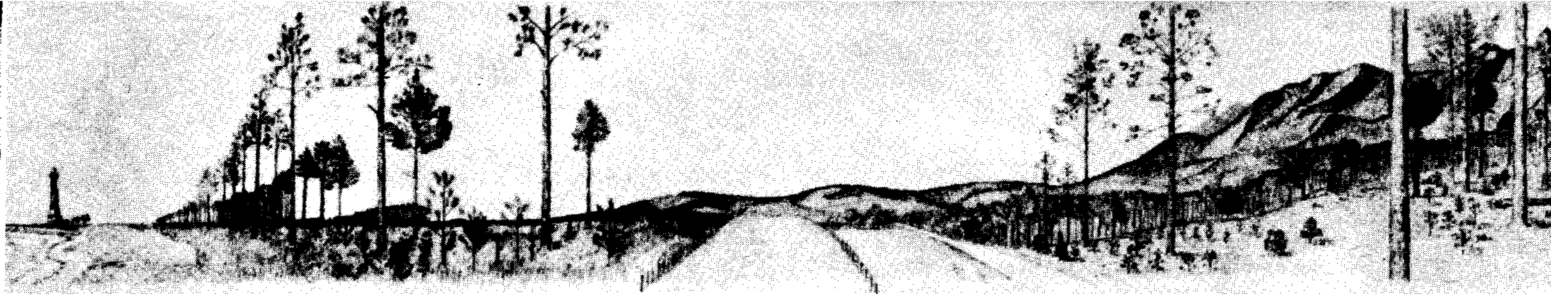

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ECTOMYCORRHIZAL COLONIZATION OF CONTAINER-GROWN NORTHERN RED OAK AS AFFECTED BY FERTILITY

by John L. **Ruehle**[1]

ABSTRACT.-The effects of different fertility regimes on ***Pisolithus tinctorius*** ectomycorrhizal development on northern red oak grown in containers was tested in the greenhouse. Artificial infestation of a milled pine bark-sewage sludge growing medium produced the best ectomycorrhizal development (63 percent) when a nutrient solution with 100 **μg/ml** N and 20 **μg/ml** P was used every 3 weeks. A commercially available nutrient solution (NP-500 **μg/ml**) produced the largest seedlings, those rated suitable for planting, but caused a marked reduction in ectomycorrhizal development (Pt = 20 percent).

The results indicate that the production of large and vigorous northern red oak seedlings in containers with abundant ***Pisolithus*** ectomyconizae is strongly dependent on the levels of available nutrients in the growing medium.

Keywords: ***Pisolithus tinctorius, Quercus rubra.***

Northern red oak (Quercus *rubra* L.) grows best in upland coves and middle and lower slopes having a fine-textured soil and topography favoring a high water table (Core 197 1). Intense competition makes it difficult to reproduce red oak stands on the better sites. There is a high mortality of newly established oak seedlings in this region (Clark and Watt 1971). Although reducing competition helps artificial oak regeneration, mowing and use of herbicides are not feasible in many areas in the uplands within the range of northern red oak (Russell 1971).

Conventional nursery stock has not demonstrated the ability to grow fast enough to compete with woody vegetation on forest sites (Johnson 1979). The advent of containerization in recent years offers a possible means of improving early seedling growth of oaks. Initial trials by Johnson (1974) and Wendel(l979) showed growth rates of containerized northern red oak to be too slow to be competitive. Johnson speculated that a larger container and a longer propagation period offered the best opportunities for improving the growth of outplanted tublings.

The use of ectomycorrhizal fungi ecologically adapted to the planting site in container operations may also improve survival and early seedling growth (Ruehle and Marx 1979). These symbiotic fungi assist the plant in water and mineral uptake by increasing the effective absorbing area of the feeder roots. Good nonmycorrhizal oak seedlings can be grown in containers in the greenhouse because conditions are controlled to minimize stress of all kinds (Tinus and McDonald 1979). However, such seedlings often fail to survive or grow well after planting, especially during a period of environmental stress. Pine seedlings colonized with *Pisolithus tinctorius* survive and grow better on routine reforestation sites than seedlings colonized with *Thelephora terrestris* or other fungi (Marx and others 1977a). Since this symbiont also colonizes oaks, it would be a good candidate for tailoring the roots of oaks before outplanting (Marx 1979). Previous work (Ruehle. unpublished data) has shown that northern red

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oak became colonized with ***P. tinctorius*** when inoculum was added to the growing medium. However, the colonization was erratic and usually quite low (below 5 percent) in these tests where trees received a commercially available soluble fertilizer (NPK at 500 p/m each concentration) every 3 weeks. Before field trials can be conducted to evaluate the performance of containerized seedlings colonized with specific ectomycorrhizae selected to adapt the seedlings to the site, greenhouse experiments must be conducted to determine the optimum growing medium, inoculation techniques, and fertility levels needed to produce acceptable plantable seedlings. Current cultural practices for growing containerized oaks are often not suitable for colonization of roots by certain ectomycorrhizal fungi. Frequent watering of seedlings in containers with poor drainage (growing medium that is too fine often becomes waterlogged) prevents feeder-root development. Lack of feeder roots prevents adequate ectomycorrhizal development. Frequent application of high concentrations of water-soluble fertilizer also prevents mycorrhizal development by many symbiotic fungi (Marx and others 1977b).

The study was conducted to determine the best fertility regime for developing ***P. tinctorius*** ectomycorrhizae on northern red oak grown in containers.

METHODS AND MATERIALS

Milled pine bark used as a growing medium for containerized southern pine seedlings promotes extensive secondary-root development and a high percentage of ectomycorrhizae (Ruehle and Marx 1977), and was used in this study. Processed sewage sludge was added to milled pine bark because it permits less frequent application of soluble fertilizer. Sewage sludge used in this study was obtained from two primary plants in Athens, Georgia, screened and dried 48 hours at 80°C. The nutrient content and organic matter of this sludge was discussed by Berry and Marx (1976). The milled pine bark was fumigated with methyl bromide and then mixed 4: 1 v/v with the processed sewage sludge to create the basic growing medium for this study. Inoculum of ***P. tinctorius*** (isolate 185) prepared according to standard procedures (Marx and Bryan 1975) was dried at room temperature to approximately 20 percent moisture, and mixed with the growing medium at a 1: 15 v/v ratio.

Fifty plywood boxes were constructed with screen bottoms. Each was large enough to hold five Super-45 Spencer-LeMaire Rootrainers® (Spencer-LeMaire Industries Ltd., Edmonton, Alberta, Canada). Each Rootrainer had three cavities; each cavity was 575 cm". After each box was filled with Rootrainers (15 cavities/box), 200 cm" of growing medium was added to each cavity. The growing medium mixed with inoculum was then added (350 cm") to each cavity to fill it to within 3 cm of the top.

A mixed lot of northern red oak seed collected in western North Carolina was Clorox® treated, sorted by flotation, and stored in plastic bags in a refrigerator for 2 months. The seed was planted (one per cavity) in March 1978. Additional seed were planted in trays to provide transplants for cavities lacking seedlings. Three weeks after seeding, those cavities lacking seedlings received transplants from the trays.

A nutrient solution modified from one employed by Marx and others (1977b) and a Rapid-gro® solution (control) was used in this test to provide various levels of N and P (table 1). Treatments assigned to each group of 10 boxes are listed in table 2.

Table 1 .-Modified nutrient solutions for fertility study on northern red oak grown in containers

Element	Source	Concentration
		µg/ml
N	NH_4NO_3	12.5, 25, 50, or 100
P	Na_2HPO_4	5 or 20
K	KC1	40
Ca	$CaCl_2$	40
Mg	$MgSO_4 \cdot 7H_2O$	20
Fe	Chelated Fe 10%	5.5
Mo	$NaMoO_4 \cdot 7H_2O$	.001
cu	$CuSO_4 \cdot 5H_2O$	.006
Bo	H_3Bo_3	.09
Zn	$ZnSO_4 \cdot 7H_2O$	.1
Mn	$MnCl_2 \cdot 4H_2O$	.7

The solutions were added to saturation (≃ 50 ml) to each cavity at 2, 5, 8, 11, 14, and 17 weeks after germination of seedlings. The treatments were completely randomized on the greenhouse bench with each treatment replicated 10 times; each replicate was a box of 15 seedlings.

After 20 weeks, five randomly selected seedlings were removed from each box and the growing medium was gently washed from the roots.

Table 2.-Treatments for fertility study on northern red oak grown in containers, by nutrient concentration

Treatment	Concentration N	Concentration P	K
	....$\mu g/ml$		
MNS[1]			
1	12.5 (N_1)	5 (P_1)	40
2	25 (N_2)	5 (P_1)	40
3	50 (N_4)	20 (P_4)	40
4	100 (N_8)	20 (P_4)	40
Rapidgro®	500	500	500

[1]MNS—Modified nutrient solution with various levels of NPK.

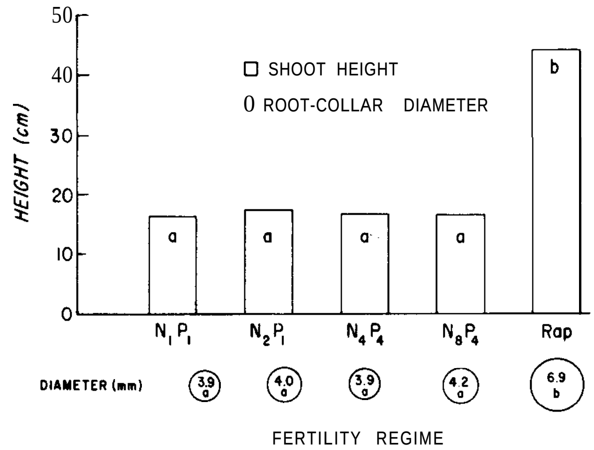

Figure 1 .-Shoot height and root-collar diameter of northern red oak seedlings at 15 weeks under five fertility regimes. Bars or circles bearing the same letter are not significantly different (P = 0.05).

Height, root-collar diameter, and fresh top and root weight were determined for each seedling. The roots were visually assessed for degree of ectomycorrhizal development.[2]

Tops and roots of 10 seedlings per replicate wereovendried at 80°C for 60 hours and weighed. Leaves were removed and analyzed for total N by the Kjeldahl method, and for the other elements by dry-ash methods (Wells and others 1973).[3]

RESULTS

Seedlings receiving Rapidgro® produced greater height and root-collar diameter than those receiving the modified nutrient solutions (fig. 1). There was no difference in the two parameters among seedlings receiving the modified nutrient solutions. Top dry weight followed the same pattern between treatments with seedlings receiving the Rapidgro® solution producing four times more top dry weight than those receiving other nutrient solutions (fig. 2). Seedlings fertilized with the Rapidgro® solution also had the greatest amount of root dry weight. Seedlings receiving N_8P_4 nutrient solution had significantly greater root weight than those receiving treatment 1 or Rapidgro®.

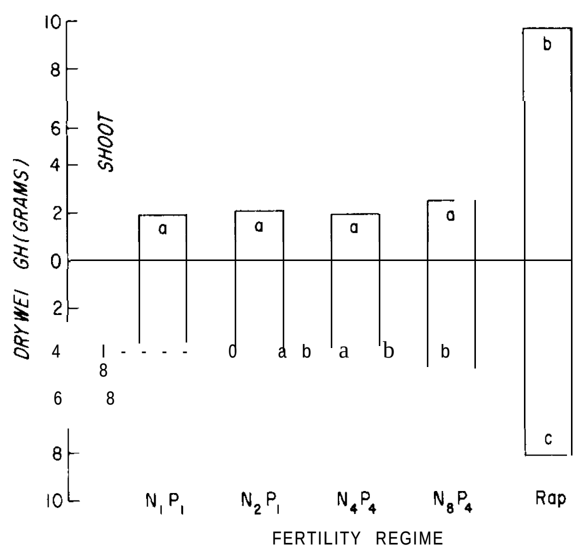

Figure 2.-Shoot and root dry weight under five fertility regimes. Bars bearing the same letter are not significantly different (P = 0.05).

The percentage of N in the foliage related well with the increasing amounts of N in the nutrient solutions (table 3). The level of N in the foliage of seedlings receiving Rapidgro® was quite high, probably approaching the luxuriant consumption range. The level of foliar P was slightly affected by fertility regime but levels of K, Ca, Mg, and Fe were not.

The highest percentage of ectomycorrhizae developed on seedlings in N_4P_4 and N_8P_4 treatments (table 4); significantly less formed on seedlings receiving the highest levels of N and P (Rapidgro®). These findings agree with those of

[2]Ectomycorrhizal assessments were made by Donald H. Marx, Institute for Mycorrhizal Research and Development, Forestry Sciences Laboratory, Athens, Georgia.

[3]Analyses of foliar samples were performed by Carol G. Wells, USDA Forest Service, Forestry Sciences Laboratory. Research Triangle Park. N.C.

Table 3.-Foliar analysis of **15-week-old** northern red oak grown under five fertility regimes in containers,' by nutrient

Treatment[2]	N	P	K	Ca	Mg	Fe
	....	....	....	*Percent*	....	....
N_1P_1	0.89d[3]	0.46b	1.18a	0.93a	0.23a	0.01a
N_2P_1	.92cd	.47b	1.25a	.91a	.22a	.01a
N_4P_4	1.03c	.53a	1.23a	1.02a	.25a	.01a
N_8P_4	1.17b	.44b	1.12a	1.00a	.22a	.01a
Rapidgro®	2.02a	.42b	1.00a	.92a	.25a	.01a

'Values are means of 10 replicates; each replicate was a composite of leaves from 10 seedlings.

[2]**Concentrations** of nitrogen as **μg/ml** – Nl = 12.5, N2 = 25, N_4 = 50, N_8 = 100; phosphorus as **μg/ml** – Pl = 5, P_4 = 20; **Rapidgro®** – NPK = 500 **μg/ml** each.

[3]**Means** within columns not followed by the same letter are significantly different (**P** = 0.05).

Table 4.-Percentage of seedlings with **ecto-mycorrhizae** and degree of ectomycorrhizal development by *Pisolithus tinctorius* **(Pt)**[1]

Treatment[2]	**Seedlings with Pt**	**Pt ecto-mycorrhizae**
	.... *Percent*	....
N_1P_1	99	37.0b[3]
N_2P_1	100	40.7b
N_4P_4	100	58.6c
N_8P_4	100	63.5c
Rapidgro®	89	20.7a

'Values are means of five seedlings from each of 10 replicates.

'Concentrations of nitrogen as **μg/ml** – N 1 = 12.5. N2 = 25. N_4 = 50, N_8 = 100: phosphorus as **μg/ml** – P1 = 5. P_4 = 20: **Rapidgro®** – NPK = 500 **μg/ml** each.

[3]**Means** not followed by the same letter are significantly different (**P** = 0.05).

Marx and others (1977b) working with *P. tinctorius* on pine seedlings. Naturally occurring ectomycorrhizae (mostly *Thelephora terrestris)* formed approximately 5 to 10 percent of the total mycorrhizal development on all seedlings.

CONCLUSIONS

Northern red oak seedlings will grow to plantable size in containers filled with 730 **cm³** of pine bark-sludge growing medium in **3½** to 4 months when fertilized with **Rapidgro®** every 3 weeks. However, ectomycorrhizal development by *Pisolithus tinctorius* is less than satisfactory under this fertility regime. More *Pisolithus* **ecto-mycorrhizae** developed with a balanced nutrient solution with lower concentrations of N and P. Treatments 3 (**N_4P_4**) and 4 (**N_8P_4**) produced seedlings with excellent ectomycorrhizal development under the conditions of this study. Unfortunately, seedlings under these fertility regimes were still slightly chlorotic (2.5 **GY/7.5/5/9**) compared to the dark-green foliage on plants receiving **Rapidgro®** (7.5 **GY/4.5/5**).[4] Seedlings under treatment 3 or 4 also lacked appropriate stem height and root collar diameter. **Tinus** and McDonald (1979) state that container-grown bur oak (*Quercus macrocarpa* Michx.) should be 20 to 25 cm tall before outplanting. Northern red oak probably should be at least this tall before planting, and possibly up to 35 to 40 cm, the height of seedlings receiving **Rapidgro®**.

Work is being continued to find the fertility regime that will produce northern red oak in containers having the robust tops and roots of seedlings receiving abundant N and P and the *P. tinctorius* colonization of those receiving less amounts of N and P. The use of sewage sludge also contributes approximately 10 to 20 **μg/ml** of N and P to the fertility regime when used in the growing medium. A chemical analysis of the growing medium before and after the addition of nutrient solutions needs to be done to define the actual levels of available N and P.

[4]**Foliage-color** rating with Munsell color charts for plant tissue **(hue/value/chroma).**

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