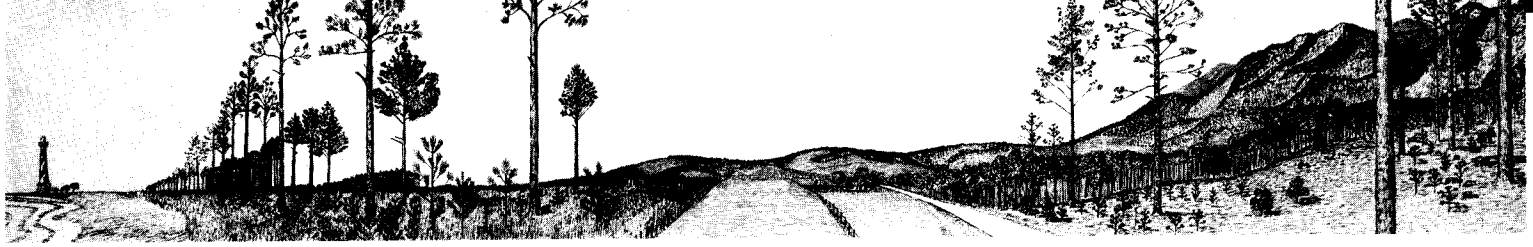

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SYNTHESIS OF *PISOLITHUS* ECTOMYCORRHIZAE ON PECAN SEEDLINGS IN FUMIGATED SOIL

by Donald H. Marx[1]

ABSTRACT.—Curtis variety of pecan (*Carya illinoensis*) seedlings were grown for 8 months in fumigated soil infested at sowing with mycelial inoculum of *Pisolithus tinctorius*. *Pisolithus* ectomycorrhizae were formed on all inoculated seedlings and significantly improved their growth over control seedlings. Inoculated and control seedlings also formed ectomycorrhizae with naturally occurring *Thelephora terrestris* which produced abundant fruit bodies on seedling stems; most were formed on control seedlings. The results showed that the techniques developed to form *Pisolithus* ectomycorrhizae on pine seedlings are also applicable to pecan seedlings except that inoculum should be incorporated 20 cm deep rather than 10 cm, which is used for pine.

Keywords; Regeneration of hardwoods, symbiotic association, nursery seedling production, *Thelephora terrestris*, *Carya illinoensis*.

Pecan (*Carya illinoensis* Wang. K. Koch) is a widely planted shade and orchard tree in the southern part of the United States and in other parts of the world (Smith and others 1974). Pecan cultivars are usually grafted onto seedling rootstocks in the nursery, but clonal rootstocks can also be produced by using various rooting and air-layering techniques (Pokorny and Sparks 1967; Sparks and Pokorny 1966). The planting stock is usually grown for 2 or more years in fumigated nursery soil, then transplanted to orchards either as bareroot or root-balled seedlings. Even with good cultural practices, many seedlings do not survive transplanting, and those that survive often do not grow appreciably in height until the second or third year. Although slow growth after outplanting is common to most hardwood trees (Russell 1971), the cause is unknown. There is reason to believe, however, that the problem may be at least partially alleviated by producing nursery seedlings that are heavily ectomycorrhizal with highly beneficial fungi.

Pecan trees in urban or orchard plantings have abundant ectomycorrhizae. Woodroof (1933) described the ectomycorrhizae of pecan in Georgia orchards and listed several species of fungi associated with them. More recently in Georgia, Marx and Bryan (1969) found that *Scleroderma bovista* Fr. formed abundant ectomycorrhizae following soil application of various fungicides and nematocides in treatment of root-diseased pecan trees. The appearance of this specific ectomycorrhizae was concurrent with the disappearance of foliar disease symptoms on the trees. Nothing is known of the significance of ectomycorrhizae to the physiological activity of roots of transplanted pecan seedlings.

Recently, techniques were developed to tailor pine and oak seedlings with *Pisolithus tinctorius* (Pers.) Coker and Couch ectomycorrhizae in fumigated soils of tree nurseries (Marx and others 1976; Marx and Artman 1978; Marx and others 1978; Marx 1979a, 1979b). Pine seedlings with abundant *Pisolithus* ectomycorrhizae formed in the nursery survive and grow better after outplanting on various reforestation sites in the South than seedlings with ectomycorrhizae formed by naturally occurring fungi in the nursery (Marx 1978; Marx and others 1977; Berry and Marx 1978). *Pisolithus* basidiocarps have been observed in pecan orchards in Arizona (Marx

[1]Marx is Plant Pathologist and Director, Institute for Mycorrhizal Research and Development, Forestry Sciences Laboratory, Athens, Ga. 30602.

1977) and southern California (Menge, personal communication). These observations indicate that *P. tinctorius* is probably ectomycorrhizal on pecan.

The research reported here had two purposes: (1) to determine whether the techniques developed to tailor pine and oak seedlings with *Pisolithus* ectomycorrhizae in nurseries would also be effective on pecan, and (2) to determine the significance of soil infestation with mycelial inoculum of *P. tinctorius* to growth and ectomycorrhizal development of pecan seedlings in an experimental nursery. Since these tests were successful, field trials on the significance of *Pisolithus* ectomycorrhizae to survival and growth of pecan seedlings should be undertaken.

MATERIALS AND METHODS

Ten wood-frame microplots, each 1.2 × 0.5 × 0.3 m deep, were placed 1 m apart on level ground. The frames and surrounding soil were fumigated in place. Each frame was filled with a fumigated mixture (2:1:1 volume ratio) of forest clay loam, sand, and pine bark. The soil mixture had a pH of 4.9 and contained 8 p/m of available P and 59, 190, and 32 p/m of exchangeable K, Ca, and Mg. Total nitrogen was 180 p/m and the organic matter content was 1.9 percent.[2] All fumigation was done with methyl bromide (MC-2, Dow Chemical Co., Midland, Michigan) at recommended rates under clear polyethylene.

Vegetative inoculum of *P. tinctorius* (isolate 138) was grown for 3 months in a nutrient culture of vermiculite and peat moss and processed as previously described (Marx and Bryan 1975). Leached inoculum (3 liters/m^2 of soil surface), commercial 10-10-10 fertilizer (280 kg/ha) and hydrated lime (1008 kg/ha) were broadcast over the soil surface of each of five microplots and mechanically mixed into the upper 10 cm of soil. Autoclaved inoculum (control) and the fertilizer and lime at the same rates were mixed similarly into the soil of the five remaining microplots.

Stratified pecan nuts of the Curtis variety were cracked to hasten germination and planted 10 cm apart in two 1.2-m-long rows (11 seeds per row) per microplot. Fumigated pine straw mulch was placed 2 cm deep over the soil in each microplot. The study was installed in April 1976. During the 1976 growing season, seedlings were fertilized once with 36 kg N/ha (as NH_4NO_3) in June and July and were irrigated at least twice weekly after seed germination. Starting in July, all microplots were examined weekly for fruit bodies of *P. tinctorius* and other ectomycorrhizal fungi and their incidence recorded.

In December 1976, the seedlings were vertically cut between rows and undercut 20 cm with a shovel. Seedlings were removed by hand and washed in water. Heights, root-collar diameters, and top (without leaves) and root fresh weights were recorded for each seedling. The percentage of feeder roots that were ectomycorrhizal were visually estimated without magnification (Marx and Bryan 1975). Randomly selected roots were free-hand sectioned, mounted in phloxine-lactophenol and examined at 100× to confirm the presence or absence of the Hartig net and to measure the thickness of the fungus mantle. All data were subjected to analysis of variance at $P = 0.05$.

RESULTS

Pisolithus formed ectomycorrhizae on all pecan seedlings in inoculated plots. Most of these ectomycorrhizae were on the upper half (10 cm) of the root system. Seedlings with *Pisolithus* ectomycorrhizae had significantly greater heights, root-collar diameters, and root and top fresh weights than the control seedlings (table 1). The ectomycorrhizae formed by *P. tinctorius* were morphologically similar (elongated coralloid) to those formed on pecan by *Scleroderma bovista* (Marx and Bryan 1969), but were yellowish brown rather than white. Many yellow-brown hyphal strands were attached to these ectomycorrhizae. The hyphae of *P. tinctorius* forming the Hartig net penetrated between at least two layers of cortex cells; fungus mantles were from 22 to 56 μ thick. No fruit bodies of *P. tinctorius* were observed in inoculated plots.

Noninoculated control seedlings also formed abundant ectomycorrhizae, but with naturally occurring fungi. Forty-one fruit bodies of *Thelephora terrestris* Ehrh. ex Fr. occurred on seedling stems in the control plots and 19 were observed on seedlings in inoculated plots. Due to its numerous associations with ectomycorrhizae of trees (Marx 1978), *T. terrestris* was assumed to be the most prevalent naturally occurring fungus forming ectomycorrhizae on the pecan seedlings. These *Thelephora* ectomycorrhizae were light brown

[2]Soil analysis done by Carol G. Wells, U.S. Department of Agriculture Forest Service, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Research Triangle Park, N. C. 27709.

Table 1.—Growth and ectomycorrhizal development of pecan seedlings (Curtis variety) after 8 months in fumigated soil artificially infested with *Pisolithus tinctorius* (Pt). Each number is the mean of 17 to 21 seedlings from each of five plots per treatment.

Treatment	Height	Root-collar diameter	Fresh weights			Feeder roots ectomycorrhizal with:		
			Top	Root	Total	Pt	Other	Total
	cm		*g*			*Percent*		
Pisolithus tinctorius	32.8*	0.71*	5.2*	22.0*	27.2*	24*	35*	60*
Noninoculated	28.9	0.64	3.9	18.0	21.9	0	56	56

*Significantly different from noninoculated seedlings (P = 0.05).

and were primarily bifurcate without much repeated branching. Few hyphal strands were apparent on these roots. The hyphae of the Hartig net usually penetrated between only one cortex cell layer; fungus mantles varied in thickness from 25 to 32 μ.

DISCUSSION

It is apparent that the techniques developed for forming *Pisolithus* ectomycorrhizae on pine seedlings are also applicable for pecan seedlings. However, the location of most *Pisolithus* ectomycorrhizae on the upper half of the root system of the pecan seedlings in this study suggests that the inoculum should be incorporated deeper than the 10 cm used for pine. The majority of the feeder roots of pecan was produced in the upper 20 cm of soil; therefore, inoculum should be mixed to a 20-cm depth. Incorporating inoculum to this depth should increase the amount of *Pisolithus* ectomycorrhizae over that reported here. This increase should benefit pecan seedlings since pine seedlings with the greatest amount of *Pisolithus* ectomycorrhizae at planting generally survive and grow better than those with a smaller amount of *Pisolithus* ectomycorrhizae (Marx and others 1977).

Since growth of pecan seedlings was stimulated by *Pisolithus* ectomycorrhizae in the nursery, the potential of this fungus to improve survival and growth of pecan in orchards should be determined.

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