THE INCIDENCE OF ECTOMYCORRHIZAE BY *PISOLITHUS TINCTORIUS* ON *QUERCUS RUBRA* SEEDLINGS FERTILIZED WITH SODIUM NITRATE AND AMMONIUM CHLORIDE

by

Peter Rains Beckjord

Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

in

Forestry and Forest Products

APPROVED:

Robert E. Adams, Co-Chairman

David Wm. Smith, Co-Chairman

Orson K. Miller, Jr.

David C. Martens

John M. Skelly

November, 1978

Blacksburg, Virginia 24061

ACKNOWLEDGEMENTS

First, through this message, I am deeply grateful for the love and comfort my wife, , has provided me during this three and a half year period of doctoral studies.

I extend my appreciation to my major professors, Dr. Robert E. Adams and Dr. David Wm. Smith, for their guidance and support throughout the course of my studies.

I also thank Dr. Orson K. Miller, Jr. for his gentle but strong encouragement and Dr. David C. Martens and Dr. John M. Skelly for their help and advice during my graduate studies.

I also thank

, for their contributions, suggestions, and assistance during the course of my graduate research activities.

Furthermore, I am grateful for the understanding and interests my parents, , have extended to me and my family.

Finally, I extend my love and devotion to my two sons, and , who have inspired me to become a happier man; and, it is to my sons that I dedicate this dissertation.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (continued)

Page

LIST OF TABLES (continued)

Page

THE INCIDENCE OF ECTOMYCORRHIZAE BY *PISOLITHUS TINCTORIUS* ON *QUERCUS RUBRA* SEEDLINGS FERTILIZED WITH SODIUM NITRATE AND AMMONIUM CHLORIDE

INTRODUCTION

Studies with mycorrhizal seedlings are of interest because of the benefits provided by the symbiotic relationship between the mycorrhizal fungus and the host plant (Marx and Barnett, 1974). A critical factor in the successful establishment of seedlings in the field is the relationship between the roots of planted seedlings and the new soil environment. Use of mycorrhizal seedlings increases the success in seedling establishment because the effective surface area of the infected root may be increased up to one hundred fold, thereby increasing nutrient and water absorption (Marx and Bryan, 1973). Survival and growth are also enhanced because mycorrhizae and associated mycelia increase root longevity, protect against mechanical and chemical damage, increase drought resistance, and improve soil aggregation (Hatch, 1937; Hacskaylo, 1971; Bowen, 1973; Marx, 1973; and Mikola, 1973).

Ectomycorrhizae are common among the families Pinaceae, Betulaceae, Salicacae and Fagaceae. Ectomycorrhizae of the pines have been studied extensively (Marx, 1976; Marx *et al*, 1976); but, the ectomycorrhizal relationships of oaks have not received as much attention despite the fact that oaks are generally mycorrhizal in forest soils (Shemakhanova, 1962). The culturing of mycorrhizal seedlings is one method of

determining the capacity of fungi to form mycorrhizae and the contribution of the fungi to absorption of nutrients. To date, little work of this type has been done with oak (Howe, 1964, Phares, 1964).

Northern red oak (Quercus rubra L.) is a commercially important tree species noted for its poor survival and slow growth after outplanting (Russell, 1971). Park (1971) also noted poor survival and slow growth with hardwood seedlings (basswood, oak, elm, hickory and others) in the reforestation of old fields in southern Ontario. He hypothesized that the growth of seedlings may be limited by an absence of mycorrhizae.

Pisolithus tinctorius (Pers.) Coker and Couch, a gastromycete, is an ectomycorrhiza-forming fungus. This species has been reported to occur naturally in 33 countries and 38 states of the United States (Marx, 1978). P. tinctorius has been proven to be a mycobiont with at least 45 tree species including Q. rubra. Marx (1978) stated that this fungal symbiont has "great potential for practical forestation efforts" because of its ease in culturing, availability, ability to improve seedling survival and growth, near world-wide distribution on a variety of sites, and its broad host range.

Mycorrhizal oak seedlings may offer a solution to some of the problems associated with the establishment of oak plantings on old field sites. However, several questions need to be answered concerning the production of mycorrhizal northern red oak seedlings. These include: (1) What inoculation technique is most effective in promoting mycorrhizal incidence? (2) What form and amount of nitrogen fertilizer

is compatible with promoting mycorrhizal incidence? (3) When should fertilizer be applied in order to promote mycorrhizal incidence, seedling vigor, and seedling growth?

In order to better understand the effects of nitrogen fertilization on the incidence of ectomycorrhizae by Pisolithus tinctorius on Quercus rubra seedlings, a greenhouse study was initiated to:

I. Determine the effects of amount and application time of sodium nitrate and ammonium chloride on P. tinctorius inoculated and non-inoculated Q. rubra seedlings, and on ectomycorrhizal incidence on the inoculated seedlings.

II. Evaluate the development at various ages of P. tinctorius inoculated and non-inoculated Q. rubra seedlings fertilized with sodium nitrate and ammonium chloride.

III. Evaluate ectomycorrhizal incidence at various ages on P. tinctorius inoculated Q. rubra seedlings fertilized with sodium nitrate and ammonium chloride.

LITERATURE REVIEW

Doak (1955) studied the effects of deficiencies of nitrogen, phosphorus, and potassium on seedlings of bur oak (Quercus macrocarpa Mich.) in sand cultures free from mycorrhizae and in humus cultures with mycorrhizal fungi present. Doak used ammonium nitrate and calcium nitrate as the nitrogen source. Although the fungus was not identified, he found that mycorrhizal development was inhibited in humus cultures by the addition of a complete fertilizer. Bond (1971) attempted to study the effect of seven species of mycorrhizal fungi on potassium uptake by seedlings of willow oak (Quercus phellos L.) in monoxenic culture using vermiculite and feldspar as potassium sources. None of the seedlings became mycorrhizal and the study was unsuccessful. Miller (1973) inoculated white oak (Quercus alba L.) seedlings with three fungal species, Russula veternosa Fr., Amanita cothurnata Atk., and an unknown. Out of the three fungal species tested, none were successful in producing mycorrhizae. Studies with ectomycorrhizal white oak seedlings infected with Pisolithus tinctorius and outplanted on Kentucky coal spoils are underway to evaluate seedling survival and growth potential (Maronek and Hendricks, 1978, personal comm.).

Even fewer reports are available concerning studies of mycorrhizal development of northern red oak in relation to soil nutrient status. Phares (1964) found that inoculated northern red oaks grown in

containers from seed for one year did not have significantly different total seedling dry weights than non-inoculated seedlings. But in another study Phares (1964) found one-year-old inoculated seedlings had 12% greater dry weight than non-inoculated seedlings. Foliage of inoculated seedlings had 14% more N, 30% more P and 5% more K than non-inoculated seedlings. Pisolithus tinctorius was isolated from Quercus spp. by Klyushnik (1952) and Marx (1977a, 1977b) successfully inoculated northern red oak by this fungus. Grand (personal comm. 1976) stated that the infection rate of northern red oak by P. tinctorius should range from 30-40% and Melhuish (personal comm. 1976) stated that there should be few problems in obtaining successful infection of northern red oak by this fungal species. Ruehle (1978) (personal comm.) has grown northern red oak inoculated with P. tinctorius in a greenhouse for four months. Using an artificial medium containing 20% sewage sludge, various amounts of ammonium nitrate and phosphorus were applied every three weeks. His unpublished data indicate that with adequate soil nitrogen and phosphorus, large, healthy oak seedlings with 50% or more ectomycorrhizal root systems can be produced.

Carrodus (1966) found that excised beech (Fagus sylvatica L.) mycorrhizae absorbed ammonium more readily than nitrate. Carrodus did not identify the fungus nor is it known if the fungus possessed nitrate reductase. Bowen (1973) stated that even though several species of fungi may not possess nitrate reductase, the generalization that mycorrhizae cannot absorb and pass nitrate to the host should not

be made. To clarify this question, Trappe (1978, personal comm.) is presently engaged in studies related to the identification of fungal species that contain nitrate reductase.

Taber and McFee (1972) studied the influence of nitrate and ammonium on phosphorus uptake by roots and shoots of mycorrhizal and non-mycorrhizal Pinus radiata D. Don (Monterey Pine) seedlings. Six-week-old seedlings grown in a mixture of 50% peat and 50% silt loam (pH 6.3) were pretreated for 10 days in a nutrient solution and then pretreated an additional 24 hours with either 7mM NaCl (control) or 100 ppm N as either sodium nitrate or ammonium chloride. All root systems were then immersed for 6 hours in a 2 ppm solution of phosphorus containing ^{32}P. Regardless of the pretreatment, mycorrhizal seedlings had the same phosphorus content in both the roots and shoots whereas non-mycorrhizal seedlings had significantly less phosphorus in roots and shoots with the ammonium pretreatment than with the nitrate pretreatment. Ammonium did stimulate more phosphorus uptake in roots and shoots of mycorrhizal pines than non-mycorrhizal pines. Non-mycorrhizal pines treated with nitrate had more phosphorus in roots and shoots than mycorrhizal pines. Taber and McFee concluded that ammonium treatment of mycorrhizal seedlings increased phosphorus uptake because of an increase in metabolic rate of mycorrhizal seedlings when compared to non-mycorrhizal seedlings.

Previous work by McFee and Stone (1968) had indicated no such depressive effect of ammonium on phosphorus uptake in non-mycorrhizal Monterey pine seedlings. Taber and McFee tested various levels of

ammonium on phosphorus uptake, Results indicated that at ammonium levels ranging from 6 ppm to 50 ppm nitrogen, phosphorus uptake was significantly enhanced leading to the hypothesis that high ammonium (100 ppm N) applications were insufficiently utilized in growth functions and interfered with phosphorus absorption.

Richards and Wilson (1963) found that mycorrhizal development with caribbean pine (Pinus caribaea Mor.) was reduced by increasing the level of soil nitrate. Richards (1965) initiated a study to investigate the influence of soil nitrate on mycorrhizal development in loblolly pine (Pinus taeda L.). He applied four levels of nitrogen as sodium nitrate to containerized transplanted seedlings: nil, 20.5, 41.1, and 82.1 kg/ha. Seedlings were removed 21 and 45 weeks after fertilizer applications and root systems evaluated for mycorrhizal development. He found the increments of sodium nitrate applied produced a progressive reduction in the ratio of mycorrhizal seedlings to non-mycorrhizal seedlings at 21 weeks. There was no significant difference in mycorrhizal percent, (the number of mycorrhizae expressed as a percentage of the total number of short roots per seedling) at 20.5 and 41.1 kg N/ha. Richards suggested that inadequate replication or lack of precision in the sampling technique may have caused the lack of significant differences in mycorrhizal percent. At 21 weeks, mycorrhizal development varied inversely and linearly with the total N content of the seedling roots. A highly significant linear correlation existed between the mycorrhizal infection percent (arcsin multiplied by the square root of infection percent) and

root nitrogen percent (as 1+log N%) which was consistent with a previous study with P. caribaea. Richards extrapolated his regression equations and suggested that mycorrhizal development would be completely inhibited when root nitrogen concentration was 2.32% in P. taeda and 2.31% for P. caribaea. Richards stated that the negative correlation between nitrogen and mycorrhizal percent was evidence of a positive correlation between carbohydrate-nitrogen balance in the roots and mycorrhiza development. He further assumed from work by Bjorkman (1942) and Harley and Waid, (1955) that extensive development of mycorrhizae occurs when carbohydrate synthesis exceeds carbohydrate utilization and soluble carbohydrate accumulates in the roots. Richards concluded that a moderate deficiency of nitrogen, severe enough to limit the synthesis of protein but not cause chlorosis, could retard growth of the seedling and permit the accumulation of soluble sugars in the root tissues and hence mycorrhizal development could be expected to increase.

Marx et al (1977) provided evidence that high levels of nitrogen and phosphorus in the growing medium reduced the nonreducing sugar, sucrose, in the root. This effectively reduced the susceptability of loblolly pine to ectomycorrhizal infection by P. tinctorius. Marx and his co-workers support Bjorkman's hypothesis that the susceptability of short roots to ectomycorrhizal infection is increased as the concentration of soluble root sugar increases.

Park (1971) conducted a study of some ecological factors affecting the formation of Cenococcum mycorrhizae on basswood (Tilia americana L.)

in southern Ontario. He collected 639 seedlings with their roots and adhering soil from 22 forests and analyzed the seedlings for percent mycorrhizal infection and the soil for nitrate, ammonium, pH, and clay content. He found a significant negative correlation between infection and nitrate. The range of nitrate was 2.2 ppm to 11.6 ppm N. No correlation was evident between infection and the ammonium concentrations which ranged from 2.6 to 5.6 ppm N. There was also an inverse correlation between pH and infection. Seedling infection percents ranged from 9 to 53.

Theodorou and Bowen (1969) studied the influence of pH and nitrate on the colonization of four week-old P. radiata seedlings by the mycorrhizal fungus, Rhizopogon luteolus Fr. and Nordh. Using a podzolized soil at adjusted pH levels of 5.0 and 8.0, sodium nitrate solutions were added to give 12 and 115 ppm nitrate. Monoxenic culturing followed using germinating pine seed and fungus inoculum discs. Results indicated that there was a marked inhibition of fungal colonization at pH 8 with both nitrate levels when compared to roots taken from the pH 5 soils. Increasing nitrate from 12 to 115 ppm at pH 5.0 significantly decreased colonization of roots. Theodorou and Bowen concluded that although high nitrate may retard fungal growth in the rhizosphere, colonization and subsequent mycorrhizal formation would occur as long as the soil remained acidic. Furthermore, the poor infection in alkaline conditions was probably due to the inhibition of the infection process by high soil nitrate as suggested by Richards (1961) and Richards and Wilson (1963).

In a summary of the carbohydrate physiology of ectomycorrhizae, Hacskaylo (1973) stated that since ectomycorrhizal fungi depend primarily on the roots of the host plants for carbohydrates, the status of the soluble carbohydrates in the roots must be considered as a major factor in the formation of ectomycorrhizae and the growth of vegetative hyphae. The factors that alter the availability of root sugars such as photosynthetic activity, availability of soil nutrients, especially nitrogen and phosphorus, are of major importance to carbohydrate metabolism of associated fungi.

Menge (1975) conducted a greenhouse study to determine the effects of nitrogen and phosphorus combinations on the development of mycorrhizal tips on containerized P. taeda seedlings. He found significantly fewer mycorrhizal tips (10-20% less) on 120-day old seedlings that were fertilized with 56 kg N/ha and 112 kg N/ha + 22 kg P/ha than on seedlings planted in a non-fertilized mix. The forest soil was analyzed before the study and contained no nitrate. The seedlings developed mycorrhizae by several species of fungi which evidently were present in the soil as either spores and/or mycelium. Menge also fertilized plots in young P. taeda plantations and used soil/root core sampling techniques to assess the effects of fertilizer application rate on mycorrhizal development. The data showed that the fertilizer effects on mycorrhizal development in the plantation study were similar to the results from the greenhouse study. Menge's study supports the conclusions of other investigators (Hatch, 1937;

Bjorkman, 1942; Harley and Waid, 1955; Richards and Wilson, 1963; Richards, 1965; and Park, 1971) that high levels of nitrogen fertilizers inhibit the development of mycorrhizae.

PROCEDURES AND METHODS

Inoculum Synthesis and Media Preparation:

An isolate of *P. tinctorius* (#185 from *Pinus taeda* L., Nov., 1975) was furnished by Marx[1] in August, 1976. Inoculum was prepared using a modification of the methods described by Marx and Bryan (1975). Ten starter plates were prepared with 20 ml per plate of Hagem's agar modified according to Modess (1941). The fungus was grown 43 days at 22°C in darkness (Appendix Table 1). One square centimeter discs were removed from the outer edge of the fungal mass and centered on 36 fresh plates. These cultures grew 60 days at 22°C in darkness. The outer 3 cm edge of each plate was divided into 6 sections with 3 sections being placed on each of 72 fresh plates. These transition plates were held for 5 days, then used to infest a peat moss-vermiculite substrate.

Seventy-two, 1-liter Erlenmeyer flasks were filled with an 800 ml (116 gm) mixture of ground peat moss (Wiley milled to pass a 20 mesh screen) and coarse vermiculite (1:30 v/v). Four hundred ml of a modified Melin-Norkrans (MN) nutrient solution (Norkrans, 1949) containing 5 gm D-glucose as the carbon source and 50 ugm thiamine-HCl per liter (solution pH = 5.9) (Appendix Table 1) were added to each

---

[1]Marx, D., Institute Director, Southeastern Forest Experiment Station, Forest Sciences Laboratory, Athens, Georgia 30602.

flask. Flasks were stoppered with cotton, covered with aluminum foil, and autoclaved 30 minutes at 15 psi and 121°C. After cooling, the substrate was infested with 3 transition fungal/agar sections. Sterile nutrient/agar sections were introduced into control substrate. All the flasks were held in total darkness at 22°C for 100 days. During production of the inoculum, foil coverings were loosened 2 days per week to allow for CO_2/O_2 exchange. Fungal hyphae penetrated the substrate producing bright mustard yellow rhizomorphs and prolific mycelial growth. Sclerotia were observed in ten flasks. Substrate from all the flasks was independently tested for contamination by culturing 1 cc of the substrate on Hagem's nutrient agar. Contaminated substrate was discarded (5 flasks from each treatment).

Inoculum was rinsed from the flasks with tap water onto several layers of cheesecloth, then 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 infested growing medium. The peat moss was autoclaved prior to mixing with the vermiculite. Non-infested growing medium was produced in the same manner except that control substrate was incorporated in the peat/vermiculite mixture. One liter of growing medium (145 gms dry wt.) was placed in 1-liter plastic containers (14.5 cm x 11 cm dia., perforated with 5 small drainage holes)[1] and supplied with second modification of MN solution (Appendix Table 1) at the rate of 350 ml per container.

---

[1]Drainage holes totaling 1.05 cm^2.

Seed Planting:

Quercus rubra acorns were collected under a tree located[1] in Blacksburg, Virginia during September, 1976. The acorns were floated, examined for weevil holes and rinsed in a fungicide and water solution (8 ml of 50% Orthocide powder + 4 liters tap water), and stored in polyethylene bags at 1°C. On April 6, 1977, acorns were rinsed in 5% Clorox solution, rinsed again with water, placed on paper towels soaked with the Clorox solution, covered with foil, and germinated for 2 days in darkness at 22°C. Acorns with undamaged radicals were weighed, planted in the containers and covered with a 1 cm Perlite mulch. The containers were located on greenhouse benches 15 cm from center to center on a gravel layer rinsed with 20% Clorox.

Care of Seedlings and Environmental Conditions:

Growing medium moisture was kept between 0.3 and 1.0 bars matric suction (approximately 50-75 ml tap water added every 3 days). The moisture content was determined by soil mositure retention curves and controlled by weight differences due to water loss. Greenhouse temperatures varied from 10°C to 43°C. The exterior of the greenhouse was sprayed with shading compound to reduce interior radiation intensity.

---

[1] 20.7m S8°W from the SW corner of Price Hall.

Study Design and Statistical Procedures:

The study design was a 2 X 2 X 2 X 4 X 3 (five factor) factorial. The study consisted of 2 fungus treatments, 2 nitrogen forms, 2 application times, 4 nitrogen levels, and 3 growth periods equalling 96 treatment combinations each of which was replicated ten times. Each replication of all treatment combinations was a block. The study, therefore, consisted of 960 seedlings and was conducted using a randomized complete block design where 320 seedlings were removed for examination, measurement, and destructive sampling at the completion of 60, 100, and 140 days of growth. Table 1 shows the study design. The first factor was the seedling treatment, P. tinctorus inoculated or non-inoculated seedlings. The second factor was the nitrogen fertilizer supplied, as either sodium nitrate ($NaNO_3$) or ammonium chloride (NH_4Cl). The fertilizer treatments were further divided according to time of application; early (applied 15 days after planting) or late (applied 40 days after planting). The fourth factor consisted of four nitrogen application levels; control (0.0 gms N), low nitrogen (.0133 gms N), medium nitrogen (.0266 gms N), or high nitrogen (.0532 gms N). The fifth factor was the length of the time the seedlings were grown; 60 days, 100 days, or 140 days.

Data were analyzed using the Analyses of Variance (ANOVA) and Duncan's Multiple-Range Tests (Barr et al, 1976). Statistical

Table 1. Study design for Pisolithus tinctorius inoculated and non-inoculated greenhouse-grown Quercus rubra seedlings fertilized with sodium nitrate and ammonium chloride at four levels of nitrogen applied at two times and analyzed after three periods of growth.

Seedling treatment	Nitrogen fertilizer supplied	Nitrogen applied per container (gms)	Day fertilizer applied after planting	Number seedlings removed at the end of each growth period		
				60 days	100 days	140 days
INOCULATED WITH PISOLITHUS TINCTORIUS	NH_4Cl	.0000	15(twb)[1]	10	10	10
			40(twb)	10	10	10
		.0133	15	10	10	10
			40	10	10	10
		.0266	15	10	10	10
			40	10	10	10
		.0532	15	10	10	10
			40	10	10	10
	$NaNO_3$	.0000	15(twb)	10	10	10
			40(twb)	10	10	10
		.0133	15	10	10	10
			40	10	10	10
		.0266	15	10	10	10
			40	10	10	10
		.0532	15	10	10	10
			40	10	10	10
NON-INOCULATED	NH_4Cl	.0000	15(twb)	10	10	10
			40(twb)	10	10	10
		.0133	15	10	10	10
			40	10	10	10
		.0266	15	10	10	10
			40	10	10	10
		.0532	15	10	10	10
			40	10	10	10
	$NaNO_3$	.0000	15(twb)	10	10	10
			40(twb)	10	10	10
		.0133	15	10	10	10
			40	10	10	10
		.0266	15	10	10	10
			40	10	10	10
		.0532	15	10	10	10
			40	10	10	10
Totals				320	320	320

[1](twb) = tap water blank.

correlations were performed to illucidate possible relationships among selected variables.

Fertilization and Media Nutrient Status:

No attempt was made to standardize the amount of sodium and chlorine among the treatments. All controls received a tap water blank. All other containers received nitrogen amendments in 50 ml solutions. Growing medium including the base nutrient solution (2nd modification of MN excluding the various nitrogen sources) was tested by the VPI & SU Agronomy Lab Testing Services for pH and base nutrient status (Table 2).

Prior to seed planting, growing medium pH was determined for all fertilizer treatment combinations. The range of the pH was 5.3 to 5.5 regardless of nitrogen source or level. The nutrient status for nitrogen and phosphorus was also determined for the growing media based on the media solution, media dry weight, and the weight and volume of an acre-furrow-slice[1] (Table 3).

Data Collection Procedures:

At the end of each growing period, the following data were collected:

1. Stem diameter 2 cm above the root collar to nearest .01 mm.
2. Leaf area to nearest mm^2.

---

[1]Standard acre-furrow-slice is one acre of soil taken to the depth of 6 2/3 inches; the volume is 24,219 ft^3 with dry soil weight of 2 x 10^6 lb. The bulk density is 1.323 gm/cc.

Table 2. Nutrient status of the growing medium including base nutrient supplement used for growing Quercus rubra seedlings. Analyses performed by VPI & SU Soil Testing Laboratory.

Extractable nutrient	kg/ha	Agronomic rating
Phosphorus (P)	109	High +
Potassium (K)	259	High
Calcium (Ca)	656	Low +
Magnesium (Mg)	266	Very high
Soluble salts	511	Medium -

Table 3. Quantity of nitrogen and phosphorus added to growing medium used to grow Quercus rubra seedlings based on medium solution (350 ml), medium dry weight (145 gms), and an acre-furrow-slice.[1]

Quantity of nitrogen or phosphorus applied per container.	Concentration of nutrient in the medium based on solution weight and medium dry weight.		Equivalent weight per hectare of nutrient added to the medium based on the weight and volume of an acre-furrow-slice.	
	Solution weight ppm	Medium dry weight ppm	Weight basis kg/ha	Volume basis kg/ha
Control N	0	0.0	0	0.00
Low N	38	91.7	204	22.25
Medium N	76	183.4	408	44.50
High N	152	366.8	816	89.00
Phosphorus .057 gm	163	393.3	874	96.00

[1]Acre-furrow-slice bulk density is 1.323 gm/cc which is 9.125 times greater than the growing medium bulk density of .145 gm/cc.

3. Green leaf area percent by estimate.
4. Ectomycorrhizal incidence percent per root system.
5. Root dry weight to nearest .01 gm.
6. Total seedling dry weight to nearest .01 gm (roots, stem, and leaves).
7. Leaf nitrogen and phosphorus percents.
8. Leaf nitrogen and phosphorus weights.

Average leaf color of seedlings in each treatment group was noted using Munsell color charts.[1] Leaf areas were measured by an Automatic Area Meter, Type AAM -5, Hayashi Denko Co., LTD., Tokyo, Japan. Visual estimates of green leaf tissue to nearest 10% were checked by the area meter once dead tissue was separated from the green tissue. All tissue was dried for 48 hours at 62°C for dry weight measurements. Roots were carefully washed with cool tap water and examined in a black bowl filled with water for ectomycorrhizal incidence estimates.

Ectomycorrhizal formation by P. tinctorius was confirmed by microscopic examination of the Hartig net and septate hyphae. P. tinctorius was isolated from excised ectomycorrhizae from five seedlings picked at random. Mycorrhizal incidence percent was defined 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 per root system.

---

[1]Munsell Color Company Inc., 2441 North Calvert Street, Baltimore, Md.

Leaves were oven dried, ground in a Wiley-Mill to pass a 20-mesh screen, re-dried, and cooled 30 minutes in dessicators prior to weighing 0.2 gm samples for nitrogen and phosphorus analyses. Nitrogen content was determined by a modified micro-Kjeldahl technique (Peterson and Chesters, 1964). Phosphorus content was determined by the molybdivanadophosphoric acid method using a Baush and Lomb Spectronic 21 at a wavelength of 470 nm (Kitson and Mellon, 1944).

After the removal of the seedlings from containers at the end of 100 and 140 days of growth, the residual medium was mixed within each container and samples were taken for individual pH determinations using a 1:2.6 v/v fresh soil to water mixture. Seedlings that flushed during the season were noted. Roots growing out of drainage holes were removed weekly and discarded.

RESULTS

I. Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings

Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings (fertilized and control seedlings grouped) at the End of Each of Three Growth Periods:

Seedlings with ectomycorrhizae were classified as mycorrhizal seedlings regardless of the number of ectomycorrhizae present. The approximate minimum numbers of ectomycorrhizae counted per seedling ranged between 20-30. Seedlings with these few ectomycorrhizae generally had a mycorrhizal incidence between 1% and 5%.

Means for the growth variables for mycorrhizal and non-mycorrhizal seedlings are presented in Table 4 for three growth periods. Data from treatments involving type of nitrogen fertilizer, time of fertilization, and level of nitrogen application were pooled for this analysis. These data indicate that leaf area for mycorrhizal seedlings was significantly less than for non-mycorrhizal seedlings at the end of all three growth periods. Green leaf area percent[1] for mycorrhizal seedlings was significantly less by 9%, 5%, and 4% after 60, 100, and 140 days of growth, respectively, than for non-mycorrhizal seedlings. Basal area for mycorrhizal seedlings was significantly less by 10% after both 100 and 140 days of growth than for non-mycorrhizal

[1]Leaf necrosis occurred during early leaf development when the maximum air temperatures in the greenhouse ranged between 32°C and 43°C for 6 days.

Table 4. Means[1] of growth variables for *Pisolithus tinctorius* mycorrhizal and non-mycorrhizal *Quercus rubra* seedlings after 60, 100, and 140 days of growth. Means within a row having a common superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

Growth Variable		60 days old		100 days old		140 days old	
		Myco	Non-Myco	Myco	Non-Myco	Myco	Non-Myco
Basal area	mm^2	6.4^{c}	6.6^{c}	7.2^{b}	8.0^{a}	7.2^{b}	8.0^{a}
Total seedling weight	gm	2.5^{d}	2.8^{d}	5.3^{c}	5.8^{b}	6.2^{b}	6.8^{a}
Leaf area	cm^2	174^{bc}	196^{a}	172^{c}	189^{ab}	169^{c}	191^{a}
Green leaf area percent	%	81^{c}	90^{a}	82^{c}	87^{ab}	84^{bc}	88^{a}
Root weight	gm	1.2^{e}	1.4^{e}	3.8^{d}	4.2^{c}	4.6^{b}	5.0^{a}
Leaf N weight	mg	20.1^{b}	19.6^{b}	17.1^{c}	17.5^{c}	22.4^{a}	21.8^{a}
Leaf N percent	%	2.0^{a}	1.9^{ab}	1.6^{c}	1.5^{c}	2.0^{ab}	1.8^{b}
Leaf P weight	mg	3.1^{d}	3.8^{b}	3.5^{bc}	5.1^{a}	3.3^{cd}	5.4^{a}
Leaf P percent	%	$.32^{cd}$	$.38^{b}$	$.34^{c}$	$.47^{a}$	$.30^{d}$	$.46^{a}$
Mycorrhizal incidence	%	30^{a}	0^{b}	31^{a}	0^{b}	35^{a}	0^{b}

[1] 160 seedlings per mean.

seedlings. Total seedling weight for mycorrhizal seedlings was significantly less by 9% and 10% after 100 and 140 days of growth, respectively, than for non-mycorrhizal seedlings. Root weight of mycorrhizal seedlings was significantly less by 10% and 9% after 100 and 140 days of growth, respectively, than for non-mycorrhizal seedlings. At the end of each growth period, there were no significant differences in leaf nitrogen weight and percent between mycorrhizal and non-mycorrhizal seedlings except leaf nitrogen percent was significantly greater for 60-day-old mycorrhizal seedlings than for non-mycorrhizal seedlings of the same age. Leaf phosphorus weight and percent for mycorrhizal seedlings were significantly less than for non-mycorrhizal seedlings at the end of all three growth periods. Mycorrhizal seedlings had significantly greater mycorrhizal incidence percents than non-mycorrhizal seedlings at the end of all three growth periods. No differences were observed between average leaf colors of mycorrhizal and non-mycorrhizal seedlings.

Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings (fertilized and control seedlings grouped) Across Three Growth Periods:

Basal areas for mycorrhizal and non-mycorrhizal seedlings increased with age. Mycorrhizal seedlings had no significant differences in basal areas between 100 and 140 days of growth (7.2 vs 7.2 mm^2, Table 4), nor were there any significant differences in basal areas of non-mycorrhizal seedlings between these times (8.0 vs 8.0 mm^2, Table 4). Mycorrhizal seedlings at 140 days had significantly less basal area than non-mycorrhizal seedlings at 100 days (7.2 vs 8.0 mm^2, Table 4).

Total seedling weight also increased with seedling age. Non-mycorrhizal seedlings at 140 days had significantly greater total seedling weight than all other treatments regardless of age. There was no significant difference in total seedling weight between mycorrhizal seedlings at 140 days and non-mycorrhizal seedlings at 100 days.

Root weight increased significantly with seedling age with both mycorrhizal and non-mycorrhizal seedlings. Mycorrhizal seedlings had significantly lower root weights than non-mycorrhizal seedlings after 100 and 140 days of growth. Leaf nitrogen weights of 100-day-old mycorrhizal and non-mycorrhizal seedlings were significantly less than for seedings after 60 and 140 days of growth and the oldest (140-day-old) seedlings had the highest leaf nitrogen weight. After 60, 100, and 140 days of growth, non-mycorrhizal seedlings had significantly higher leaf phosphorus weight and percent than any mycorrhizal treatment. Mycorrhizal incidence percents for mycorrhizal seedlings were not significantly different with seedling age.

Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings Grown Without Nitrogen Fertilization:

After 60, 100, and 140 days of growth, basal area, root weight, and leaf nitrogen weight for unfertilized mycorrhizal seedlings were not significantly different than for unfertilized non-mycorrhizal seedlings (Table 5). Leaf area was significantly less (23%) for mycorrhizal seedlings than for non-mycorrhizal seedlings after 140 days of growth. Green leaf area percent was not significantly

Table 5. Means[1] of growth variables for unfertilized Pisolithus tinctorius mycorrhizal and non-mycorrhizal Quercus rubra seedlings after 60, 100, and 140 days of growth. Means within a row having a common superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

Growth Variable		60 days old		100 days old		140 days old	
		Myco	Non-Myco	Myco	Non-Myco	Myco	Non-Myco
Basal area	mm^2	6.6^{a}	6.5^{a}	6.4^{a}	6.4^{a}	6.2^{a}	6.9^{a}
Total seedling weight	gm	2.7^{d}	2.6^{d}	4.5^{c}	4.4^{c}	5.6^{b}	6.2^{a}
Leaf area	cm^2	185^{ab}	189^{a}	163^{abc}	158^{bc}	155^{c}	190^{a}
Green leaf area percent	%	84^{b}	90^{ab}	85^{b}	89^{ab}	86^{b}	92^{a}
Root weight	gm	1.3^{c}	1.3^{c}	3.1^{b}	3.0^{b}	4.1^{a}	4.6^{a}
Leaf N weight	mg	18.6^{a}	16.7^{a}	12.9^{b}	11.7^{b}	17.8^{a}	16.3^{a}
Leaf N percent	%	1.9^{a}	1.7^{a}	1.3^{b}	1.3^{b}	1.8^{a}	1.4^{b}
Leaf P weight	mg	3.4^{c}	4.1^{b}	3.5^{c}	4.2^{b}	3.4^{c}	5.3^{a}
Leaf P percent	%	$.35^{b}$	$.44^{a}$	$.37^{b}$	$.46^{a}$	$.33^{b}$	$.46^{a}$
Mycorrhizal incidence	%	18^{a}	0^{b}	13^{a}	0^{b}	18^{a}	0^{b}

[1] 40 seedlings per mean.

different between mycorrhizal and non-mycorrhizal seedlings after 60 and 100 days of growth; however, it was significantly less (6%) for mycorrhizal seedlings after 140 days of growth. Mycorrhizal seedlings had significantly greater leaf nitrogen percent than non-mycorrhizal seedlings after 140 days of growth, however the leaf nitrogen weight did not differ.

Total seedling weight and root weight increased significantly with seedling age for mycorrhizal and non-mycorrhizal seedlings. Total seedling weight for mycorrhizal seedlings at 140 days was significantly less than for non-mycorrhizal seedlings at 140 days (5.6 vs 6.2 gms, Table 5). Leaf nitrogen weight for 100-day-old seedlings was significantly less than for 60- and 140-day-old seedlings regardless of whether mycorrhizal were present or absent. Leaf phosphorus weight and percent for mycorrhizal seedlings of all ages were significantly less than for non-mycorrhizal seedlings of corresponding ages. Leaf phosphorus weight of 140-day-old non-mycorrhizal seedlings was significantly higher than all other treatments regardless of age. Mycorrhizal incidence percents for mycorrhizal seedlings were not significantly different with seedling age.

After 60, 100, and 140 days of growth, all unfertilized seedlings had an average Munsell color value for living leaf tissue of 5GY-5/8, 5GY-6/8, and 2.5GY-6/8 respectively. Leaves were chlorotic throughout all growth periods and chlorosis increased as the seedlings aged with the leaves exhibiting an increasing amount of yellow (green-yellow became yellowish-green-yellow).

II. Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings Fertilized with Sodium Nitrate and Ammonium Chloride

Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings Fertilized with Sodium Nitrate and Ammonium Chloride at the End of Each of Three Growth Periods:

Means of growth variables for mycorrhizal and non-mycorrhizal seedlings fertilized with sodium nitrate and ammonium chloride after 60, 100, and 140 days of growth are presented in Tables 6, 7, and 8, respectively. Data from treatments involving time of fertilization and levels of nitrogen applied were pooled.

Mycorrhizal Seedlings

Few differences in growth occurred between mycorrhizal seedlings fertilized with sodium nitrate and those fertilized with ammonium chloride. Mycorrhizal seedlings fertilized with sodium nitrate had significantly less (6% and 8%) green leaf area percent after 100 and 140 days of growth (Tables 7 and 8) and significantly higher leaf phosphorus percent after 140 days of growth (Table 8) than those fertilized with ammonium chloride. Mycorrhizal incidence percent was not significantly different between these two treatments at the end of any of the three growth periods (Tables 6, 7 and 8).

Non-Mycorrhizal Seedlings

Few differences in growth occurred between non-mycorrhizal seedlings fertilized with sodium nitrate and those fertilized with ammonium chloride. However, seedlings fertilized with ammonium chloride had greater (significant only at 100 days) total seedling

Table 6. Means[1] of growth variables for 60-day-old *Pisolithus tinctorius* mycorrhizal and non-mycorrhizal *Quercus rubra* seedlings fertilized with sodium nitrate and ammonium chloride. Means within a row having a common superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

Growth variable		Mycorrhizal		Non-Mycorrhizal	
		Sodium nitrate	Ammonium chloride	Sodium nitrate	Ammonium chloride
Basal area	mm^2	6.5^{a}	6.3^{a}	6.3^{a}	6.8^{a}
Total seedling weight	gm	2.6^{ab}	2.5^{b}	2.7^{ab}	2.8^{a}
Leaf area	cm^2	169^{b}	180^{ab}	188^{ab}	204^{a}
Green leaf area percent	%	79^{b}	83^{b}	90^{a}	89^{a}
Root weight	gm	1.2^{a}	1.2^{a}	1.4^{a}	1.3^{a}
Leaf N weight	mg	19.9^{a}	19.4^{a}	19.4^{a}	20.8^{a}
Leaf N percent	%	2.1^{a}	2.0^{ab}	1.9^{bc}	1.9^{c}
Leat P weight	mg	3.2^{b}	3.0^{b}	3.7^{a}	3.9^{a}
Leaf P percent	%	$.33^{b}$	$.31^{b}$	$.38^{a}$	$.38^{a}$
Mycorrhizal incidence	%	31^{a}	29^{a}	0^{b}	0^{b}

[1] 80 seedlings per mean.

Table 7. Means[1] of growth variables for 100-day-old Pisolithus tinctorius mycorrhizal and non-mycorrhizal Quercus rubra seedlings fertilized with sodium nitrate and ammonium chloride. Means within a row having a common superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

Growth variable		Mycorrhizal		Non-Mycorrhizal	
		Sodium nitrate	Ammonium chloride	Sodium nitrate	Ammonium chloride
Basal area	mm^2	7.5^{ab}	6.8^{b}	7.5^{ab}	8.4^{a}
Total seedling weight	gm	5.6^{b}	5.1^{b}	5.4^{b}	6.3^{a}
Leaf area	cm^2	174^{ab}	169^{b}	180^{ab}	198^{a}
Green leaf area percent	%	79^{b}	85^{a}	87^{a}	87^{a}
Root weight	gm	4.0^{b}	3.6^{b}	3.8^{b}	4.5^{a}
Leaf N weight	mg	17.4^{a}	16.7^{a}	16.7^{a}	18.2^{a}
Leaf N percent	%	1.6^{a}	1.6^{a}	1.6^{a}	1.5^{a}
Leaf P weight	mg	3.6^{b}	3.4^{b}	5.3^{a}	4.9^{a}
Leaf P percent	%	$.34^{c}$	$.33^{c}$	$.51^{a}$	$.44^{b}$
Mycorrhizal incidence	%	31^{a}	31^{a}	0^{b}	0^{b}

[1] 80 seedlings per mean.

Table 8. Means[1] of growth variables for 140-day-old *Pisolithus tinctorius* mycorrhizal and non-mycorrhizal *Quercus rubra* seedlings fertilized with sodium nitrate and ammonium chloride. Means within a row having a common superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

Growth variable		Mycorrhizal		Non-Mycorrhizal	
		Sodium nitrate	Ammonium chloride	Sodium nitrate	Ammonium chloride
Basal area	mm^2	7.1^{b}	7.3^{b}	7.6^{b}	8.4^{a}
Total seedling weight	gm	6.0^{b}	6.5^{ab}	6.5^{ab}	7.1^{a}
Leaf area	cm^2	164^{b}	174^{b}	185^{ab}	198^{a}
Green leaf area percent	%	80^{b}	88^{a}	87^{a}	89^{a}
Root weight	gm	4.3^{b}	4.8^{ab}	4.8^{ab}	5.2^{a}
Leaf N weight	mg	22.6^{a}	22.3^{a}	22.3^{a}	22.5^{a}
Leaf N percent	%	2.0^{a}	2.0^{a}	1.8^{b}	1.8^{b}
Leaf P weight	mg	3.5^{b}	3.1^{b}	5.6^{a}	5.2^{a}
Leaf P percent	%	$.33^{c}$	$.28^{d}$	$.48^{a}$	$.43^{b}$
Mycorrhizal incidence	%	33^{a}	38^{a}	0^{b}	0^{b}

[1] 80 seedlings per mean.

weight at the end of each growth period (4%, 17% and 9% greater, respectively) than for seedlings fertilized with sodium nitrate (Tables 6, 7 and 8). After 100 and 140 days of growth, leaf phosphorus percent was significantly greater for non-mycorrhizal seedlings fertilized with sodium nitrate than for those fertilized with ammonium chloride (Tables 7 and 8). However, there were no significant differences in leaf phosphorus weight between these two treatments at these respective ages. Leaf weight for non-mycorrhizal seedlings fertilized with sodium nitrate at 100 days was significantly less than for those seedlings fertilized with ammonium chloride (1.09 vs 1.23 gms, not depicted in Table 7). Leaf weight for non-mycorrhizal seedlings fertilized with sodium nitrate at 140 days was not significantly different than for those seedlings fertilized with ammonium chloride (1.17 vs 1.32 gms, not depicted in Table 8).

Sodium Nitrate and Ammonium Chloride Fertilized Seedlings

Total seedling weight of mycorrhizal seedlings fertilized with sodium nitrate was not significantly different from non-mycorrhizal seedlings fertilized with sodium nitrate at any age (Tables 6, 7 and 8). Mycorrhizal seedlings fertilized with ammonium chloride had significantly less total seedling weight at the end of 60 and 100 days of growth (12% and 24% less, respectively) than non-mycorrhizal seedlings fertilized with ammonium chloride (Tables 6 and 7). Leaf phosphorus weight and percent were significantly less for mycorrhizal seedlings at the end of each growth period when compared to

non-mycorrhizal seedlings regardless of fertilizer treatment (Tables 6, 7, and 8). Leaf nitrogen percent was significantly greater for mycorrhizal seedlings after 60 and 140 days of growth than for non-mycorrhizal seedlings within each fertilizer treatment (Tables 6 and 8). However there were no significant differences in leaf nitrogen weight between these two treatments at their respective ages.

Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings Fertilized with Sodium Nitrate and Ammonium Chloride Across Three Growth Periods:

Means of growth variables for mycorrhizal and non-mycorrhizal seedlings fertilized with sodium nitrate and ammonium chloride are presented in Tables 6, 7, and 8 for each growth period respectively. Data from treatments involving time of fertilization and levels of nitrogen applied are pooled. Superscripts for significant differences for comparisons of growth variables across the three growth periods are not embodied in these tables.

Basal areas for non-mycorrhizal seedlings (8.4 and 8.4 mm^2, Tables 7 and 8) fertilized with ammonium chloride at 100 and 140 days were not significantly different but they were significantly greater than those for all other treatments over all growth periods. Total seedling weight for non-mycorrhizal seedlings fertilized with ammonium chloride at 140 days was significantly larger (7.1 gms, Table 8) than for all other treatments over all growth periods. Green leaf area percents for 60-, 100-, and 140-day-old mycorrhizal seedlings fertilized with sodium nitrate were not significantly different among themselves but were significantly less than those for all other treatments

over all growth periods. All 100-day-old seedlings had significantly less leaf nitrogen percent than all 60- and 140-day-old seedlings. All mycorrhizal seedlings regardless of age and fertilizer treatment had significantly less leaf phosphorus percent than those for any of the other non-mycorrhizal treatments. Mycorrhizal incidence percents were not significantly different among 60-, 100-, and 140-day-old mycorrhizal seedlings fertilized with sodium nitrate and ammonium chloride.

Means and standard deviations of growing-medium pH from containers of mycorrhizal and non-mycorrhizal seedlings fertilized with sodium nitrate and ammonium chloride after 100 and 140 days of growth are presented in Table 9. The acidities of the growing media for mycorrhizal and non-mycorrhizal seedlings fertilized with sodium nitrate were greater than for those seedlings fertilized with ammonium chloride after 100 and 140 days of growth. The average pH determinations for mycorrhizal seedlings fertilized with sodium nitrate and ammonium chloride (combined) were 5.1 at the end of 100 days and 5.8 at the end of 140 days. Regardless of the treatment, the growing-medium pH was greater after 140 days than for 100 days.

III. Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings Fertilized with Sodium Nitrate and Ammonium Chloride Fifteen Days and Forty Days After Planting

Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings Fertilized with Sodium Nitrate and Ammonium Chloride Fifteen Days and Forty Days After Planting at the End of Each of Three Growth Periods:

Means of growth variables for mycorrhizal and non-mycorrhizal seedlings fertilized with sodium nitrate and ammonium chloride fifteen

Table 9. Means[1] and standard deviations of growing-medium pH of 100- and 140-day-old Pisolithus tinctorius mycorrhizal and non-mycorrhizal Quercus rubra seedlings fertilized with sodium nitrate and ammonium chloride.

Age of growth medium in days	Sodium nitrate		Ammonium chloride	
	Mycorrhizal	Non-Mycorrhizal	Mycorrhizal	Non-Mycorrhizal
100	5.5 ± .4	5.7 ± .4	4.7 ± .4	4.8 ± .5
140	6.1 ± .3	6.1 ± .5	5.5 ± .3	5.4 ± .3

[1] 60 samples per mean (control samples have been omitted).

days and forty days after planting are presented in Tables 10, 11, and 12 for the three growth periods. Data from treatments involving levels of nitrogen fertilizer applied were pooled.

Mycorrhizal Seedlings Fertilized with Sodium Nitrate and Ammonium Chloride

The oldest (140-day-old) mycorrhizal seedlings fertilized early within each fertilizer treatment had significantly greater basal area, leaf nitrogen weight, and leaf nitrogen percent than mycorrhizal seedlings fertilized late (Table 12). Green leaf area percent was lowest (but not significantly so in all cases) for mycorrhizal seedlings fertilized early with sodium nitrate than for any of the other treatments at the end of each growth period (Tables 10, 11, and 12). Mycorrhizal incidence percents at the end of all three growth periods were significantly greater for seedlings fertilized late with sodium nitrate than for those fertilized early with sodium nitrate (43 vs 19% for 60-day-old seedlings, 41 vs 20% for 100-day-old seedlings, and 45 vs 21% for 140-day-old seedlings). Note that the mycorrhizal incidence percents of 60-, 100-, and 140-day-old seedlings fertilized early with sodium nitrate remained constant but did not have more than one-half the mycorrhizal incidence percents for those seedlings fertilized late with sodium nitrate. Mycorrhizal incidence was significantly greater for 140-day-old seedlings fertilized late with ammonium chloride (45%) than for those fertilized early with ammonium chloride (31%) (Table 12).

Table 10. Means[1] of growth variables for 60-day-old Pisolithus tinctorius mycorrhizal and non-mycorrhizal Quercus rubra seedlings fertilized with sodium nitrate and ammonium chloride 15 days (early) and 40 days (late) after planting. Means within a row having a common superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

Growth variable		Mycorrhizal				Non-Mycorrhizal			
		Sodium Nitrate		Ammonium chloride		Sodium nitrate		Ammonium chloride	
		Early	Late	Early	Late	Early	Late	Early	Late
Basal area	mm^2	6.7^{abc}	6.3^{bcd}	6.8^{ab}	5.9^{d}	6.6^{abc}	6.0^{cd}	7.2^{a}	6.4^{bcd}
Total seedling weight	gm	2.6^{abc}	2.5^{bc}	2.7^{abc}	2.4^{c}	2.8^{ab}	2.6^{abc}	3.0^{a}	2.6^{abc}
Leaf area	cm^2	170^{b}	168^{b}	197^{ab}	164^{b}	194^{ab}	183^{b}	220^{a}	187^{ab}
Green leaf area percent	%	77^{d}	80^{cd}	84^{bcd}	82^{cd}	89^{ab}	92^{a}	91^{a}	88^{abc}
Root weight	gm	1.2^{a}	1.2^{a}	1.3^{a}	1.2^{a}	1.4^{a}	1.3^{a}	1.4^{a}	1.3^{a}
Leaf N weight	mg	20.8^{abc}	18.9^{abc}	20.3^{abc}	18.5^{bc}	21.3^{ab}	17.4^{c}	22.6^{a}	18.9^{abc}
Leaf N percent	%	2.1^{a}	2.1^{a}	2.0^{ab}	2.1^{a}	2.0^{ab}	1.8^{b}	1.9^{ab}	1.9^{ab}
Leaf P weight	mg	3.7^{ab}	2.7^{d}	3.0^{cd}	3.0^{cd}	3.5^{bc}	3.9^{ab}	4.1^{a}	3.8^{ab}
Leaf P percent	%	$.38^{bc}$	$.29^{e}$	$.29^{e}$	$.33^{cde}$	$.33^{cde}$	$.42^{a}$	$.37^{bcd}$	$.39^{ab}$
Mycorrhizal incidence	%	19^{c}	43^{a}	27^{bc}	31^{b}	0^{d}	0^{d}	0^{d}	0^{d}

[1] 40 seedlings per mean.

Table 11. Means[1] of growth variables for 100-day-old Pisolithus tinctorius mycorrhizal and non-mycorrhizal Quercus rubra seedlings fertilized with sodium nitrate and ammonium chloride 15 days (early) and 40 days (late) after planting. Means within a row having a common superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

Growth variable		Mycorrhizal				Non-Mycorrhizal			
		Sodium nitrate		Ammonium chloride		Sodium nitrate		Ammonium chloride	
		Early	Late	Early	Late	Early	Late	Early	Late
Basal area	mm^2	8.2^{bc}	6.9^{cd}	7.0^{cd}	6.6^{d}	8.4^{b}	6.6^{d}	9.6^{a}	7.2^{bcd}
Total seedling weight	gm	5.8^{ab}	5.5^{b}	4.9^{b}	5.3^{b}	5.5^{b}	5.3^{b}	6.7^{a}	5.9^{ab}
Leaf area	cm^2	184^{ab}	164^{b}	169^{b}	169^{b}	181^{ab}	179^{ab}	212^{a}	183^{ab}
Green leaf area percent	%	74^{b}	84^{a}	84^{a}	87^{a}	84^{a}	90^{a}	87^{a}	87^{a}
Root weight	gm	4.0^{abc}	4.0^{abc}	3.4^{c}	3.7^{bc}	3.9^{bc}	3.8^{bc}	4.8^{a}	4.3^{ab}
Leaf N weight	mg	19.1^{a}	15.6^{b}	17.0^{ab}	16.5^{ab}	17.7^{ab}	15.7^{b}	18.5^{ab}	18.0^{ab}
Leaf N percent	%	1.6^{a}	1.6^{ab}	1.6^{a}	1.6^{ab}	1.6^{a}	1.5^{ab}	1.4^{b}	1.6^{a}
Leaf P weight	mg	4.0^{b}	3.3^{bc}	3.3^{c}	3.5^{bc}	5.4^{a}	5.3^{a}	5.2^{a}	4.7^{a}
Leaf P percent	%	$.34^{d}$	$.34^{d}$	$.34^{d}$	$.33^{d}$	$.49^{b}$	$.54^{a}$	$.43^{c}$	$.45^{bc}$
Mycorrhizal incidence	%	20^{b}	41^{a}	29^{ab}	31^{a}	0^{c}	0^{c}	0^{c}	0^{c}

[1] 40 seedlings per mean.

Table 12. Means[1] of growth variables for 140-day-old Pisolithus tinctorius mycorrhizal and non-mycorrhizal Quercus rubra seedlings fertilized with sodium nitrate and ammonium chloride 15 days (early) and 40 days (late) after planting. Means within a row having a common superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

Growth variable		Mycorrhizal				Non-Mycorrhizal			
		Sodium nitrate		Ammonium chloride		Sodium nitrate		Ammonium chloride	
		Early	Late	Early	Late	Early	Late	Early	Late
Basal area	mm^2	7.7^{bc}	6.6^{d}	7.8^{b}	6.8^{cd}	7.6^{bc}	7.6^{bc}	9.0^{a}	7.9^{b}
Total seedling weight	gm	6.3^{ab}	5.7^{b}	6.9^{a}	6.1^{ab}	6.4^{ab}	6.5^{ab}	7.1^{a}	7.1^{a}
Leaf area	cm^2	177^{abc}	151^{c}	185^{ab}	162^{bc}	191^{ab}	178^{abc}	204^{a}	191^{ab}
Green leaf area percent	%	78^{c}	81^{bc}	88^{ab}	88^{ab}	83^{bc}	91^{a}	89^{a}	89^{a}
Root weight	gm	4.4^{ab}	4.2^{b}	5.0^{ab}	4.5^{ab}	4.6^{ab}	4.9^{ab}	5.1^{a}	5.2^{a}
Leaf N weight	mg	26.3^{a}	19.0^{d}	25.1^{ab}	19.4^{d}	22.3^{bcd}	20.1^{cd}	23.7^{abc}	21.4^{cd}
Leaf N percent	%	2.1^{a}	1.9^{b}	2.1^{a}	1.9^{b}	1.8^{b}	1.8^{b}	1.8^{b}	1.8^{b}
Leaf P weight	mg	3.9^{c}	3.2^{d}	3.1^{d}	3.1^{d}	6.1^{a}	5.2^{b}	5.2^{b}	5.1^{b}
Leaf P percent	%	$.34^{d}$	$.32^{d}$	$.26^{e}$	$.30^{de}$	$.50^{a}$	$.47^{ab}$	$.41^{c}$	$.44^{bc}$
Mycorrhizal incidence	%	21^{b}	45^{a}	31^{b}	45^{a}	0^{c}	0^{c}	0^{c}	0^{c}

[1] 40 seedlings per mean.

Non-Mycorrhizal Seedlings Fertilized with Sodium Nitrate and Ammonium Chloride

Leaf phosphorus percent was significantly greater for non-mycorrhizal seedlings fertilized late with sodium nitrate than for non-mycorrhizal seedlings fertilized early with sodium nitrate at the end of 60 and 100 days of growth (Tables 10 and 11). Most other growth variables at the end of each growth period were not significantly different between non-mycorrhizal seedlings fertilized early and those fertilized late with sodium nitrate. Basal area of non-mycorrhizal seedlings fertilized early with ammonium chloride was significantly greater (13%, 33%, and 14%, respectively) after all three growth periods than for non-mycorrhizal seedlings fertilized late with ammonium chloride (Tables 10, 11, and 12). Most other growth variables at the end of each growth period were not significantly different between non-mycorrhizal seedlings fertilized early and those fertilized late with ammonium chloride.

Seedling Flushes and Leaf Color

During the 140-day-growing season, 157 seedlings flushed with new growth. Seventy-eight percent (123 seedlings) of these 157 seedlings that flushed with new growth were fertilized early. Forty-six percent (73 seedlings) out of the total number (157) of seedlings that flushed with new growth were mycorrhizal seedlings. Fifty-six percent (88 seedlings) out of the total number (157) of seedlings that flushed with new growth were seedlings fertilized with sodium nitrate.

Regardless of mycorrhizal condition, type of nitrogen fertilizer, and time of nitrogen application, average leaf colors of 140-day-old seedlings were conspicuously darker in the value factor of the hue (green-yellow) as the rate of nitrogen applied increased. The average leaf colors of 140-day-old seedlings (from control, low, medium to high nitrogen rates) were 2.5GY-6/8, 5GY-6/6, and 5GY-4/6, respectively.

Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings Fertilized with Sodium Nitrate and Ammonium Chloride Fifteen Days and Forty Days after Planting Across Three Growth Periods:

Means of growth variables for mycorrhizal and non-mycorrhizal seedlings fertilized with sodium nitrate and ammonium chloride fifteen and forty days after planting are presented in Tables 10, 11, and 12 for each growth period, respectively. Data from treatments involving levels of nitrogen applied are pooled. Superscripts for significant differences for comparisons of growth variables across the three growth periods are not embodied in these tables.

There are 24 treatments from the combination of the treatments from Tables 10, 11, and 12. Differences between these 24 treatments are obscure and difficult to interpret. The mean basal area of 100-day-old non-mycorrhizal seedlings (9.6 mm^2) fertilized early with ammonium chloride was significantly greater than the basal areas of all other treatments except for 140-day-old non-mycorrhizal seedlings fertilized early with ammonium chloride (9.0 mm^2). Greatest total seedling weights were attained by 140-day-old non-mycorrhizal seedlings fertilized with ammonium chloride. Seedlings that had the

greatest green leaf area percent were generally non-mycorrhizal and those with the lowest green leaf percent were mycorrhizal seedlings fertilized with sodium nitrate.

Regardless of mycorrhizal condition, fertilizer type applied, and time of fertilizer application, average leaf colors of 100-day-old seedlings with control, low, medium, and high rates of nitrogen fertilizer were lighter in color (greater value factor) than 60-day-old seedlings. Changes in color were subtle between 100-day-old and 140-day-old seedlings. The 100-day-old seedlings had leaves with a greater chroma factor (departure from neutral) than the leaves of the 140-day-old seedlings. The 100-day-old seedlings did not look as green as the younger or older seedlings.

IV. Ectomycorrhizal Incidence and Comparisons of Individual Treatments

Ectomycorrhizal Incidence and Relationships:

Ectomycorrhiza infection and mantle formation was observed as early as 18 days after seed planting. After each growth period, all inoculated seedlings were ectomycorrhizal. Ectomycorrhizal incidence ranged from 1-95% on individual root systems. Ectomycorrhizae other than those characteristically produced by Pisolithus tinctorius were not observed. All non-inoculated seedlings were free of ectomycorrhizae after each growth period.

Early applications of nitrogen fertilizer (especially sodium nitrate) reduced the incidence of mycorrhizae during all growth periods (Tables 10, 11, and 12). Sixty-day-old seedlings supplied

with sodium nitrate late in the season had significantly higher mycorrhizal incidence percents than the other treatments (Table 10). Oldest seedlings (140-days-old) that received late applications of either sodium nitrate or ammonium chloride had the highest but similar mycorrhizal incidence percents (Table 12).

Figures 1, 2, and 3 illustrate mean ectomycorrhizal incidence percents for each period of growth for nitrogen fertilizer type, application time, and nitrogen fertilizer rate. At the end of each growth period, mycorrhizal incidence percents of control, early-medium nitrate, early-medium ammonium, and early-high ammonium treatments were significantly lower than for all other nine treatments shown in Figures 1, 2, and 3. Seedlings fertilized with an early application of sodium nitrate had lower, and in most cases significantly lower, mycorrhizal incidence percents than corresponding seedlings fertilized with late applications of sodium nitrate throughout the season. Seedlings fertilized early with medium and high rates of ammonium chloride had significantly lower mycorrhizal incidence percents throughout the season than those seedlings fertilized late. Seedlings fertilized early with the low rate of ammonium chloride had no significant differences between mycorrhizal incidence percents from those seedlings at any age that had been fertilized late with ammonium chloride at the low rate.

Ectomycorrhizal incidence percents of all 100-day-old mycorrhizal seedlings correlated with residual growing medium pH with an $r = -.0042$.

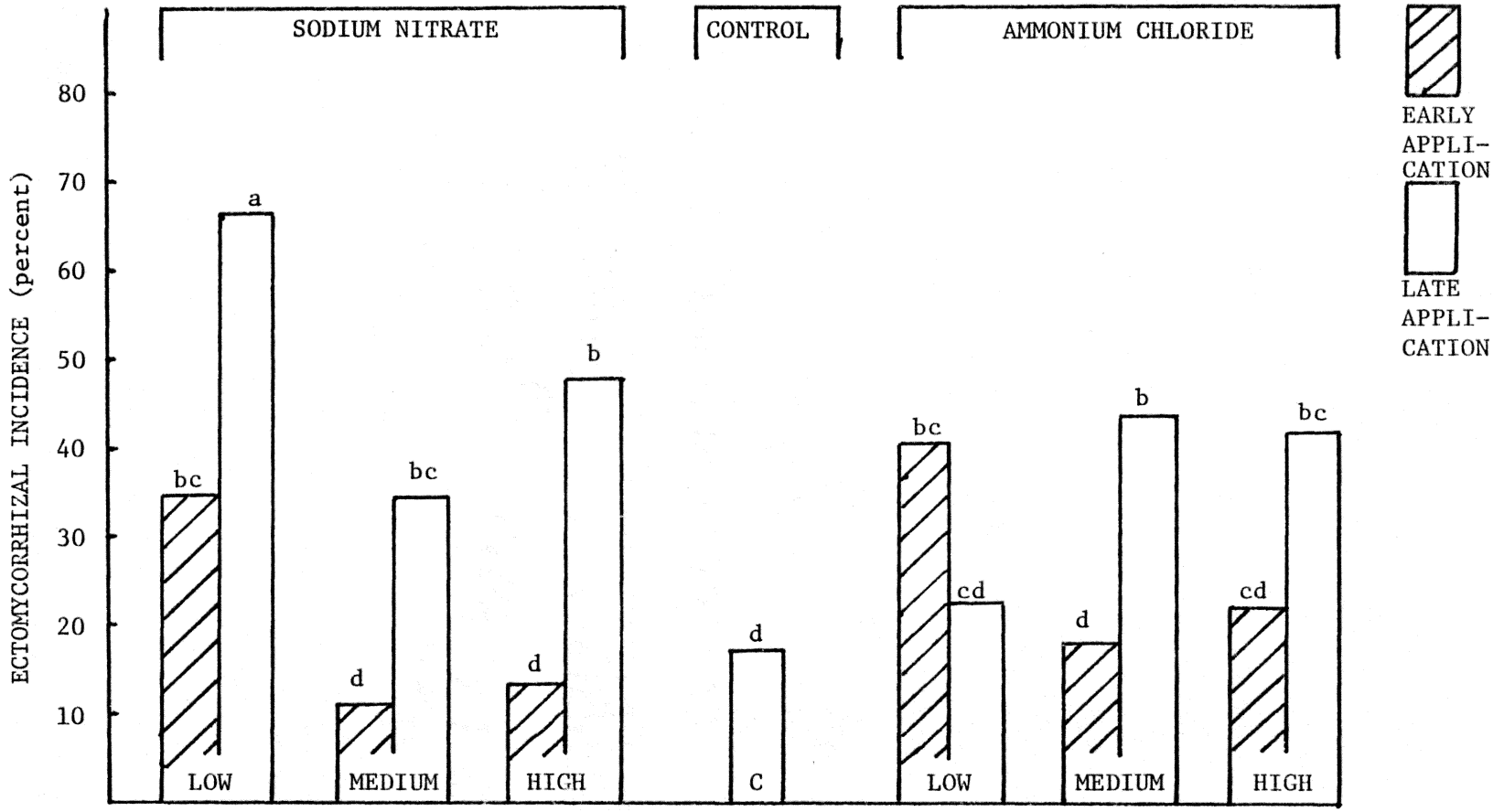

Figure 1. Means of ectomycorrhizal incidence percents of 60-day-old Pisolithus tinctorius mycorrhizal Quercus rubra seedlings fertilized with sodium nitrate and ammonium chloride at two different times and at four different nitrogen application rates. Each treatment mean calculated from ten seedlings (control calculated from forty seedlings). Means that have the same superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

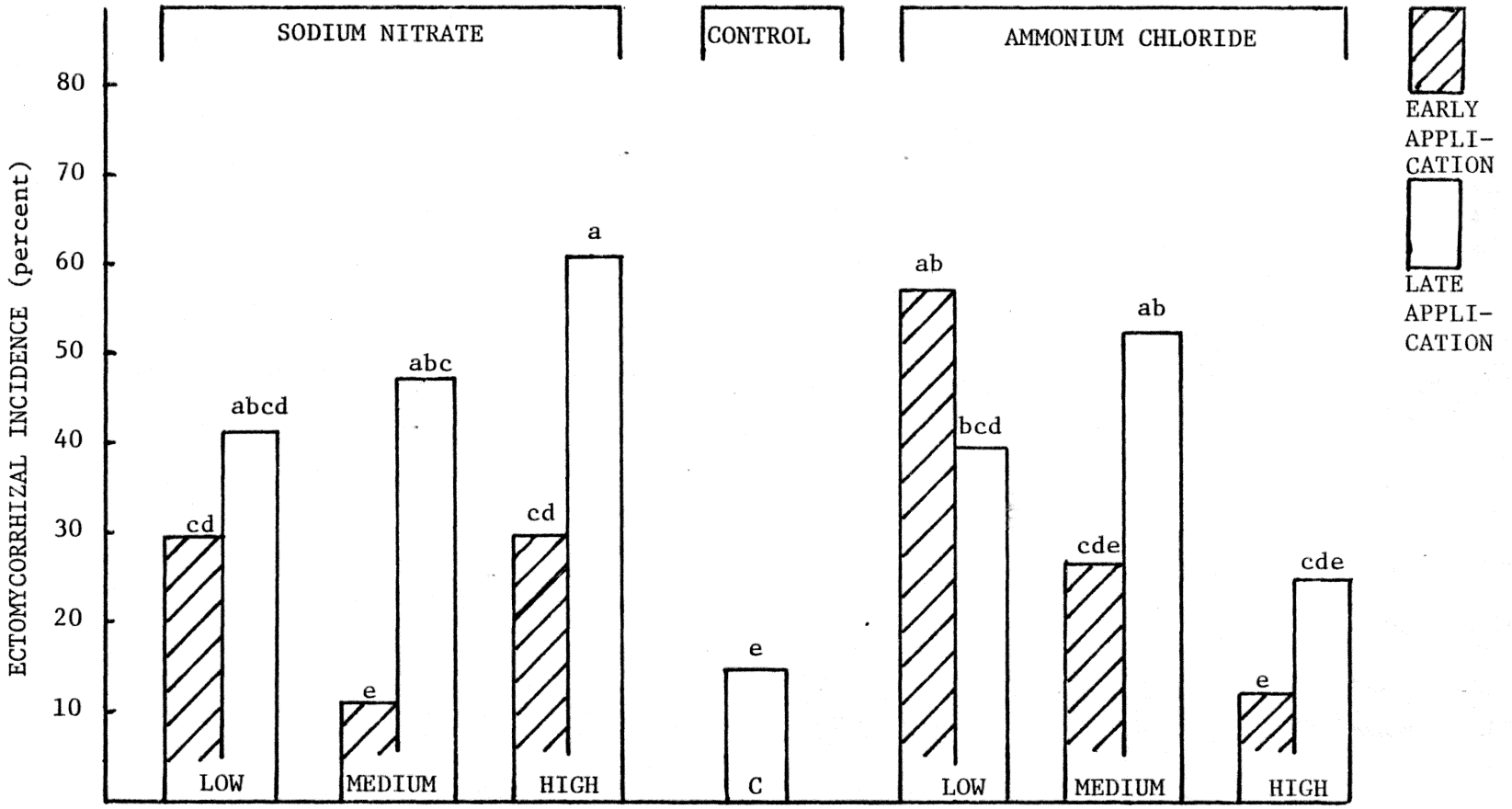

Figure 2. Means of ectomycorrhizal incidence percents of 100-day-old Pisolithus tinctorius mycorrhizal Quercus rubra seedlings fertilized with sodium nitrate and ammonium chloride at two different times and at four different nitrogen application rates. Each treatment mean calculated from ten seedlings (control calculated from forty seedlings). Means that have the same superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

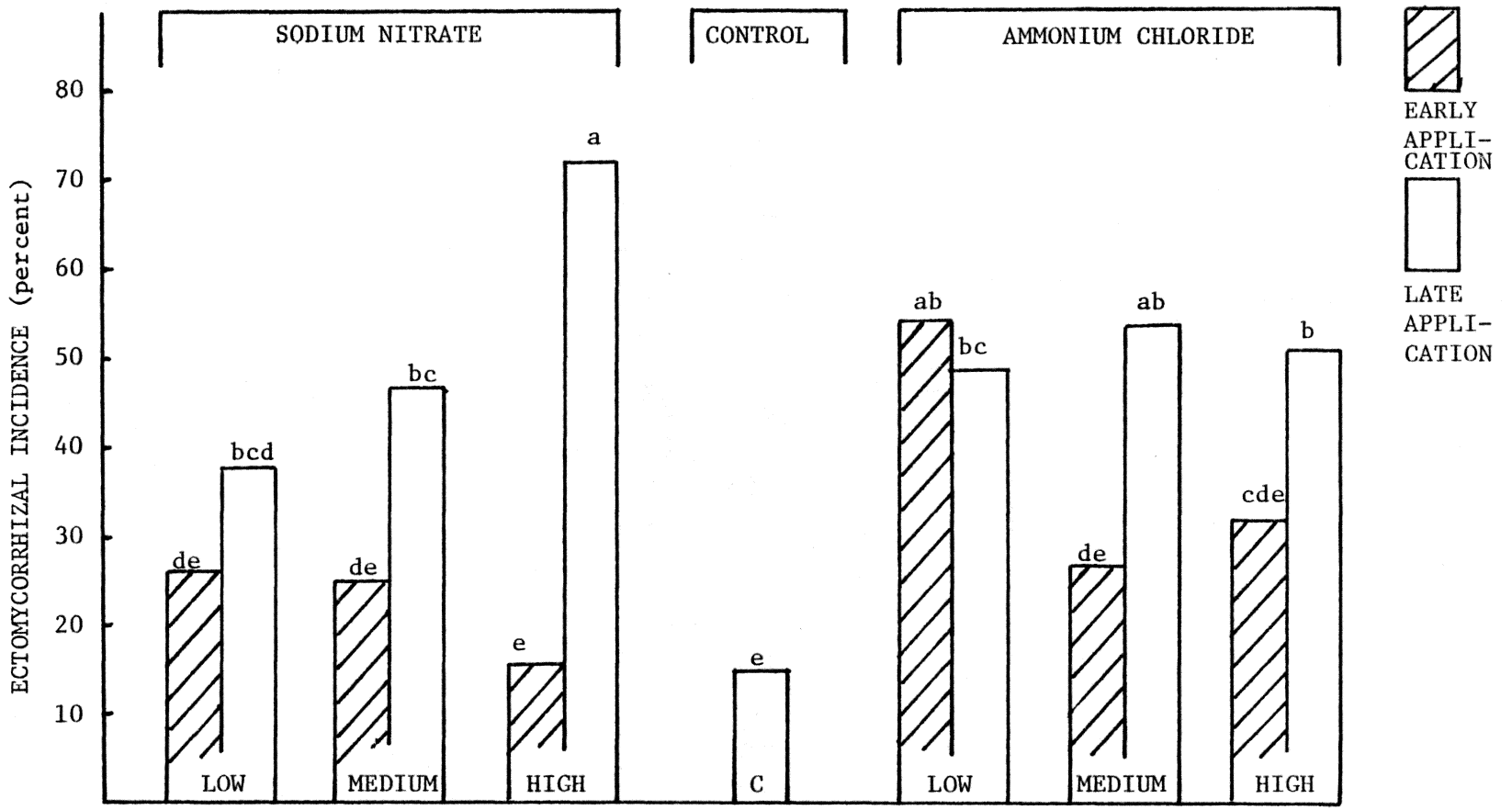

Figure 3. Means of ectomycorrhizal incidence percents of 140-day-old Pisolithus tinctorius mycorrhizal Quercus rubra seedlings fertilized with sodium nitrate and ammonium chloride at two different times and at four different nitrogen application rates. Each treatment mean calculated from ten seedlings (control calculated from forty seedlings). Means that have the same superscript are not significantly different at the .05 probability level in the Duncan's multiple-range test.

No correlations with an $r \geq .6$ or $r \leq -.6$ resulted between mycorrhizal incidence percents and growing-medium pH within each 100-day-old treatment.

Mycorrhizal incidence percent and the transformation to the arcsin of the square root of incidence percent were correlated with the variables leaf nitrogen percent, leaf phosphorus percent, leaf nitrogen/phosphorus ratio, the numerical values of (1 + log N%), and the numerical values of (1 + log P%) for the following mycorrhizal treatments for each growth period:

(1) All mycorrhizal seedlings,

(2) Sodium nitrate and ammonium chloride treatments, and

(3) Early-nitrate, late-nitrate, early-ammonium, and late-ammonium treatments.

The following are those correlations with a resultant $r \geq .49$ or $r \leq -.49$: 100-day-old seedlings fertilized late with sodium nitrate had a correlation between mycorrhizal incidence percent and leaf nitrogen percent with $r = +.49$, $P = .0013$ (40 seedlings contributing).

Individual Treatments with Some Specific Comparisons:

Means of the growth variables for each individual treatment are presented in Tables 13, 14, and 15 for all three growth periods. Data which follow refer to tests of significance that are not embodied in these tables.

Table 13. Means[1] of growth variables for 60-day-old *Pisolithus tinctorius* mycorrhizal and non-mycorrhizal *Quercus rubra* seedling/fertilizer treatments.[2]

Seedling treatments			Basal area mm^2	Total seedling weight gm	Leaf area cm^2	Green leaf area percent %	Root weight gm
MYCORRHIZAL	SODIUM NITRATE	EARLY	C	7.0	2.9	201	91	1.4
			L	6.0	2.3	143	77	1.1
			M	6.7	2.6	165	75	1.2
			H	7.2	2.6	166	67	1.7
		LATE	C	6.9	2.8	195	81	1.3
			L	6.1	2.7	179	83	1.4
			M	5.6	2.1	143	77	1.1
			H	6.7	2.4	157	79	1.2
	AMMONIUM CHLORIDE	EARLY	C	6.5	2.6	182	83	1.4
			L	6.8	2.7	172	84	1.4
			M	6.4	2.3	164	75	1.1
			H	7.4	3.0	268	93	1.4
		LATE	C	5.9	2.3	159	82	1.1
			L	5.7	2.6	144	79	1.2
			M	5.5	2.2	151	76	1.1
			H	6.4	2.6	203	91	1.3
NON-MYCORRHIZAL	SODIUM NITRATE	EARLY	C	6.8	2.7	200	95	1.4
			L	5.8	2.7	175	94	1.4
			M	6.7	2.7	183	80	1.4
			H	7.2	3.1	218	86	1.3
		LATE	C	6.1	2.7	186	93	1.4
			L	5.6	2.5	174	92	1.3
			M	6.0	2.4	182	92	1.3
			H	6.3	2.8	188	90	1.4
	AMMONIUM CHLORIDE	EARLY	C	7.0	2.4	179	82	1.2
			L	6.5	3.0	214	94	1.5
			M	7.4	3.1	203	92	1.5
			H	7.8	3.3	282	94	1.4
		LATE	C	6.0	2.7	191	89	1.4
			L	6.0	2.4	173	87	1.2
			M	7.4	3.0	226	89	1.5
			H	6.2	2.5	160	85	1.3

[1] 10 seedlings per mean.

[2] Seedling/fertilizer treatments are arranged by fertilizer (sodium nitrate and ammonium chloride), time of fertilization (early and late), and level of nitrogen applied (control = C, low = L, medium = M, and high = H).

Table 13. Continued

Seedling treatments			Leaf N weight mg	Leaf N percent %	Leaf P weight mg	Leaf P percent %	Mycorrhizal incidence %
MYCORRHIZAL	SODIUM NITRATE	EARLY	C	19.6	1.8	4.6	.45	13
			L	15.7	1.7	3.0	.32	37
			M	22.9	2.2	3.4	.33	11
			H	25.2	2.4	3.7	.35	13
		LATE	C	21.4	2.0	3.8	.35	18
			L	17.6	1.8	3.1	.31	67
			M	17.7	2.3	2.1	.28	37
			H	19.1	2.2	2.2	.22	49
	AMMONIUM CHLORIDE	EARLY	C	16.7	1.8	2.1	.23	28
			L	17.1	1.7	2.9	.30	42
			M	17.6	2.0	2.5	.28	18
			H	29.6	2.3	4.4	.35	22
		LATE	C	16.5	1.9	3.3	.36	16
			L	18.7	2.2	2.2	.26	22
			M	15.9	1.9	2.2	.27	44
			H	22.8	2.2	4.2	.42	42
NON-MYCORRHIZAL	SODIUM NITRATE	EARLY	C	17.6	1.7	3.8	.38	0
			L	17.0	1.8	3.0	.31	0
			M	21.4	2.1	3.6	.34	0
			H	29.2	2.2	3.4	.26	0
		LATE	C	18.1	1.9	3.8	.40	0
			L	13.8	1.6	3.9	.46	0
			M	14.3	1.6	4.0	.45	0
			H	23.6	2.3	3.8	.50	0
	AMMONIUM CHLORIDE	EARLY	C	13.9	1.6	4.6	.50	0
			L	16.2	1.5	3.0	.28	0
			M	23.9	1.5	4.8	.39	0
			H	36.3	2.0	4.1	.28	0
		LATE	C	17.1	1.7	4.4	.49	0
			L	18.1	1.8	3.6	.36	0
			M	22.8	2.0	3.6	.33	0
			H	17.7	1.9	3.4	.37	0

Table 14. Means[1] of growth variables for 100-day-old Pisolithus tinctorius mycorrhizal and non-mycorrhizal Quercus rubra seedling/fertilizer treatments.[2]

Seedling treatments			Basal area mm^2	Total seedling weight gm	Leaf area cm^2	Green leaf area percent %	Root weight gm
MYCORRHIZAL	SODIUM NITRATE	EARLY	C	6.7	4.6	187	87	3.3
			L	7.6	6.9	224	75	5.0
			M	7.9	5.7	150	72	4.1
			H	10.4	5.6	176	63	3.5
		LATE	C	6.7	5.1	184	86	3.6
			L	6.2	4.9	150	85	3.5
			M	7.2	5.2	167	83	4.2
			H	7.7	6.3	155	81	4.7
	AMMONIUM CHLORIDE	EARLY	C	5.8	3.6	127	83	2.5
			L	6.2	4.5	162	87	3.1
			M	6.2	4.6	149	78	3.3
			H	9.6	6.9	237	89	4.8
		LATE	C	6.2	4.3	156	84	2.9
			L	5.5	4.7	148	88	3.4
			M	7.7	6.7	190	85	5.0
			H	7.1	5.3	184	91	3.7
NON-MYCORRHIZAL	SODIUM NITRATE	EARLY	C	6.0	3.8	127	89	2.6
			L	6.6	4.2	152	82	3.0
			M	9.1	6.7	218	80	4.6
			H	11.9	7.4	226	84	5.3
		LATE	C	5.6	4.0	150	87	2.8
			L	6.0	4.9	166	91	3.5
			M	7.2	6.5	194	95	5.0
			H	7.7	5.7	206	88	3.8
	AMMONIUM CHLORIDE	EARLY	C	7.3	4.5	186	87	3.0
			L	9.0	6.5	177	81	4.8
			M	9.1	7.8	227	88	5.7
			H	13.1	8.0	257	94	5.6
		LATE	C	6.8	5.2	170	93	3.8
			L	6.8	5.3	174	79	3.8
			M	7.8	6.9	212	91	5.1
			H	7.3	6.2	178	85	4.6

[1] 10 seedlings per mean.

[2] Seedling/fertilizer treatments are arranged by fertilizer (sodium nitrate and ammonium chloride), time of fertilization (early and late), and level of nitrogen applied (control = C, low = L, medium = M, and high = H).

Table 14. Continued

Seedling treatments			Leaf N weight mg	Leaf N percent %	Leaf P weight mg	Leaf P percent %	Mycorrhizal incidence %
MYCORRHIZAL	SODIUM NITRATE	EARLY	C	13.0	1.2	4.4	.41	10
			L	17.1	1.3	4.2	.32	30
			M	19.6	1.8	3.7	.35	11
			H	26.9	2.0	3.6	.26	30
		LATE	C	13.8	1.3	3.6	.37	10
			L	15.0	1.6	3.8	.38	42
			M	13.4	1.5	2.6	.30	48
			H	20.2	1.8	3.0	.28	63
	AMMONIUM CHLORIDE	EARLY	C	11.2	1.5	2.7	.36	20
			L	14.4	1.5	2.9	.29	59
			M	16.0	1.7	2.7	.30	27
			H	26.3	1.7	5.0	.34	12
		LATE	C	13.7	1.4	3.3	.33	14
			L	13.0	1.4	2.6	.29	39
			M	18.1	1.5	3.7	.31	54
			H	21.3	1.8	4.2	.36	26
NON-MYCORRHIZAL	SODIUM NITRATE	EARLY	C	11.1	1.5	3.0	.39	0
			L	12.0	1.5	4.7	.54	0
			M	22.3	1.6	7.0	.50	0
			H	25.3	1.8	6.7	.45	0
		LATE	C	10.7	1.8	4.6	.54	0
			L	14.4	1.5	5.4	.55	0
			M	16.4	1.6	6.2	.60	0
			H	21.5	1.8	5.1	.43	0
	AMMONIUM CHLORIDE	EARLY	C	12.1	1.2	4.6	.44	0
			L	15.2	1.3	5.8	.51	0
			M	20.5	1.5	5.1	.36	0
			H	26.2	1.7	5.2	.34	0
		LATE	C	12.7	1.3	4.5	.45	0
			L	17.1	1.6	4.5	.41	0
			M	20.5	1.7	4.9	.41	0
			H	21.8	1.9	5.0	.45	0

Table 15. Means[1] of growth variables for 140-day-old Pisolithus tinctorius mycorrhizal and non-mycorrhizal Quercus rubra seedling/fertilizer treatments.[2]

Seedling treatments			Basal area mm^2	Total seedling weight gm	Leaf area cm^2	Green leaf area percent %	Root weight gm
MYCORRHIZAL	SODIUM NITRATE	EARLY	C	6.7	6.2	168	86	4.7
			L	6.9	5.6	153	75	4.1
			M	7.7	6.7	196	79	4.7
			H	9.6	6.6	193	74	4.3
		LATE	C	5.6	4.7	143	80	3.3
			L	7.2	5.4	156	81	3.7
			M	6.3	6.5	141	80	5.1
			H	7.2	6.2	165	83	4.7
	AMMONIUM CHLORIDE	EARLY	C	6.5	5.8	147	82	4.4
			L	6.5	5.7	152	88	4.2
			M	7.4	7.9	183	93	6.2
			H	10.9	8.2	264	90	5.4
		LATE	C	6.3	5.8	161	95	4.2
			L	6.4	4.6	125	80	3.3
			M	7.1	7.7	187	90	6.1
			H	7.3	6.1	177	86	4.4
NON-MYCORRHIZAL	SODIUM NITRATE	EARLY	C	6.1	5.6	157	91	4.2
			L	6.6	6.0	164	83	4.5
			M	8.9	7.7	237	89	5.4
			H	8.6	6.6	207	68	4.4
		LATE	C	6.0	5.6	183	91	4.0
			L	7.4	7.1	199	95	5.5
			M	7.3	7.8	177	96	6.3
			H	9.6	5.7	153	82	3.9
	AMMONIUM CHLORIDE	EARLY	C	8.4	6.7	217	90	4.8
			L	8.2	6.1	163	83	4.5
			M	9.5	8.5	234	93	6.1
			H	9.7	7.2	201	91	5.2
		LATE	C	7.0	7.2	205	96	5.4
			L	7.5	5.8	182	84	4.0
			M	9.4	8.9	228	94	6.8
			H	7.6	6.4	161	82	4.8

[1] 10 seedlings per mean.

[2] Seedling/fertilizer treatments are arranged by fertilizer (sodium nitrate and ammonium chloride), time of fertilization (early and late), and level of nitrogen applied (control = C, low = L, medium = M, and high = H).

Table 15. Continued

Seedling treatments			Leaf N weight mg	Leaf N percent %	Leaf P weight mg	Leaf P percent %	Mycorrhizal incidence %
MYCORRHIZAL	SODIUM NITRATE	EARLY	C	19.9	1.9	4.4	.43	13
			L	24.1	2.2	3.3	.30	27
			M	29.8	2.1	4.0	.29	26
			H	31.5	2.1	4.1	.30	16
		LATE	C	16.0	1.7	2.9	.30	22
			L	19.7	1.8	4.0	.36	38
			M	20.8	2.1	2.8	.29	48
			H	19.2	1.9	2.9	.29	72
	AMMONIUM CHLORIDE	EARLY	C	20.7	2.1	2.5	.28	10
			L	20.8	2.0	2.1	.20	55
			M	22.9	1.9	2.6	.22	28
			H	36.2	1.9	5.0	.26	32
		LATE	C	14.5	1.3	3.6	.31	26
			L	18.2	2.1	2.6	.28	50
			M	23.8	2.0	3.0	.28	54
			H	21.2	1.9	3.4	.31	52
NON-MYCORRHIZAL	SODIUM NITRATE	EARLY	C	13.8	1.4	4.2	.44	0
			L	20.5	2.0	5.3	.50	0
			M	29.3	1.9	7.9	.51	0
			H	25.5	1.7	6.9	.46	0
		LATE	C	17.6	1.5	5.3	.46	0
			L	20.1	1.8	5.6	.50	0
			M	21.8	2.1	5.1	.49	0
			H	20.8	1.9	4.8	.39	0
	AMMONIUM CHLORIDE	EARLY	C	18.6	1.4	5.9	.46	0
			L	19.6	1.9	4.6	.43	0
			M	31.2	1.9	5.1	.32	0
			H	25.3	1.9	5.4	.41	0
		LATE	C	15.3	1.2	5.8	.46	0
			L	21.6	1.9	5.0	.46	0
			M	26.3	1.9	5.1	.36	0
			H	22.3	2.0	4.6	.43	0

Mycorrhizal Seedlings At 100 Days

Ectomycorrhizal seedlings at 100 days had 80% green leaf area and the greatest total seedling weight (6.9 gms) when fertilized early with the highest level of ammonium chloride (Table 14). These seedlings had a low mycorrhizal incidence percent (12%) which ranked 13th out of the sixteen 100-day-old mycorrhizal treatments. Mycorrhizal incidence percent (62%) was greatest with 100-day-old seedlings fertilized late with the highest level of sodium nitrate (Table 14 and Figure 2). These seedlings had a total seedling weight (6.3 gms) that ranked 4th and 81% green leaf area. Figure 2 indicates this mycorrhizal treatment was not significantly different in mycorrhizal incidence percent than mycorrhizal seedlings at 100 days fertilized late with the medium level of ammonium chloride (62% vs. 54%, respectively). However, the total seedling weights between these latter two treatments (6.3 vs. 6.8 gms, respectively) are significantly different.

Non-Mycorrhizal Seedlings at 100 Days

Information from Table 14 indicates that non-mycorrhizal seedlings at 100 days fertilized early with the high level of ammonium chloride had 94% green leaf area and the greatest total seedling weight (8.0 gms). Non-mycorrhizal seedlings of the same age but fertilized early with the medium rate of ammonium chloride had a total seedling weight of similar magnitude (7.8 gms) and 88% green leaf area. Seedlings with the lowest total seedling weight mean (3.8 gms) were unfertilized non-mycorrhizal seedlings.

Mycorrhizal Seedlings At 140 Days

Mycorrhizal seedlings at 140 days had the greatest total seedling weight (8.2 gms) when fertilized early with the highest level of ammonium chloride and a mycorrhizal incidence (32%) which ranked 8th. However, seedlings fertilized late with the highest level of sodium nitrate had the greatest mycorrhizal incidence (72%) and ranked 7th out of sixteen treatments in total seedling weight (6.2 gms). The total seedling weights were not significantly different between these two treatments, however the mycorrhizal incidence percents were significantly different. Seedlings fertilized late with ammonium chloride at the medium level had a significantly greater total seedling weight at the .15 level than seedlings having a 72% mycorrhizal incidence (7.7 vs. 6.2 gms). Mycorrhizal incidence was not significantly different between these two treatments (72% vs. 54%).

Non-Mycorrhizal Seedlings at 140 Days

One hundred-forty-day-old non-mycorrhizal seedlings had 94% green leaf area and were the largest (8.9 gms) when fertilized late with the medium level of ammonium chloride (Table 15). Smallest non-mycorrhizal seedlings were unfertilized.

Some Specific Comparisons Between 100- and 140-Day-Old Mycorrhizal and Non-Mycorrhizal Treatments:

Neither mycorrhizal incidences (54% vs 54%) nor total seedling weights (6.7 vs 7.7 gms) were significantly different between 100-day-old and 140-day-old mycorrhizal seedlings fertilized late with the medium level of ammonium chloride.

Neither total seedling weights (7.8 vs 8.9 gms) nor green leaf area percents (88% vs 94%) were significantly different between 100-day-old non-mycorrhizal seedlings fertilized early with the medium rate of ammonium chloride and 140-day-old non-mycorrhizal seedlings fertilized late with the medium level of ammonium chloride.

DISCUSSION

I. Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings

Three major differences encountered between mycorrhizal and non-mycorrhizal seedlings are categorized below as:

(1) Less leaf phosphorus weight and percent for mycorrhizal seedlings than for non-mycorrhizal seedlings;

(2) Less leaf area and green leaf area percent for mycorrhizal seedlings than for non-mycorrhizal seedlings; and

(3) Less total seedling weight, root weight, and basal area for mycorrhizal seedlings than for non-mycorrhizal seedlings (Table 4).

Phosphorus:

Numerous studies have shown that mycorrhizae, associated mycellia, and microorganisms enhance nutrient uptake for host use (Hatch, 1937; Finn, 1942; McComb, 1943; Gray and Gerdemann, 1969; Bjorkman, 1970; Bowen, 1973; De la Cruz, 1974; and Berry and Marx, 1976). Phares (1964) found that foliage of mycorrhizal *Quercus rubra* seedlings contained 14% more nitrogen, 30% more phosphorus, and 5% more potassium than the foliage of non-inoculated seedlings. However, Fisher and Cox (1978) found no significant differences between phosphorus contents in the tops of mycorrhizal and non-mycorrhizal *Q. rubra* seedlings. Fisher and Cox concluded that mycorrhizae had no apparent influence on phosphorus uptake by the plant. In the present study, higher leaf phosphorus content in non-mycorrhizal *Q. rubra*

seedlings was probably a result of less competition between the seedling root system and soil micro-flora for the uptake and use of phosphorus.

The mycorrhizosphere and the surface of the mycorrhizal mantle provide a complex ecological niche for soil-inhabiting organisms (Marx and Bryan, 1973). Rambelli (1973) found nitrite- and nitrate-forming bacteria were stimulated in the vicinity of mycorrhizae of Monterey pine seedlings. The acidities of the growing medium for mycorrhizal and non-mycorrhizal seedlings (Table 9) are within the range (pH 3.0 - 8.0) for fungal and bacterial growth (Alexander, 1961). Competition by Pisolithus tinctorius, mycorrhizae and associated mycelia, bacteria, and other soil organisms in the growing medium of mycorrhizal seedlings for phosphorus may have resulted in the reduction of the available-phosphorus pool. Thus, seedling uptake and translocation of phosphorus to the leaves may have been reduced. The competition for phosphorus by all organisms in the growing medium of mycorrhizal seedlings was undoubtedly greater than for non-mycorrhizal seedlings. Although leaf phosphorus content was less in mycorrhizal seedlings, this should not be interpreted to mean that the total seedling phosphorus content was less than for non-mycorrhizal seedlings. Phosphorus uptake by both mycorrhizal root systems and non-mycorrhizal root systems may have been the same, but phosphorus may have been differentially translocated to the seedling tops among the treatments. Mycorrhizal sheaths and/or fungal vacuoles could have actively stored incoming (absorbed) phosphorus throughout the season reducing the

quantity of phosphorus translocated to mycorrhizal seedling leaves (Jennings, 1964; Harley 1969).

Leaf Area and Green Leaf Area:

Lower leaf areas and green leaf area percents of mycorrhizal seedlings (Tables 4 and 5) were a result of extensive leaf damage. Leaf damage in the form of leaf margin necrosis was detected when maximum greenhouse-air temperatures ranged between 32°C and 43°C. This occurred during the period when leaves were developing and expanding, and when root infestation and mycorrhizal formation by P. tinctorius was taking place. It is hypothesized that the young leaves of mycorrhizal seedlings suffered more heat damage than leaves of non-mycorrhizal seedlings because the former had a higher percentage of succulent tissue (a higher percentage of juvenile tissue which does not have as high a degree of secondary cell-wall thickening). Succulency may have been increased and/or prolonged in three ways:

(1) During mycorrhizal formation, the mycobiont is dependent on the host for carbohydrates (Bjorkman, 1942) which are translocated from the leaves to the root for fungal use (Melin and Nilsson, 1957). Reduction of leaf carbohydrates (photosynthates) may have delayed secondary cell-wall thickening within the leaves, thus increasing leaf succulency.

(2) Slankis (1973) found that with increased IAA concentrations added to the medium, better developed stems and considerably longer needles were obtained in aseptically grown Pinus sylvestris L.

seedlings. He stated that the growth stimulation of seedlings is induced by fungal auxins. However, other fungal hormones, growth regulators such as vitamins, or other metabolites in fungal exudates may also stimulate the growth of seedling parts. In this study, auxins and/or other growth regulators in the fungal exudates of P. tinctorius may have stimulated early leaf growth and extended the duration of leaf succulency.

(3) The rapid uptake of nutrients and water by mycorrhiza and infested roots may have promoted rapid leaf-cell division and expansion, thereby increasing leaf succulency. The utilization of nutrients in protoplasm synthesis during rapid growth would also require carbohydrates as an energy and carbon source, further delaying cell-wall thickening in the leaves.

Total Seedling Weight, Root Weight, and Basal Area:

Total seedling weight, root weight, and basal area were in most cases significantly less for mycorrhizal seedlings than for non-mycorrhizal seedlings (Table 4). The reduced growth of mycorrhizal seedlings in this study does not conform to the doctrine that mycorrhizal seedlings are generally larger than non-mycorrhizal seedlings when grown in soils of "low" fertility (Hatch, 1937; Harley, 1969). Several factors could account for lower values of the growth variables in mycorrhizal seedlings.

First, reduced green-leaf area of mycorrhizal seedlings probably provided less carbohydrate for root and top growth. Not only did

damaged leaves have reduced photosynthetic areas, but the damage to the margins of the leaves probably partially destroyed the leaf meristematic tissues. Leaf expansion was restricted, thus leaf weights were also reduced thereby decreasing the total seedling weight.

Second, P. tinctorius utilizes host carbohydrates therefore possibly reducing the quantities of available carbohydrates for vegetative growth of seedling parts. In normal protein synthesis and resultant growth, there is probably an optimum carbohydrate/nitrogen (C/N) ratio that would be expected for optimum growth based on the type of cell being produced. When this ratio deviates from the "optimum," one component may become limiting. This ratio is probably much easier to elucidate in fungi and bacteria because of the relative simplicity and homogeneity of these organisms and their cellular structure. Oak seedlings on the other hand have many plant components and during the physiological development of the entire plant, the plant parts would undoubtedly have diverse physiological processes which could be influenced by various C/N ratios. This could result in the mycorrhizal seedlings not having the proper C/N ratios conducive to optimum growth.

Third, mycelia and associated microorganisms may have immobilized one or more nutrients necessary for plant growth. Based on the results, it is doubtful that the macro-nutrients supplied in this study were growth limiting. (Tables 2 and 3). The leaf nitrogen content of both mycorrhizal and non-mycorrhizal seedlings was not significantly

different in most cases (Tables 4 and 5). However, the leaf phosphorous content was significantly less in mycorrhizal seedlings (Tables 4 and 5). Walsh and Beaton (1973) have reported that the medium range of phosphorus content in the leaves of oak species is between .13 and .28%. Leaf phosphorus percents of all the treatments (Tables 13, 14, and 15) are within or well above this range, therefore suggesting that phosphorus was probably not limiting growth. However, the nitrogen/phosphorus ratios (N/P) are quite variable between mycorrhizal and non-mycorrhizal seedlings with mycorrhizal seedlings having a greater N/P ratio in all cases. The N/P ratio will probably be quite variable in leaf tissue and have little effect on growth and physiological processes provided that the nitrogen and/or the phosphorus content does not fall below the critical level when that element(s) is actually limiting the growth of the plant part or the entire plant.

It is suggested that neither nitrogen nor phosphorus limited seedling growth and that the other macro-nutrients supplied were also in adequate supply for growth. However, micro-nutrients were not added and one or more of these may have been in critical supply for seedling growth. Competition between the symbionts for carbohydrates and other nutrients undoubtedly occurred. This competition was intensified by the physical restriction placed upon the organisms. The root systems and mycelial extensions of mycorrhizal seedlings were unable to explore beyond the container walls for other nutrients as they would normally do in nursery or field soils. Mycorrhizal

seedlings were also grown in containers which were infested with a large quantity (112 ml) of inocula. Literally, two plants were being cultured in one container. Thus, the reduced growth of the host was the effect of the symbiotic relationship.

Inoculated seedlings grown without nitrogen fertilization had very low mycorrhizal incidence percents at the end of each growth period (Table 5) when compared to all mycorrhizal seedlings in Table 4. Figures 1, 2, and 3 also show that low mycorrhizal incidence percents of control seedlings occurred in comparison to most fertilized seedling treatments. Low mycorrhizal incidence was probably due to limited growing-media nitrogen for fungal growth and seedling growth and development. Low or deficient nitrogen would not only cause decreased mycelia growth but also would indirectly reduce chlorophyll production and photosynthesis in the seedlings resulting in the chlorotic appearance of unfertilized seedlings. The results indicate that unfertilized seedlings had lighter green leaves when compared to the leaves of fertilized seedlings which indirectly supports the suggestion that growing-media nitrogen was limiting.

The nitrogen required for mycelia growth and subsequent mycorrhizae formation on unfertilized seedlings was undoubtedly supplied by the nitrogen acquired during inoculum synthesis and endogenous cotyledon nitrogen. Nitrogen may also have been supplied by nitrogen-fixing organisms within the growing medium.

Leaf Nitrogen and Mycorrhizal Incidence:

Leaf nitrogen weight and percent for 100-day-old mycorrhizal and non-mycorrhizal seedlings was significantly less than 60- and

140-day-old seedlings (Tables 4 and 5). The nitrogen decline may have been due to the translocation of nitrogenous compounds from the leaves for root growth, bud formation, and other biomass production during the rapid period of growth that occurred between 60 and 140 days. This is substantiated by the increase in root and total seedling weight between 60 and 100 days (Tables 4 and 5). The translocation of nitrogenous compounds back into the leaves of 140-day-old seedlings could have occurred after the demand for these compounds during the period of major growth had abated. Between 100 and 140 days of growth, the root systems were extensive and able to explore for nitrogen in the growing media more effectively, thus providing more nitrogen to the entire seedling. The results indicate that the leaves of 100-day-old seedlings did not look as green as the leaves of younger or older seedlings. This indirectly supports the suggestion that leaf nitrogen of 100-day-old seedlings was translocated.

II. Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings Fertilized With Sodium Nitrate and Ammonium Chloride

The effects of the nitrogen fertilizer on the growth variables are presented in the data in Tables 6, 7, and 8. The major differences between the treatments (excluding mycorrhizal incidence percents) included leaf phosphorus content, green leaf area percent, and total seedling weight.

Differences in leaf phosphorus content have been previously discussed. However leaf phosphorus percent of non-mycorrhizal seedlings

fertilized with sodium nitrate was greater than that of non-mycorrhizal seedlings fertilized with ammonium chloride (Tables 7 and 8). This is probably not biologically significant because leaf weight was less in non-mycorrhizal seedlings fertilized with sodium nitrate, but the absolute amount of phosphorus was not significantly different between the fertilizer treatments. This concentration effect of phosphorus between these treatments is analogous to the concentration effect of leaf nitrogen between mycorrhizal and non-mycorrhizal seedlings fertilized with either sodium nitrate or ammonium chloride after 60 and 140 days of growth (Tables 6 and 8). Leaf nitrogen percents were greater for mycorrhizal seedlings in these cases due to the concentration effect of an absolute amount of nitrogen in less leaf tissue than for an equivalent amount of nitrogen in the larger leaves of non-mycorrhizal seedlings.

Mycorrhizal seedlings had less green leaf area percents than non-mycorrhizal seedlings within each fertilizer treatment at the end of each growth period (Tables 6, 7, and 8). However, green leaf area percents of mycorrhizal seedlings fertilized with sodium nitrate were significantly less than any of the other treatments compared across all growth periods.

The leaf damage to mycorrhizal seedlings fertilized with sodium nitrate may be due to a greater increase in leaf succulency when compared to the other treatments. Leaf succulency may have been increased by three factors. First, rapid absorption and utilization of nitrogen may increase vegetative growth early in seedling growth

(Black, 1968), thereby increasing the length of time the new tissue remains in the succulent stage. Nitrate anions are highly soluble and mobile in soil solution and probably more available for absorption by the root than ammonium cations. The quantity and uptake of nitrogen in the form of nitrate may have been greater than in the form of ammonium thereby increasing the succulency of the leaves and predisposing them to heat injury.

Second, nitrate absorption may have enhanced oak mycorrhizae respiration. Willis and Yemm (1955) found a significant increase in the respiratory quotient and respiratory rate of barley roots during "nitrate-respiration" than found with barley roots in solution-culture without nitrogen. An increase in respiration rate reflects a probable increase in the utilization of carbohydrates. The carbohydrates supplied by the leaves of the host could have been utilized in order to facilitate the respiration of the mycorrhizae. Other researchers have reported that mycorrhizae have higher respiration rates than non-mycorrhizal roots (Routien and Dawson, 1943; Kramer and Hodgson, 1954; and Mikola, 1967). A reduction in leaf carbohydrates may have prolonged leaf succulency.

Third, P. tinctorius utilized nitrate (Beckjord, 1978; unpublished data) in pure culture but a coffee-brown discoloration of the nutrient-agar media was observed. Metabolites from the fungal exudates in response to nitrate assimilation may have interfered with the physiological development of the seedling leaves. The levels of sodium in the growing media of mycorrhizal seedlings fertilized with sodium

nitrate were low and probably did not promote root respiration. Willis and Yemm (1955) found that wide concentrations of Na^{+}, Ca^{++}, K^{+}, Cl^{-}, and PO_4^{-} had little effect on barley-root respiration. P. tinctorius produced the observed dark stain in nutrient-agar when supplied with either potassium nitrate (KNO_3) and sodium nitrate ($NaNO_3$). This response was likely due to nitrate assimilation and not potassium or sodium assimilation.

In most cases non-mycorrhizal seedlings fertilized with ammonium chloride had consistantly greater values for measured growth variables than non-mycorrhizal seedlings fertilized with sodium nitrate. Young Q. rubra seedlings may have preferentially utilized ammonium over nitrate for growth.

III. Comparisons of Mycorrhizal and Non-Mycorrhizal Seedlings Fertilized With Sodium Nitrate and Ammonium Chloride 15 Days and 40 Days After Planting

Regardless of fertilizer type, mycorrhizal and non-mycorrhizal seedlings fertilized early (15 days after planting) had greater total seedling weight than those seedlings fertilized late (40 days after planting) (Tables 10, 11, and 12). Nitrogen additions 40 days after planting were probably too late to be utilized as effectively as early nitrogen additions. This is verified by a four-fold increase in the number of seedlings that flushed with new growth when fertilized early.

Mycorrhizal incidence was enhanced when seedlings were fertilized late (Tables 10, 11, and 12). When nitrogen is supplied and absorbed during early seedling growth, carbohydrates are utilized for energy and biomass production. Competition for carbohydrates between the

host and mycobiant probably occurred with the host utilizing the greater portion of the carbohydrates (Harley and Lewis, 1969). This resulted in lower mycorrhizal incidence percents with treatments fertilized early (Tables 10, 11, and 12). This evidence is in agreement with the carbohydrate theory concerning mycorrhizal formation as proposed by Bjorkman (1942) and elucidated by Harley and Wade (1955) and Richards (1965) which suggests that mycorrhizal development occurs when carbohydrate synthesis exceeds carbohydrate utilization.

IV. Ectomycorrhizal Incidence and Comparisons of Individual Treatments

Ectomycorrhizae formation by Pisolithus tinctorius on Quercus rubra seedlings occurred with all inoculated seedlings. Ectomycorrhiza formation was observed during the period of leaf expansion and as early as 18 days after seed planting. This is in agreement with Morrison's (1956) observations with the development of ectomycorrhizae on silver beech. Morrison found that a weft of hyphae formed around the rootlet about the time the first leaves appeared. Soon thereafter, intercellular penetration occurred and a Hartig net was formed. Similar observations were made by Clowes (1950) for Fagus. Ectomycorrhizae were predominantly monopodial as observed with white oak by Maronek and Hendricks (1978, personal comm.) with frequent pinnate and compound pinnate structures present.

An attempt was made to duplicate the correlations Richards (1965) and Richards and Wilson (1963) performed between mycorrhizal infection percents and root nitrogen and phosphorus percents. In

this study, the correlation between leaf nitrogen percent and mycorrhizal incidence percent of 100-day-old seedlings fertilized late with sodium nitrate was r = .49. It is suggested that correlations of this nature be conducted between root nitrogen and/or phosphorus and mycorrhizal incidence. Correlations between growing-medium pH and mycorrhizal incidence percents were also not significant. A knowledge of rhizoplane pH prior to or at the onset of infestation by mycelia would be more appropriate as a means of identifying factors in the infection process whereas a knowledge of mycorrhizoplane pH may be more useful in studies of nutrient availabilities and ion exchanges between the mycorrhiza and soil solution.

The influence of sodium and chlorine on mycorrhizal development is unknown. Richards (1961) found that sodium did not affect mycorrhizal development with loblolly pine. Little information is available concerning the effects of chlorine on mycorrhizal development. Smith (1972) however, found that chloride uptake by excised beech mycorrhizae was less than phosphate uptake and that 45% of the chloride absorbed was found in the fungal sheath.

Results of treatments were compared in order to select one suitable for the production of large, healthy, Q. rubra seedlings. In this study large, healthy seedlings with ectomycorrhizae were produced in 100 days using a late application of ammonium chloride at the medium rate. The production of large, healthy non-mycorrhizal seedlings was obtained by the use of an early application of ammonium

chloride at the medium rate and a 100-day growth period. Field outplanting tests would be the next step in determining which treatment provides for the best survival and rapid growth of Q. rubra seedlings.

SUMMARY AND CONCLUSIONS

Quercus rubra seedlings were subjected to five treatment factors in a 2 x 2 x 2 x 4 x 3 factorial, replicated ten times in a randomized complete block design. The factors included: (1) *Pisolithus tinctorius* inoculated seedlings or non-inoculated seedlings; (2) nitrogen fertilizer supplied as sodium nitrate or as ammonium chloride; (3) fertilization at 15 days or at 40 days after planting of seed; (4) rates of nitrogen applied at 0, 0.0133, 0.0266 or 0.0532 gm N per seedling; and (5) the seedlings grown for 60, 100, or 140 days.

Mycorrhiza formation was observed as early as 18 days after seed planting. All inoculated seedlings were ectomycorrhizal with *P. tinctorius*. Ectomycorrhizae other than those characteristically produced by *P. tinctorius* were not observed and all non-inoculated seedlings were free of ectomycorrhizae.

The formation of mycorrhizae was low in the unfertilized seedlings. Unfertilized seedlings had chlorotic leaves which may have reduced carbohydrate synthesis sufficiently to hinder mycorrhizal infection. The nitrogen deficiency in the growing media of unfertilized seedlings also restricted seedling growth and probably fungal growth. Mycorrhizal infection was significantly enhanced with late applications of nitrogen fertilizer. However, growth of all seedlings was enhanced with higher rates of fertilizer as well as with early fertilizer applications.

Mycorrhizal seedlings had less basal area, total seedling weight, and root weight than non-mycorrhizal seedlings. This was primarily due to the competition by P. tinctorius and soil organisms for seedling carbohydrates and growing-media nutrients. Mycorrhizal seedlings also had less leaf area which probably reduced carbohydrate synthesis necessary for optimum growth. Mycorrhizal seedlings had less leaf phosphorus weight and percent than non-mycorrhizal seedlings. P. tinctorius and soil organisms were strong competitors for the growing-media phosphorus.

Mycorrhizal seedlings had less green leaf area percent than non-mycorrhizal seedlings due to increased heat damage to young succulent leaves. Leaf succulence of mycorrhizal seedlings may have been enhanced by delayed secondary wall thickening of leaf cells. P. tinctorius may have reduced the leaf carbohydrate content sufficiently to retard cellulose synthesis in the leaf.

Non-mycorrhizal seedlings fertilized with ammonium chloride generally had greater basal area, total seedling weight, and root weight than non-mycorrhizal seedlings fertilized with sodium nitrate. Non-mycorrhizal Q. rubra seedlings may have preferentially utilized ammonium over nitrate for early growth.

High quality Q. rubra seedlings well infected with mycorrhizae can be produced under greenhouse conditions in 100 days with a late application of ammonium chloride at 0.0266 gms N/seedling. High quality non-mycorrhizal seedlings can be produced in 100 days with an early application of ammonium chloride at 0.0266 gms N/seedling.

These seedlings appeared sufficiently well developed to provide good survival and growth in a field planting.

LITERATURE CITED

Alexander, M. 1961. Introduction to Soil Microbiology. John Wiley & Sons, Inc. N.Y. 472 p.

Barr, J., A. Goodnight, J. Sall, and J. Helwig. 1976. Stat. Anal. Sys. (SAS-76) SAS Institute Inc., P.O. Box 10066, Raleigh, N.C. 27605.

Beckjord, P. R. 1978. Unpublished studies.

Berry, C. and D. Marx. 1976. Sewage sludge and Pisolithus tinctorius ectomycorrhizae: Their effects on growth of pine seedlings. For. Sci. 22(3):351-358.

Bjorkman, E. 1942. Uber die Bedigungen der Mykorrhizabildung bei kiefer und Fichte. Symb. Bot. Upsal. 6(2):1-191.

Bjorkman, E. 1970. Mycorrhiza and tree nutrition in poor forest soils. Studia Forest. Suec. 83:1-23.

Black, C.A. 1968. Soil-Plant Relationships. John Wiley & Sons, Ind. N.Y. 792 p.

Bond, J. J. 1971. Mycorrhizal fungi of oak in North Carolina and their influence on uptake of potassium by oak seedlings. Ph.D. Dissertation. North Carolina State University, Raleigh, N.C. 43 p.

Bowen, G. D. 1973. Mineral nutrition of ectomycorrhizae. (Chap. 5. p. 151-205). In Ectomycorrhizae: Their Ecology and Physiology. G. C. Marks and T. T. Kozlowski (eds.) Academic Press, N.Y. 444 p.

Carrodus, B. B. 1966. Absorption of nitrogen by mycorrhizal roots of beech: I. Factors affecting the assimilation of nitrogen. New Phytol. 65:358-371.

Clowes, F. A. L. 1950. The structure of mycorrhizal roots of Fagus sylvatica. New Phytol. 49:248.

De La Cruz, R. E. 1974. The effects of mycorrhizal treatments and nitrogen-phosphorus fertilization on field growth of slash pine seedlings. Ph.D. Dissertation, University of Florida. 188 p.

Doak, K. D. 1955. Mineral nutrition and mycorrhizal association of bur oak. Lloydia. 18(3):101-108.

Finn, R. F. 1942. Mycorrhizal inoculation of soil of low fertility. Black Rock For. Paper (New York). 1(19):115-117.

Fisher, J.A. and G.S. Cox. 1978. Phosphorus nutrition of mycorrhizal and non-mycorrhizal red oak seedlings. (p. 480-493). Central Hardwood Forest Conference II - Proceedings held at Purdue Univ. (P. Pope, ed.). 549 p.

Grand, L. 1976. Personal communication.

Gray, L. E. and J. W. Gerdemann. 1969. Uptake of phosphorus-32 by besicular arbuscular mycorrhizae. Plant and Soil. 30(3): 415-422.

Hacskaylo, E. 1971. Metabolite exchanges in ectomycorrhizae. (Paper #15, p. 175-182) In Mycorrhizae. E. Hacskaylo (ed.) Proc. 1st N. Am. Conf. on Mycorrhizae. April, 1969. USDA For. Serv. Misc. Pub. 1189. 255 p.

Hacskaylo, E. 1973. Carbohydrate physiology of ectomycorrhizae. (Chap. 6, p. 207-230) In Ectomycorrhizae: Their Ecology and Physiology. G. C. Marks and T. T. Kozlowski (eds.) Academic Press, N.Y. 444 p.

Harley, J. L. 1969. The biology of mycorrhiza. Plant Sci. Monogr. Leonard Hill Ltd., London. 233 p.

Harley, J.L. and D.H. Lewis. 1969. The physiology of ectotropic mycorrhizas. (p. 53-81). Advances in microbial physiology. 3:

Harley, J. L. and J. S. Waid. 1955. The effect of light upon the roots of beech and its surface population. Plant and Soil. 7:96-112.

Hatch, A. B. 1937. The physical basis of mycotrophy in the genus Pinus. Black Rock For. Bull. No. 6. p. 1-188.

Howe, V. K. 1964. Mycorrhizal fungi of white oak. Ph.D. Dissertation, Iowa State Univ. of Science and Technology, Ames, Iowa. 115 p.

Jennings, D. H. 1964. Changes in the size of or the phosphate pools in mycorrhizal roots of beech with reference to absorption of the ion from the external medium. New Phytol. 63:181-193.

Kitson, R. E. and M. G. Mellon. 1944. Colorimetric determination of phosphorus as molybdivanadophosphoric acid. Ind. and Eng. Chem. (Anal. Ed.) 16:379-383.

Klyushnik, P. I. 1952. Oak mycorrhizal fungi. Lesn. Khoz. 5(8): 63-65.

Kramer, P. J. and R. H. Hodgson. 1954. Differences between mycorrhizal and non-mycorrhizal roots of loblolly pine. Proc. Internat. Bot. Congr., 8th (Paris). 13:133-134.

Marks, G.C. and R.C. Foster. 1973. Structure, morphogenisis, and ultrastructure of ectomycorrhizae. (Chap. 1, p. 1-41). In Ectomycorrhizae: Their Ecology and Physiology. G. C. Marks & T. T. Kozlowski (eds.). Academic Press, N. Y. 444 p.

Maronek, D. and J. Hendricks. 1978. Personal communication.

Marx, D. H. 1973. Mycorrhizae and feeder root diseases (Chap. 9, p. 351-382) In Ectomycorrhizae: Their Ecology and Physiology. A. C. Marks and T. T. Kozlowski (eds.). Academic Press, N.Y. 444 p.

Marx, D. H. 1976. Synthesis of ectomycorrhizae on loblolly pine seedlings with basidiospores of Pisolithus tinctorius. For. Sci. 22:13-20.

Marx, D. H. 1977a. Comparison of Abbott's and Athen's dried inoculum of Pisolithus tinctorius at different densities with good or poor soil fumigated for ectomycorrhizal development on loglolly pine and northern red oak seedlings in microplots at Whitehall (p. 19). In Abstracts: 3rd N. Am. Conf. on Mycorrhizae (Aug. 23-25, 1977) Athens, Ga. 115 p.

Marx, D. H. 1977b. Ectomycorrhizal development by different isolates and inoculum rates of Pisolithus tinctorius on northern red oak seedling. (p. 20) In Abstracts: 3rd N. Am. Con. on Mycorrhizae (Aug. 23-25, 1977), Athens, Ga. 115 p.

Marx, D. H. 1978. Tree host range and world distribution of the ectomycorrhizal fungus Pisolithus tinctorius (unpublished to date).

Marx, D. H. and J. P. Barnett. 1974. Mycorrhizae and containerized forest tree seedlings. (p. 85-92). In Proc. N. Am. Cont. For. Tree Seedling Symp., Great Plains Agr. Coun. Pub. No. 68: 453 p.

Marx, D.H. and W.C. Bryan. 1973. The significance of mycorrhizae to forest trees (Chapter 7, p. 107-117). In Forest Soils and Forest Land Mgt., Proc. 4th Am. For. Soils Conf., Laval Unic., Quebec.

Marx, D. H. and W. C. Bryan. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont Pisolithus tinctorius. For. Sci. 21:245-254.

Marx, D. H., W. C. Bryan, and C. E. Cordell. 1976. Growth and ectomycorrhizal development of pine seedlings in nursery soils infested with the fungal symbiont Pisolithus tinctorius. For. Sci. 22: 91-100.

Marx, D.H., A.B. Hatch, and J. Mendicino. 1977. High soil fertility decreases sucrose content and susceptibility of loblolly pine roots to ectomycorrhizal infection by Pisolithus tinctorius. Can. J. Bot. 55:1569-1574.

McComb, A. L. 1943. Mycorrhizae and phosphorus nutrition of pine seedlings in a prairie soil nursery. Iowa Agr. Exp. Sta., Res. Bull. 314:582-612.

McFee, W. W. and E. L. Stone, Jr. 1968. Ammonium and nitrate as nitrogen sources for Pinus radiata and Picea Glauca. Soil Sci. Soc. Am. Proc. 32:879-883.

Melhuish, J. 1976. Personal communication.

Melin, E. and H. Nilsson. 1957. Transport of C^{14} labelled photoshythate to the fungal associate of pine mycorrhiza. Svensk. Botan. Tidskr. 51: 166.

Menge, J. A. 1975. The effect of forest fertilization upon mycorrhizae of loblolly pine. Ph.D. Dissertation. N.C. State Univ., Raleigh, N.C. 109 p.

Mikola, P. 1967. The effect of mycorrhizal inoculation on the growth and root respiration of Scotch pine seedlings. Proc. 14th Congr., Internat. Union For. Res. Organiz., Munich, Part V, Sect. 24:100-111.

Mikola, P. 1973. Application of mycorrhizal symbiosis in forestry practice. (Chap. 10, p. 383-411) In Ectomycorrhizae: Their Ecology and Physiology. G. C. Marks and T. T. Kozlowski (eds.). Academic Press, N.Y. 444 p.

Miller, K. C. 1973. Variant nutrient effects upon the mycorrhizal of white oak (Quercus alba L.) Ph.D. Dissertation. Iowa State Univ., Ames, Iowa, 121 p.

Modess, O. 1941. Zur Kenntnis der Mykirrhizbildner von Kiefer und Fichte. Symb. Bot. Upsal. 5:1-147.

Morrison, T. M. 1956. Mycorrhiza of silver beech. N.Z.J. For. 7(3):47-60.

Norkrans, B. 1949. Some mycorrhiza-forming Tricholoma species. Svensk. Botan. Tidskr. 43:438-490.

Park, J. Y. 1971. Some ecological factors affecting the formation of Cenococcum mycorrhizae on basswood in southern Ontario. Can. J. Bot. 49:95-97.

Peterson, L. A. and G. Chesters. 1964. A reliable total nitrogen determination on plant tissue accumulating nitrate nitrogen. Agron. J. 56(1):89-90.

Phares, R. E. 1964. Mineral nutrition of forest tree seedlings. Ph.D. Dissertation. Iowa State Univ. of Science and Technology. Ames, Iowa. 168 p.

Richards, B. N. 1961. Soil pH and mycorrhiza development in Pinus. Nature (Lond.) 190:105-106.

Richards, B. N. 1965. Mycorrhizae development of loblolly pine seedlings in relation to soil reaction and the supply of nitrate. Plant & Soil. 22(2):187-199.

Richards, B.N. and G.L. Wilson. 1963. Nutrient supply and mycorrhiza development in Caribbean pine. For. Sci. 9:405-412.

Rambelli, A. 1973. The rhizosphere of mycorrhizae. (Chap. 8, p. 299-349). In Ectomycorrhizae: Their Ecology and Physiology. G. C. Marks and T. T. Kozlowski (eds.) Academic Press, N.Y. 444 p.

Routien, J. B. and R. F. Dawson. 1943. Some inter-relations of growth, salt absorption, respiration and mycorrhizal development in Pinus echinata Mill. Amer. J. Bot. 30:440-451.

Ruehle, J. L. 1978. Personal communication.

Russell, T. E. 1971. Seeding and planting upland oaks. (p. 49-54). Oak Symp. Proc. USDA For. Serv. Northeast For. Exp. Sta. held at Morgantown, W. Va. 161 p.

Shemakhanova, N. M. 1962. Mycotrophy of Woody Plants. Akad. Mauk SSSR, Moscow. (Transl.). Israel Program for Scientific Translation Ltd. 1967. Jerusalem. 329 p.

Slankis, V. 1973. Hormonal relationships in mycorrhizal development. (Chap. 7, p. 231-298), In Ectomycorrhizae: Their Ecology and Physiology. G. C. Marks & T. T. Kozlowski (eds.). Academic Press, N.Y. 444 p.

Smith, F. A. 1972. A comparison of the uptake of nitrate, chloride and phosphate by excised beech mycorrhizas. New Phytol. 71:875-882.

Tabor, H. G. and W. W. McFee. 1972. Nitrogen influence on phosphorus uptake by Pinus radiata seedlings. For. Sci. 18(2):126-132.

Theodorou, C. and G.D. Bowen. 1969. The influence of pH and nitrate on mycorrhizal associations of Pinus radiata D. Don. Austral. J. Bot. 17:59-67.

Trappe, J. M. 1978. Personal communication.

Walsh, L. M. and J. D. Beaton (eds.) 1973. Soil Testing and Plant Analysis. Soil Sci. Soc. of Am., Inc., Madison, Wisconsin. 491 p.

Willis, A. B. and E. W. Yemm. 1955. The respiration of barley plants. VIII: Nitrogen assimilation and the respiration of the root system. New Phytol. 54(2):163-181.

APPENDIX

Table 1

Appendix Table 1. Quantity and form of nutrients used in nutrient solutions for fungus culturing, inoculum production, and in growing medium.

Chemical	Hagem's modified by Modess (fungus culture)	Melin-Norkrans (MN) solution	MN modification #1 (inoculum production)	MN modification #2 (growing medium)
KH_2PO_4	.5 gm	.5 gm	.5 gm	.714 gm
$MgSO_4 \cdot 7H_2O$	.5 gm	.15 gm	.15 gm	.214 gm
NH_4Cl	.5 gm	--	--	(1)
$(NH_4)_2HPO_4$	--	.25 gm	.25 gm	(1)
NaCl	--	.025 gm	.025 gm	.0375 gm
$CaCl_2$	--	.05 gm	.05 gm	.0714 gm
D-Glucose	5.0 gm	2.5 gm	5.0 gm	--
$CaSO_4 \cdot 2H_2O$	--	--	--	.357 gm
Thiamine - HCl	--	25.0 ugm	50.0 ugm	--
Malt Extract	5.0 gm	--	--	--
Bacto-agar	15.0 gm	--	--	--
$FeCl_3$ 1% solu	.5 ml	1.2 ml	1.2 ml	1.71 ml
H_2O distilled	to 1000 ml	to 1000 ml	to 1000 ml	to 1000 ml (tap water)

(1) Nitrogen applied as $NaNO_3$ and NH_4Cl in accordance with study design.

The vita has been removed from the scanned document

THE INCIDENCE OF ECTOMYCORRHIZAE BY *PISOLITHUS TINCTORIUS* ON *QUERCUS RUBRA* SEEDLINGS FERTILIZED WITH SODIUM NITRATE AND AMMONIUM CHLORIDE

by

Peter Rains Beckjord

(ABSTRACT)

Quercus rubra seedlings were subjected to five treatment factors in a factorial design replicated ten times in a randomized complete block design. The factors included: (1) *Pisolithus tinctorius* inoculated seedlings or non-inoculated seedlings; (2) nitrogen fertilizer supplied as sodium nitrate or as ammonium chloride; (3) fertilization at 15 days or at 40 days after planting of seed; (4) rates of nitrogen applied at 0.0, 0.0133, 0.0266, or 0.0532 g N per seedling; and (5) the seedlings grown for 60, 100, or 140 days.

All 960 seedlings were grown in a peatmoss/vermiculite medium in one-liter containers in a greenhouse and fertilized at the time of planting with a nutrient solution excluding nitrogen. Growing-medium moisture was maintained between 0.3 and 1.0 bars matric suction throughout the growing periods. Details are given with regards to inoculum synthesis, seed planting, care for seedlings, fertilization and media nutrient status, and data collection procedures.

Ectomycorrhiza formation was observed as early as 18 days after planting. All inoculated seedlings (480) were ectomycorrhizal with P. tinctorius. Ectomycorrhizae other than those characteristically produced by P. tinctorius were not observed and all non-inoculated seedlings (480) were free of ectomycorrhizae. Ectomycorrhiza formation was enhanced with late nitrogen applications; however, infection was low on unfertilized seedlings. Mycorrhizal seedlings generally had less basal area, total seedling weight, root weight, leaf phosphorus weight, and leaf phosphorus percent than non-mycorrhizal seedlings. Mycorrhizal seedlings also had decreased leaf areas and green leaf area percents than non-mycorrhizal seedlings.

Total seedling weight was enhanced with early applications of nitrogen fertilizer. Non-mycorrhizal seedlings fertilized with ammonium chloride generally had greater basal area, total seedling weight, and root weight than non-mycorrhizal seedlings fertilized with sodium nitrate. The probable effects of P. tinctorius in enhancing leaf damage and reducing seedling growth and leaf phosphorus contents are discussed. Several specific fertilization treatments are recommended for the production of large and healthy mycorrhizal and non-mycorrhizal northern red oak seedlings.