifugation technique of Jenkins (1964) using Ludox TM as an alternative to sucrose as a centrifugation medium. In this procedure, a volume of soil containing spores is wet sieved using two sieves. The first sieve, having larger openings (250-500 μm), is used to remove large, undesirable particles. The second sieve has smaller openings (44 μm) and is used to collect the spores. The collected material is then centrifuged in water at 1750 rpm for 5 minutes. The supernatant fluid is poured off (spores sink in water) and a sucrose solution (454 g/l) or Ludox is added. This solution is mixed thoroughly and centrifuged at 1750 rpm for 1 minute. When using Ludox, centrifugation can be for a longer time, since no damage will result to the spores. The supernatant fluid containing the spores (spores float in sucrose or Ludox solutions) is poured onto a 44 μm sieve, rinsed, and placed on lined filter paper for counting under a microscope. The procedure was repeated with solutions having specific gravities ranging from 1.020 to 1.325 g cm^{-3} (20°C). Stock solutions of sucrose (454 g/l water) and Ludox TM were diluted with distilled water to the appropriate specific gravity.

Results and Discussion

Use of Ludox TM in the wet sieving and centrifugation procedure (Jenkins, 1964) for isolation of spores resulted in the recovery of more endomycorrhizal sporocarps than when sucrose was used as a centrifugation medium (Fig. IV-1). It was most effective at specific gravities above 1.16 g cm^{-3} , although at specific gravity greater than 1.20 excessive organic debris was retained in the isolation. It is likely that some spores isolated in sucrose solutions became damaged or burst due to the osmotic pressure of the solution (Furlan et al., 1964). Destruction of spores by osmotic pressure of sucrose solution may explain the greater recovery of spores by Ludox than sucrose at a given specific gravity, since this destruction would not occur with Ludox.

Ludox TM is suitable for use in the isolation of endomycorrhizal spores. It currently is available for purchase only in very large volumes, which may prevent the use of Ludox unless considerable spore isolation is undertaken over time.

Figure IV-1. Effect of specific gravity of centrifugation medium on the number of spores recovered from soil using wet sieving and centrifugation either with sucrose or Ludox (colloidal silica) as the centrifugation medium. Six replications per treatment.

Literature Cited

- Daniels, B.A. and H.D. Skipper. 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: Methods and Principles of Mycorrhizal Research. N.C. Schenck, Ed. The Amer. Phytopath. Soc. St. Paul, Minn. pp 29-35.
- Furlan, V., H. Bartshi, and J.-A. Fortin. 1980. Media for density gradient extraction of endomycorrhizal spores. Trans. Brit. Mycol. Soc. 75(2):336-338.
- Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Rep. 48:692.
- Ohms, R.E. 1957. A flotation method for collecting spores of phycomycetous mycorrhizal parasites from soil. Phytopath. 47:751-752.
- Smith, G.W. and H.D. Skipper. 1979. Comparison of methods to extract spores of vesicular-arbuscular mycorrhizal fungi. Soil Sci. Soc. Amer. J. 43:722-725.

Chapter V

Mycorrhizal Spore Content of Highway Soils

Introduction

Soils remaining after the construction of a highway are generally characterized by low fertility, low water holding capacity, low organic matter, and low levels of beneficial endogenous microflora. Mycorrhizae have been reported to promote growth of plants transplanted into harsh environments in strip mine sites. Relatively little information is available on the mycorrhizal status of successfully revegetated highway slopes.

The objective of this experiment was to evaluate the mycorrhizal condition of highway slopes which were successfully and unsuccessfully revegetated following highway construction.

Materials and Methods

Six soil samples were taken randomly from each of four successfully revegetated and four unsuccessfully revegetated highway slopes. Within each site, the samples were taken randomly in a 5'x5' area, with some sampling occurring nearby established plants and also from spots with no vegetation. A 'bulb planter' was used to sample soil to a depth of 4-6". Three spore isolations were made from each soil sample by the techniques of Verkade and Hamilton (1981).

The slopes sampled at the intersection of I-65 and Indiana 43 were both East-facing with dry rocky soil. The poorly revegetated slope had mostly weeds as vegetative cover and had severe erosion gullies, whereas the well revegetated slope had no erosion problem and vegetative cover consisted mainly of grasses intermixed with a few weeds. The well revegetated site had

vegetation including Broomgrass, Horsetail (Equisetum arvense), Red Clover, Common Ragweed (Ambrosia artemisifolia), and Perennial Ryegrass (Lolium perenne). The site which was poorly revegetated at I-65 and Indiana 43 had vegetation including Perennial Ryegrass, Horsetail, Kentucky Bluegrass (Poa sp.), and Dock (Rumex sp.).

The slopes sampled at I-74 were all South-facing. The well revegetated slope had no erosion problems, dry soil, and vegetative cover composed mostly of grasses. The vegetation included Smartweed (Polygonum sp.), Tall Fescue, Perennial Ryegrass, Ivy-leaf Morning Glory (Ipomoea hederacea), and Horse Nettle (Solanum carolinense). The slope on I-74 which was poorly revegetated had severe erosion gullies and very dry soil. The vegetation at this site was composed of grasses and weeds including Dock, Horsetail Lambsquarter, some Ryegrass, and some Crown Vetch. At I-74, a third site was sampled, which was a successfully revegetated site composed solely of Crown Vetch.

The slopes sampled at Route 231 in Linden were West-facing. The well revegetated slope had no erosion problem and the soil samples were moist. The well revegetated site in Linden had vegetation including Queen Anne's Lace (Daucus carota), Crown Vetch, Bluegrass, Wild Barley (Hordeum jubatum), and Dock. The poorly revegetated site in Linden had moderate erosion gullies and very dry soil. The vegetative cover included some Fescue and some Ryegrass.

Results and Discussion

All sites were found to contain at least some mycorrhizal spores, indicating that mycorrhizal recolonization was possible for all sites. In all cases, sites which were successfully revegetated had substantially higher numbers of spores than sites which had poor vegetative cover.

At the highway site at the intersection of I-65 and Indiana 43, the highway slope which was successfully revegetated had 250% more mycorrhizal spores than the adjacent slope which was unsuccessfully revegetated. The highway slopes on Indiana 231 in Linden also showed substantial differences, with the successfully revegetated slope having 389% more spores than the unsuccessfully revegetated slope. On Interstate 74, the successfully revegetated slope composed mostly of grasses had 127% more spores than the unsuccessfully revegetated portion of the slope. The adjacent slope successfully revegetated with Crown Vetch had 35% more mycorrhizal spores than the site successfully revegetated with grasses and 207% more spores than the unsuccessfully revegetated.

In all cases, successful revegetation was associated with the occurrence of mycorrhizal spores. In these situations, the presence of mycorrhizae was not the cause of the successful revegetation, since they are not initially present at planting and early establishment. Although not present in large numbers upon seeding or planting, the spores recolonize from neighboring undisturbed sites and once established contribute to plant growth and revegetation of the site.

If plants which already had mycorrhizal roots were introduced into the highway landscape, or if a high population of mycorrhizal spores was reintroduced into the area at the time of planting, the beneficial effect of mycorrhizae might be taken advantage of to increase the success of revegetation efforts in highly disturbed highway landscapes.

Literature Cited

Verkade, S.D. and D.F. Hamilton. 1981. Techniques to increase survival of new highway plantings using mycorrhizal inoculation. FHWA/IN/JHRP-82-14.

Chapter VI

Effects of Mycorrhizal Inoculation on Growth of Landscape Plants Growing in Highway Soils

Introduction

Evidence exists indicating a correlation between the occurrence of mycorrhizal roots and the successful revegetation of highway slope. Substantial increases in spore numbers were observed in soils in which revegetation had been achieved, when compared to those in soils in which revegetation was relatively unsuccessful.

The causal role of mycorrhizae in the successful revegetation of highway soils has yet to be established. The objective of this experiment was to determine the effect of mycorrhizal inoculation during planting in the highway landscape on plant growth and establishment. Three plant species and two methods of inoculation were examined.

Materials and Methods

Twice transplanted seedlings of Norway maple (Acer platanoides) (55 cm tall), redstem dogwood (Cornus sericea) (78 cm tall), and arrowwood viburnum (Viburnum dentatum) (58 cm tall) were planted on land at the intersection of I-65 and Indiana 43 in October 1983. Treated plants were inoculated with mycorrhizae either by placing inoculum (containing 1000 spores, hyphae, plant roots, and soil) in three cores 12-15 cm deep around the plant, or by submerging the root system in a wet slurry of the same inoculum. The control plants received no treatment. Plants were surrounded with chicken wire cages for protection from rabbits and mice, and were thoroughly watered. Plants of each species were arranged in a completely randomized design.

Results and Discussion

At the end of one year, no differences were noted in the growth or survival rate of plants inoculated with mycorrhizal fungi. This indicates that there would be little immediate value of mycorrhizal inoculation at the time of planting on the highway site. Although there is no immediate value, inoculation at the time of planting may play a role in later development of the plants. Since mycorrhizal inoculation at the time of planting did not result in infection sufficient to enhance plant growth, plants used in highway plantings may need to become mycorrhizal prior to the time of planting to obtain any benefit from inoculation efforts.

Other environmental factors have a greater influence on plant establishment on the roadside than the presence of mycorrhizal. If the conditions are too adverse for plant establishment, (i.e., moisture and temperature stresses) plant failure results even if they are inoculated with mycorrhizal fungi.

Recommendations for Further Research

At the beginning of this research only one known company was interested in mycorrhizal spore production and it suspended operations in this area before the project was completed. This means there is no possibility of inoculation being available for application to plants either in production, on the highway or anywhere else. Until such commercial production exists or is planned, further research on application to highway sites is not recommended.

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