INFLUENCE OF *PISOLITHUS TINCTORIUS* ON NORTHERN RED OAK SEEDLINGS WITH NITRATE FERTILIZATION

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ABSTRACT

Northern red oak seedlings were grown in one-liter containers in a greenhouse for 60, 100, and 140 days. Shortly after seedling emergence, sodium nitrate was applied as the nitrogen source to both *Pisolithus tinctorius* inoculated and non-inoculated seedlings.

Ectomycorrhizae developed in all inoculated treatments and were observed as early as 18 days after acorn planting. Non-inoculated treatments were free of ectomycorrhizae throughout the study. At the termination of each growth period, tree height, tree weight, leaf weight, leaf area, green leaf area, leaf nitrogen weight, stem cross-sectional area, and root weight of mycorrhizal seedlings were not significantly different from non-mycorrhizal seedlings. Leaf nitrogen percent was significantly (α = .05) greater for mycorrhizal trees than for non-mycorrhizal trees after 100 days of growth. Total leaf phosphorus weight and percent were the same for 60-day-old trees for both groups but significantly greater (α = .01) for 100- and 140-day old non-mycorrhizal trees. Averaged mycorrhizal infection rates of root systems were the same for 60- and 100-day-old trees at 11.3% and 10.6% respectively, but significantly higher (α = .05) for 140-day-old trees (26.2%).

INTRODUCTION

Mycorrhizal inoculation studies with seedlings are of interest because of the numerous benefits provided by the symbiotic relationship between the mycorrhizal fungus and the host plant (Marx et al., 1973; Marx et al., 1974). Northern red oak (*Quercus rubra L.*) is a commercially important tree species noted for its poor survival and growth after outplanting (Russell, 1971; Beckjord, 1973). Park (1971) reported that these characteristics are especially evident with many hardwood species in the reforestation of old fields in Southern Ontario and suspected that the poor growth may be due to an absence of mycorrhizal fungi. Many studies have demonstrated that mycorrhizae increase the survival and growth of seedlings in field plantings (Vozzo et al., 1971; Marx et al., 1977).

Production of containerized hardwood seedlings appears to be increasing and they have been studied intensively as a means of improving regeneration success by increasing adaptability of seedlings to specific sites (Tinus et al., 1974). The inoculation of containerized hardwood seedlings may provide an "underground boost" (Bergstrom, 1976) as occurs with many coniferous seedlings. Questions arise as to which mycobiont is best suited to a specific host, what is the best inoculation technique, and what fertilizer amendments are needed to create a mycorrhizal seedling with sufficient size for outplanting. Little work has been reported with inoculation studies with the oaks (Shemakhanova, 1962; Howe, 1964) or northern red oak in particular (Phares, 1964). However, a few have recently been published (Marx, 1977a, 1977b).

The objective of this research was to determine the influence of *Pisolithus tinctorius* (Pers.) Coker and Couch on northern red oak seedlings fertilized with nitrate.

METHODS

An isolate of *P. tinctorius* (#185 from *Pinus taeda L.*, November, 1975) was furnished by Marx[1] and inoculum was prepared using a modification of the methods described by Marx and Bryan (1975). Cultures were grown on petridishes using Hagem's nutrient agar modified by Modess (1941) for a total of 108 days during which reculturing was employed twice. Colonized agar sections were introduced into one-liter flasks containing 800 ml (116 grams dry weight) of peat moss (Wiley milled to pass a 20 mesh screen) and coarse vermiculite mixture (1:30 v/v) wetted with 400 ml of a modified Melin-Norkrans nutrient solution (Norkrans, 1949) containing 5 gm D-glucose as the carbon source and 50 µg thiamine-HCl per liter (solution pH = 5.9). Control-flask inoculum was also prepared except introduced nutrient-agar sections were devoid of fungi. All the flasks were held in darkness at 22 C for 100 days with foil coverings loosened 2 days per week for CO_2/O_2 exchange.

Flask inoculum was tested for contamination and flasks containing contaminated inoculum were discarded. Inoculum from the uncontaminated flasks was removed with tap water onto several layers of cheesecloth, bundled, and rinsed under cool tap water for 2 minutes with frequent squeezings. It was then mixed with peat/vermiculite mixture in a ratio of 1:8 (v/v) to produce the inoculated seedling growing medium. The peat moss was ground and autoclaved prior to mixing with the vermiculite. Control growing medium was produced in the same manner using control-flask inoculum. One liter of inoculated growing medium (145 grams dry weight) was placed in 1-liter plastic cups (14.5 cm x 11 cm dia., 5 small drainage holes totaling 1.045 cm^2) and supplied with 350 ml of a second modification of Melin-Norkrans solution containing .714 gm KH_2PO_4, .214 gm $MgSO_4 \cdot 7H_2O$, .0375 gm NaCl, .0714 gm $CaCl_2$, .357 gm $CaSO_4 \cdot 2H_2O$, and 1.71 ml $FeCl_3$ 1% solution in 1-liter of solution.

Half-sib northern red oak acorns were collected in the autumn from a tree located in Blacksburg, Virginia. The acorns were floated, examined for weevil holes and other damage, and rinsed in a fungicide-water solution (8 ml of 50% Orthocide powder plus 4-liters tap water), and stored in polyethylene bags at 1 C until the spring. At this time, the acorns were rinsed in 5% Clorox solution, rinsed with water, and placed on Clorox solution-soaked paper towels, covered with foil, and germinated for 2 days in darkness at 22 C. Acorns with undamaged radicals 2 cm or less were weighed, planted in the medium-filled pots, and covered with a 1 cm Perlite mulch. Pots were located 15 cm from center to center on a gravel layer rinsed with 20% Clorox on greenhouse benches. Growing medium moisture was kept between .3 and 1.0 bars matric suction as determined by soil moisture retention curves (Kuzmic, 1977) and maintained so that no leaching occurred. Greenhouse environmental conditions were not controlled except for use of cooling fans and latex shading compound. Seedlings were randomly rotated every 3 weeks.

Sixty seedling were fertilized once with a sodium nitrate ($NaNO_3$) solution at the rate of .0266 gm N per seedling (equivalent to 44.5 kgm/Ha on a volume basis, 76 ppm N on a soil solution basis, or 183.4 ppm on a soil medium dry weight basis) 15 days after planting. The 60 seedlings consisted of 30 inoculated with *Pisolithus tinctorius* (Pt.) and 30 inoculated with the control inoculum. From each of these two groups, 10 trees were removed for examination and destructive analyses after 60, 100, and 140 days of growth. Leaf tissue nitrogen and phosphorus contents were determined using standard micro-Kjeldahl and molybdovanadate methods, respectively.

RESULTS

All 30 inoculated (Pt) seedlings were ectomycorrhizal with Pt as confirmed by microscopic examination for Hartig nets, clamp connections, and reisolation techniques. Ectomycorrhizal formation was observed as early as 18 days after acorn planting during the time initial leaves were unfolding and expanding. Ectomycorrhizae were predominantly monopodial and were detected most readily after soaking in cool tap water for 5 minutes. Infection percent was assessed (and cross-checked with microscopic techniques) as the number of infected lateral root tips 7 mm and shorter belonging to the orders of the secondaries and smaller roots divided by the total number of such root tips. Infection percent of the root systems ranged from 1 to 65 percent over all 30 seedlings. Control seedlings were free of ectomycorrhizae. Infection percent means for 60-, 100-, and 140-day-old seedling groups were 11.3, 10.6, and 26.2 respectively. There were no significant differences between 60- and 100-day-old tree groups but the 140-day-old trees had a significantly greater (α = .0222) infection percent.

Table 1 shows the group means for the growth parameters as well as information concerning leaf nitrogen and phosphorus content. At the termination of each growth period, tree height, tree weight, leaf weight, leaf area, green leaf area, stem cross-sectional area and root weight of mycorrhizal seedlings were not significantly different from non-mycorrhizal seedlings.

Total leaf nitrogen weight was not significantly different between mycorrhizal and non-mycorrhizal trees, whereas leaf nitrogen percent was significantly greater (α = .05) for mycorrhizal trees than non-mycorrhizal trees after 100 days of growth. Leaf phosphorus weight and percent were not significantly different between seedling groups after 60 days of growth but were significantly greater (α = .01) for non-mycorrhizal seedlings after 100 and 140 days of growth than mycorrhizal seedlings.

DISCUSSION

The techniques reported herein produced good control over contamination for an inoculation study utilizing open pots. The detection of mycorrhizal formation during leaf expansion was in agreement with Morrison's (1956) observations with ectomycorrhizal formation on silver beech. Although mycorrhizal seedlings did not have significantly less tree biomass, there was a trend in that direction. This trend suggested that production and growth factors which contribute to biomass (height, stem diameter, root weight and leaf weight) were sacrificed to some extent by the competition for carbohydrates by the mycobiont. Although the levels of macronutrients supplied, including nitrogen and phosphorus, appeared to be suitable for growth for both symbionts, it is possible a micronutrient may have limited the growth of the seedlingsdue to the competition of the fungus and perhaps associated micro-organisms. Future studies need to examine the critical levels of micronutrients needed for both the growth of seedlings and associated rhizo-flora.

Other factors may have caused the trend toward the development of smaller mycorrhizal seedlings. For example, oak has nitrate reductase but it is not known if Pt has this enzyme (Marx, Trappe, and Hacskaylo, 1978, personal communication). Bowen (1973) suggested that even though various fungi may not have nitrate reductase the generalization that nitrate anions cannot be utilized or passed on to the host by the fungus should not be made. Unpublished work in the authors' laboratory has shown that Pt utilized nitrate in pure culture but grew slower than when on NH_4CL-nitrogen agar medium and stained the agar brown. Substances secreted by the fungus as possible waste metabolites may not only be toxic to the fungus itself but to the host-oak as well.

Table 1. Growth variable means of mycorrhizal and non-mycorrhizal northern red oak seedlings fertilized with sodium nitrate and evaluated at three stages of development.

Growth Variable	60 days old		100 days old		140 day olds	
	NON-MYCO	MYCO	NON-MYCO	MYCO	NON-MYCO	MYCO
Stem cross-section - mm^2	6.70	6.70	9.08	7.92	8.93	7.67
Tree weight - gm	2.74	2.60	6.68	5.72	7.65	6.72
Tree height - cm	12.2	12.0	15.2	14.8	13.5	12.5
Leaf weight - gm	1.04	1.04	1.45	1.12	1.57	1.49
Leaf area - cm^2	182.	165.	218. *	150.	237.	196.
Green leaf area - cm^2	151.	126.	178. *	112.	212.	160.
Root weight - gm	1.36	1.23	4.63	4.08	5.44	4.69
Root/top ratio	.97	.90	2.23	2.52	2.58	2.38
Leaf N weight - mgm	21.42	22.94	22.31	19.59	29.29	29.78
Leaf N percent - %	2.05	2.18	1.56	1.80*	1.91	2.12
Leaf P weight - mgm	3.59	3.44	6.99**	3.69	7.86**	4.00
Leaf P percent - %	.34	.33	.50**	.35	.51**	.29
N/P ratio	6.09	6.77	3.18	5.47**	3.80	7.53**
Mycorrhizal infection - %	0	11.3	0	10.6	0	26.2 *

* = significant at .05
** = significant at .01

Oak seedlings suffered leaf damage during early growth due to a period of high temperatures in the greenhouse. Inoculated seedlings (with Pt) were damaged more (and significantly so, $\alpha = .05$ for 100-day-old trees), reducing the effective green leaf area for photosynthesis. The leaf damage may be attributed to inadequate root systems to meet the demands of rapid transpirational loss as well as a probable increase in respiration of both roots and fungus in the rhizocylinder. Routien and Dawson (1943) found that mycorrhizal roots of *Pinus echinata* Mill. respire anaerobically at a higher rate than normal roots. Willis and Yemm (1955) found an increase in the respiratory quotient of barley roots within 6 hours after the addition of sodium nitrate. Increased respiration on or about root systems could alter osmotic potential and thus influence water uptake. It is also possible that rapid uptake of readily available nitrate anions by roots and fungus may have increased the succulence of young leaves predisposing them to heat injury.

As anticipated, mycorrhizal seedlings were more efficient in nitrogen uptake than non-mycorrhizal seedlings. However, leaf phosphorus percent was considerably less in mycorrhizal seedlings. It is suggested that non-mycorrhizal seedlings underwent luxury phosphorus consumption while mycorrhizal seedlings may have: (1) shared the phosphorus pool with the mycorrhizae, associated mycelia and microorganisms such that available phosphorus was depleted from the medium and limited for uptake, and/or (2) stored P within the mycorrhizal sheath or fungal vacuoles as suggested by the work done by Harley and his associates (Harley, 1969). Stored P would remain in a "pool" not mixing with other incoming phosphate and could be released to the plant later in time. Since the leaf P% was well above the medium range of .13 to .28 reported for oak species (Walsh and Beaton, 1973) in mycorrhizal seedlings, P probably was not growth limiting.

The nitrogen uptake efficiency of mycorrhizal oak seedlings may be due to the efficiency of mycelia to: (1) extract the added N via its extensive network, (2) extract N from the peat moss (1.85% N) and vermiculite (.011% N), (3) provide a favorable habitat for other micro-organisms (such as the N-fixers), and/or (4) recycle root exudates and degraded root cell substances efficiently. However, leaf N% may have been greater in mycorrhizal seedlings because less N was incorporated into biomass. The competition for N by mycelia and associated micro-organisms for growth would undoubtedly tap the soil N pool. Thus, in order to absorb N for fungal growth as well as provide the seedling with higher leaf N%, ectomycorrhizal oak seedlings were more efficient in N acquisition than non-mycorrhizal seedlings.

CONCLUSIONS

1. The inoculum preparation and inoculation techniques reported herein are reliable for the production of ectomycorrhizal northern red oak seedlings in open pots in the greenhouse.

2. Ectomycorrhizal oak seedlings were more efficient in nitrogen uptake than non-mycorrhizal seedlings.

3. Ectomycorrhizal oak seedlings were in competition with the fungus itself so that leaf P was less than in non-mycorrhizal seedlings.

4. Mycorrhizal seedlings did not differ significantly from non-mycorrhizal seedlings in any of the growth parameters measured except leaf area. Leaf area of non-mycorrhizal seedlings was significnatly greater than mycorrhizal seedlings only at the completion of 100 days of growth.

The future holds promise for the support of studies related to containerized mycorrhizal hardwood seedlings. Not only is experimentation needed in inoculum production, inoculation techniques, and host-mycobiont compatibilities but the fertilizer prescriptions which favor the prolific production of mycorrhizae as well as large, healthy, and vigorous seedlings are necessary for numerous host-mycobiont combinations. Finally, field trials are necessary to test various prescriptions for site-specific plantings.

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