EFFECT OF GLOMUS SPP. ON THE GROWTH OF EASTERN COTTONWOOD CUTTINGS [1]

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Abstract. The rapid juvenile growth of eastern cottonwood (Populus deltoides Bartr. ex Marsh.) makes it a desirable hardwood species for revegetation of disturbed sites. In addition, revegetation may be facilitated by improved root growth in response to endomycorrhizal colonization. An experiment was conducted to identify the effect of inoculation with a mix of three Glomus spp. isolates on the root growth rate of eastern cottonwood cuttings. Results indicated that endomycorrhizal colonization of eastern cottonwood cuttings was successful using commercial endomycorrhizal spore inoculum. However, endomycorrhizal inoculation appeared to have a negative effect on growth during greenhouse production. Factors contributing to this response are discussed. Reduced root growth rate in response to endomycorrhizal inoculation, but lack of either shoot or root dry weight response, suggests that endomycorrhizal inoculation may have affected root system morphology.

Introduction

Benefits derived from endomycorrhizal associations have been documented for many hardwood tree species (Pope 1980; Kormanik et al., 1982; Melichar et al., 1986). In addition to modification of shoot growth, endomycorrhizal colonization may result in physiological and morphological alteration of root development. This potential influence of endomycorrhizal fungi on hardwood root systems may lead to improved survival and growth following outplanting of seedlings and cuttings.

[1] Paper presented at Sixth Biennial Southern Silvicultural Research Conference, Memphis, TN, Oct. 30-Nov. 1, 1990.

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Past research has suggested that endomycorrhizal symbiosis may modify root morphological features such as the number of lateral roots and root hairs, total root length and root dry weight (Kormanik 1985, Berta and Gianinazzi-Pearson 1986, Dixon 1988, Simmons and Pope 1988). The rate of root elongation, another aspect of root development which may be important to seedling survival and growth, may be characterized by a greater increase in mycorrhizal than in nonmycorrhizal plants.

An increase in root elongation rate would be beneficial following outplanting of hardwood species which are relatively intolerant of moisture stress. Eastern cottonwood (Populus deltoides Bartr. ex Marsh.) possesses rapid juvenile growth making it an excellent choice for use in revegetating disturbed sites. Unfortunately, this species is relatively intolerant of moisture stress. This was exemplified by

Blake et al. (1984) who compared the water use efficiency (WUE) and dry matter production of 17 Populus spp. clones. Results indicated that eastern cottonwood ranked only tenth in WUE and possessed the lowest biomass production rate when compared to the remaining 16 clones tested. Endomycorrhizae could prove advantageous to drought-intolerant species such as eastern cottonwood and provide a growth advantage on sites with adequate fertility but inadequate moisture.

Objectives of this study included the synthesis of endomycorrhizal eastern cottonwood cuttings using a commercial spore inoculum mix of three Glomus spp. isolates. Subsequently, effects of endomycorrhizal inoculation on shoot growth and root development of greenhouse-grown eastern cottonwood cuttings were evaluated.

Materials And Methods

Clear acrylic tubes, 61.0 cm in length and 7.6 cm in diameter, were covered at the base with 0.5 cm^2 aluminum mesh. Washed gravel was placed in tubes to an approximate depth of 5 cm. Subsequently, 6.3 L of uninoculated or inoculated growth medium was poured into tubes resulting in approximately 10 cm of unoccupied depth at the top of tubes. The growth medium of uninoculated and inoculated tubes consisted of 1:1:2 (v/v/v) sandy loam soil-peat-perlite which was sterilized with methyl bromide. The growth medium of inoculated tubes contained, in addition, a 15-ml volume of spore inoculum of each of three isolates of Glomus spp. As a result, approximately 15 thousand spores of Glomus spp., isolates 10 (Imperial Valley, CA), 25 (Vetura/Oxnard Plain, CA) and 71 (Midwestern U.S.) (Native Plants Inc., Salt Lake City, UT), were thoroughly mixed throughout the growth medium of each inoculated tube providing an inoculation intensity of 7.1 spores/ml of growth medium (8.2 spores/g).

Dormant eastern cottonwood cuttings from a single selection (miscellaneous Missouri selection, GG-1) were obtained from the George 0. White State Forest Nursery in Licking, Missouri, in April 1989. Cuttings were divided into apical, middle, and basal segments, each 20.3 cm in length. Cutting segments were planted vertically in tubes to a 10-cm depth. With the exception of the most terminal intact bud, aboveground lateral buds were excised. To facilitate adventitious root growth along the lower surface, tubes were placed at a 45° angle, 46 cm apart, on greenhouse benches. Sides of tubes were covered with black plastic to create a dark rooting environment. Black plastic was then covered with white cotton sheeting to reduce temperature fluctuation of the rooting environment.

Eastern cottonwood segments were watered when the growth medium appeared dry. Four weeks following bud break, fertilization began. Segments were fertilized weekly with 0.5 L of %-strength Hoagland's solution (Bonner and Galston 1952) throughout the 26-week cultural period. Natural lighting was utilized. Ambient greenhouse temperature ranged from 20 to 32°C.

A randomized complete block design with three blocks was used. Treatments were noninoculation or inoculation with a Glomus spp. mix (three isolates of Glomus spp., species unknown). Blocks represented the location of 20.3-cm segments within the original dormant cutting (i.e., apical, middle, and basal segments). Three replications of apical, middle, and basal 20.3-cm cutting segments received either no inoculation or inoculation with Glomus spp. and were randomly placed on greenhouse benches.

The gravitropic response of roots as well as the acrylic nature of tubes allowed root growth to be monitored throughout the cultural period. Growth increments were drawn on lower surfaces of tubes at approximately 2-day intervals with permanent marking pens. Following termination of the greenhouse phase of the experiment, marked increments were used to calculate root growth rates.

Cuttings were harvested following a 26-week cultural period. Stem length and diameter were measured following 13 weeks of growth and after harvest. Leaf surface area (Li-3000, Li-Cor Inc., Lincoln, NE) as well as root, stem and foliar dry weights (72 h, 77°C) were measured after harvest. Stem length was defined as shoot height from location of shoot emergence on the cutting segment to shoot tip. Stem diameter was defined as the diameter of the stem, 12.7 cm from the location of shoot emergence on the cutting segment.

Following harvest, a 2.0-g subsample of fresh root tissue was randomly selected from the fine roots of each cutting. Endomycorrhizal colonization was evaluated after clearing and staining roots with acid fuchsin (Kormanik and McGraw 1982). Percentage of root length infected and the number of vesicles per cm of root were estimated using the procedure of Giovannetti and Mosse (1980).

Analysis of variance was utilized for determination of relationships between inoculation with Glomus spp. and shoot and root development of eastern cottonwood cuttings. Differences between treatment means were tested at $P \leq 0.05$ and $P < 0.10$ using the least significant difference (LSD) test.

Results

Following the 26-week cultural period, inoculated eastern cottonwood cuttings were heavily colonized (63 percent ± 6 percent; 1.8 vesicles/cm root) with Glomus spp.; while uninoculated cuttings were less than 1 percent colonized.

Despite high levels of colonization, endomycorrhizal inoculation had no significant effect on stem length, stem diameter, or on stem, root, or foliar dry weights of eastern cottonwood cuttings (Table 1). The leaf surface area of inoculated cuttings was significantly less than that of uninoculated cuttings (Table 1). Moreover, the daily root growth rate of cuttings during the initial 13-week cultural period was significantly reduced by inoculation (Table 2). Rate of root growth during the last 13-week portion of the 26-week cultural period was not significantly affected by inoculation treatment.

Table 1. **Effect of a mixture of three *Glomus* spp. isolates on the growth of eastern cottonwood cuttings following a 26-week greenhouse cultural period.**

Variable	Treatment	
	Uninoculated	Inoculated
Stem length (cm)	93.4 a *	89.0 a
Stem diameter (mm)	14.6 a	13.6 a
Shoot dry weight (g)	15.2 a	13.9 a
Root dry weight (g)	11.0 a	11.9 a
Foliar dry weight (g)	23.6 a	21.6 a
Leaf surface area (cm*)	3697.8 a	3378.7 b

* Means within a variable followed by the same letter are not significantly different at $P \leq 0.05$ using the LSD test.

Table 2. **Effect of a mixture of three *Glomus* spp. isolates on the root growth rate of eastern cottonwood cuttings throughout the initial 13 weeks of a 26-week greenhouse cultural period.**

Growth interval	Treatment	
	Uninoculated	Inoculated
(wk)	(cm)	
o-3	....	....
3-5	0.94 a *	0.88 a
5-7	1.00 a	0.80 b
7-9	0.90 a	0.81 a
9-11	0.60 a	0.40 b
11-13	0.26 a	0.12 a
3 - 13 .	0.74 **a**	**0.60 b**

* Means within a growth interval followed by the same letter are not significantly different at $P \leq 0.10$ using the LSD test.

Discussion

Past research has reported the synthesis of mycorrhizae on eastern cottonwood seedlings (Vozzo and Hacskaylo 1974) and cuttings (Lodge 1989) using field soil containing both endomycorrhizal and ectomycorrhizal fungal inocula. In this experiment, we successfully synthesized endomycorrhizal eastern cottonwood cuttings using commercial endomycorrhizal spore inoculum. Shoot and root growth were either unaffected or inhibited due to endomycorrhizal inoculation.

Many studies have shown that endomycorrhizal colonization stimulates growth of greenhouse-grown hardwood species (Pope 1980; Kormanik et al., 1982; Kormanik 1985; Melichar et al., 1986; Dixon 1988). Furthermore, Navratil and Rochon (1981) demonstrated that, although ectomycorrhizae did not develop on root systems, inoculation with *Pisolithus tinctorius* [(Pers.) Coker & Couch] resulted in enhanced shoot and root growth of cuttings of four *Populus* spp. hybrids. However, our results are similar to those of others (Snellgrove et al., 1982; Hselova et al., 1989) in which potential benefits associated with endomycorrhizal inoculation were not expressed in plant growth measurements during the production phase. Negative root growth rate and leaf surface area responses of endomycorrhizal cuttings were observed in this study and may be attributed to a combination of factors.

The physiology of hardwood cuttings may provide some explanation for the growth inhibition observed. Nanda et al. (1971) reported the importance of having adequate exogenous glucose, in addition to indoleacetic acid, for rooting of *Populus* spp. cuttings. It was reported that a proper balance of nutritional and regulatory compounds determines the rooting ability of this genus. The rapid rate of shoot growth of cuttings when compared with that of seedlings suggests that starch availability for initial root growth may be more limiting in cuttings than in seedlings. Furthermore, the shoot growth rate of cuttings compared to seedlings suggests that production of growth regulators in shoot meristematic tissues may be greater in cuttings. As a result, growth responses of cuttings and seedlings inoculated with endomycorrhizal fungi may differ.

Energy for early shoot and root growth of cuttings is supplied by starch stored within upper and lower portions of the cutting, respectively (Okoro and Grace 1976). Root growth of cottonwood cuttings has been associated with both the initial starch concentration as well as the rate of starch utilization within the lower portion of the stem (Tschaplinski and Blake 1990). This information is supported by Fege and Brown (1984) who found that rooting of *Populus* spp. cuttings was directly related to size of cutting. Harley and Smith (1983) suggested that negative effects of endomycorrhizal inoculation on plant growth may occur when the intensity of infection is high. In the current experiment, respiration attributed to endomycorrhizal fungus metabolism may have reduced the availability of starch for root growth and subsequently reduced root growth.

Greenhouse environmental conditions may have also contributed to nutritional stress leading to negative growth responses of inoculated cuttings.

Okoro and Grace (1976) attributed low rates of photosynthesis by two species of Populus spp., in part, to low irradiance during greenhouse production. They reported that the rate of photosynthesis of Populus spp. was only one-tenth that obtained by Regehr et al. (1975) in which cuttings were grown under 1600 uE m^{-2} s^{-1} photosynthetically active radiation (PAR), which represented 90 percent of the PAR necessary for maximum photosynthesis. In the current experiment, maintenance of PAR at 400 uE $^{-2}$ s^{-1} may have been inadequate for maximum growth of eastern cottonwood cuttings, especially those colonized by endomycorrhizal fungi. Again, limited availability of photosynthate for root growth, due to higher metabolic requirements of plants with endomycorrhizal fungal associates than without, may have contributed to reduced growth of the host.

Furthermore, competition between endomycorrhizal fungal isolates may have played a role in the negative growth observed. Lopez-Aguillon and Mosse (1987) demonstrated the negative effect of competition between two endomycorrhizal species on the shoot growth of sorghum (Sorghum vulgare Pers.). Following a 5-month cultural period, sorghum plants inoculated with Gigaspora margarita (Becker and Hall) and Glomus fasiculatum (Thaxt.) Gerd. and Trappe were 60-80 and 80-90 percent infected, respectively; whereas, those inoculated with both G. margarita and G. fasiculatum were 10-15 percent and 60-70 percent infected, respectively. In association with this competition was an approximate 28 percent decrease in sorghum shoot dry weight.

Similar results were obtained by Lopez-Aguillon and Mosse (1987) with white clover (Trifolium repens L.). Following a 4-month cultural period, white clover inoculated with G. margarita or G. fasiculatum was 45-60 percent infected. However, white clover inoculated with both G. margarita and G. fasiculatum was 10-40 and 17-35 percent infected by G. margarita and G. fasiculatum, respectively. Shoot dry weights of plants inoculated with either G. margarita, G. fasiculatum or the two in combination were approximately 0.82, 0.80, and 0.55 g/pot, respectively. Moreover, root lengths of plants inoculated with either G. magarita, G. fasiculatum or both were 661, 663, and 410 cm, respectively: In the current experiment, shoot and root growth of eastern cottonwood cuttings may have been reduced due to effects of competition between two or more endomycorrhizal isolates as has previously been reported.

The effect of endomycorrhizal colonization on the nutrition of the eastern cottonwood cuttings during early root development and the effect of competition between endomycorrhizal isolates, in combination with low greenhouse light conditions, may explain the unexpected decrease in growth of inoculated cuttings in this study. However, observations during greenhouse production may not be a good indication of the growth potential of inoculated eastern cottonwood cuttings following outplanting. A benefit which may have been enhanced by endomycorrhizal colonization but not manifested in shoot and root growth measurements is an alteration of root morphology. In the current experiment, significant reduction in root growth rate during the initial 13 weeks of growth, but the lack of a significant effect on root dry weight following the 26-week cultural period, suggests that endomycorrhizal inoculation may have had an early effect on adventitious root system morphology. These potential changes could be beneficial to water and nutrient uptake following outplanting. Further analysis of data collected and additional testing will be necessary to identify such changes and their benefit to outplanting stock.

Literature Cited

Berta, G.; Gianinazzi-Pearson, V. 1986. Influence of mycorrhizal infection on root development in Calluna vulgaris (L.) hull seedlings. In Les mycorhizes: physiologie et genetique. ler SEM, Dijon, 1-5 July 1985. INRA, Paris, 1986.

Blake, T.J.; Tschaplinski, T.J.; Eastham, A. 1984. Stomata1 control of water use efficiency in poplar clones and hybrids. Canadian J. Botany 62: 1344-1351.

Bonner, J.; Galston, A. 1952. Principles of Plant Physiology. W.H. Freeman & Co., San Fransisco, CA. 499 p.

Dixon, R.K. 1988. Seed source and vesicular-arbusculr mycorrhizal symbiont affects growth of Juglans nigra seedlings. New Forests 2: 203-211.

Fege, A.S.; Brown, G.N. 1984. Carbohydrate distribution in dormant Populus shoots and hardwood cuttings. Forest Science 30: 999-1010.

Giovannetti, M.; Mosse, B. 1980. Au evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytopathology. 84:489-500.

Harley, J. L. ; Smith, S.E. 1983. Mycorrhizal Symbiosis. Academic Press Inc., New York, NY. 483 p.

Hselova , H.; Gryndler, M. ; Vancura, V. 1989. Influence of inoculation with VA mycorrhizal fungus Glomus sp. on growth of strawberries and runner formation. Agriculture, Ecosystems and Environment 29: 193-197.

Kormanik, P.P. 1985. Effects of phosphorus and vesicular-arbuscular mycorrhizae on growth and leaf retention of black walnut seedlings. Canadian J. Forest Resources 15: 688-693.

Kormanik, P. P. ; McGraw, A.-C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In Methods and Principles of Mycorrhizal Research. N. C. Schenck (ed.). American Phytopathology Soc., St. Paul, MN. pp. 37-46.

Kormanik, P.P.; Schultz, R.C.; Bryan, W.C. 1982. The influence of vesicular-arbuscular mycorrhizae on the growth and development of eight hardwood tree species. Forest Science 28: 531-539.

Lodge, D.J. 1989. The influence of VA-endo and ectomycorrhizae in Populus and Salix. Plant and Soil 117: 243-254.

Lopez-Aquillon, R.; Mosse, B. 1987. Experiments on competitiveness of three endomycorrhizal fungi. Plant and Soil 97: 155-170.

Melichar, M.W.; Garrett, H.E.; Cox, G.S. 1986. Mycorrhizae benefit growth and development of eastern black walnut seedlings. Northern J. Applied Forestry 3: 151-153.

Nanda, K.K. ; Jain, M.K. ; Malhotra, S. 1971. Ef fect of glucose and auxins in rooting etiolated stem segments of Populus nigra. Physiol. Plant. 24: 387-391.

Navratil, S. ; Rachon, G.C. 1981. Enhanced root and shoot development of poplar cuttings induced by Pisolithus inoculum. Canadian J. Forest Resources 11: 844-848.

Okoro, 0.0. ; Grace, J. 1976. The physiology of rooting Populus cuttings. I. Carbohydrates and photosynthesis. Physiol. Plant. 36: 133-138.

Pope, P.E. 1980. Influence of Glomus fasciculatus mycorrhizae on some physical and chemical characteristics of Platanus occidentalis seedlings. Canadian J. Botany 58: 1601-1606.

Regehr, D.L. ; Bazzaz, F.A.; Boggess, R. 1975. Photosynthesis, transpiration and leaf conductance of Populus deltoides in relation to flooding and drought. Photosynthetica 9: 52-61.

Simmons, G.L. ; Pope, P.E. 1988. Influence of soil water potential and mycorrhizal colonization on root growth of yellow-poplar and sweet gum seedlings grown in compacted soil. Canadian J. Forest Resources 18: 1392-1396.

Snellgrove, R.C.; Splittstoesser, W.E.; Stribley, D.P.; Tinker, P.B. 1982. The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytopathology. 92:75-87.

Tschaplinski, T.J.; Blake, T.J. 1990. Correlation between early root production, carbohydrate metabolism, and subsequent biomass production in hybrid poplar. Canadian J. Botany 67: 2168-2174.

Vozzo, J.A.; Hacskaylo, E. 1974. Endo- and ectomycorrhizal associations in five Populus species. Bulletin Torrey Botanical Club 101: 182-186.