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Synthesis of *Pisolithus tinctorius* Ectomycorrhizae on Seedlings of Four Woody Species[1]

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Abstract. **Fresh and stored vegetative mycorrhizal inoculum of *Pisolithus tinctorius* (Pers.) Coker & Couch were incorporated into steamed media containing slow-release fertilizer. Austrian pine (*Pinus nigra* Arnold.), Norway spruce (*Picea abies* (L.) Karst.) and Canadian hemlock (*Tsuga canadensis* (L.) Carriere) seedlings were grown in 165-ml tubes in a greenhouse for 7 months. Yellow birch (*Betula alleghaniensis* Britt.) seedlings were grown in 7.6 cm diameter pots. All species formed mycorrhizae but the percent of seedlings which formed mycorrhizae differed among species. Stored inoculum was inferior to fresh inoculum. Differential growth responses to *P. tinctorius* also occurred among the species. *P. tinctorius* significantly increased height and stem diameter of Norway spruce only, and reduced the stem diameter of Austrian pine.**

Mycorrhizal fungi are responsible for a variety of beneficial responses in plants such as increased growth, ion uptake, disease resistance and rooting of cuttings (1, 4, 15). However, plant species may vary in their response to mycorrhizal fungi (3. 6). The ecotypic variation characteristic of many tree species may also be characteristic of mycorrhizal fungi. Furthermore, we have found that isolates of the same mycorrhizal fungus may influence plant growth differently under a given set of environmental conditions (5. 6). Identification of host ranges is essential to determining whether a fungal symbiont may be capable of influencing plant performance under a given set of environmental conditions. For example, production of seedlings specifically infected with ecologically-adapted mycorrhizal isolates is essential for adequate productivity on reclaimed stripmine lands (8).

Production of mycorrhizal plants has some unique problems; simply adding mycorrhizal inoculum to the growing medium may not work. One factor which may require modification is fertilization. Fertility influences both development of mycorrhizae and the effects the fungi have on their host. Most reported ectomycorrhizal infections and subsequent beneficial effects attributed to the fungus are often found in low fertility soils. Normal fertilization practices for seedling production tend to inhibit mycorrhizal development (11). Consequently, most host-range studies involving artificial inoculations of seedlings with a mycorrhizal fungus are generally conducted under low fertility regimes. However, mycorrhizal development is not always indicative of nutritionally poor soils, but may also be dependent on a balance of nutrients (12, 14). In addition, the rate of fertilizer release may influence mycorrhizal development. We have found it possible to produce plants with abundant mycorrhizae and at an adequate growth rate by using a slow release fertilizer (5).

Our objectives were to: 1) produce by inoculation *P. tinctorius* ectomycorrhizae on Austrian pine, Norway spruce, Candian hemlock and yellow birch; 2) determine the effects of ectomycorrhizae on the development of container-grown seedlings and 3) determine the effect of storing *P. tinctorius* inoculum on its subsequent capacity to form mycorrhizae.

Materials and Methods

Seed of yellow birch and Austrian pine were collected from single trees at Lexington, Kentucky; all other seed were obtained from a commercial tree seed distributor. Seeds were screened for viability and stratified (16). One isolate of *Pisolithus tinctorius,* M3, was obtained in the summer of 1977 from sporcarp tissue collected on loblolly pine *(Pinus taeda)* on a stripmine in Laurel County, Kentucky. A second *P. tinctorius* isolate, M1, (Original no. 138) originally isolated in Georgia, was obtained from D. H. Marx, Institute of Mycorrhizal Research and Development, U.S. Forest Service, Athens, Georgia. Vegetative mycorrhizal inoculum of both isolates was produced in 3.75 liter jars by the procedures of Marx and Bryan (10). After 3 months, the inoculum was washed, excess water was removed by squeezing, and inoculum was stored at 3°C for 24 hr. The M3 inoculum was incorporated at the rate of 1:8 (by volume) into steamed 1 peat:1 perlite (by volume) medium containing 1.1 kg/m^3 of 18N-2.6P-9.9K Osmocote, a slow release fertilizer (Sierra Chemical Co., Milipitas, California) and 0.11 kg/m^3 of fritted trace elements. M1 inoculum was incorporated at a rate of 1:10 (by volume) into steamed Weblite a medium composed of expanded shale, sphagnum peat and composted pine bark (Weblite Corporation, P.O. Box 12887, Roanoke, Virginia).

Seedlings of all 4 species were initially grown in flats containing a steamed 1 peat:1 perlite (by volume) medium. Month-old Norway spruce, Canadian hemlock and Austrian pine seedlings were transplanted to 165 ml Leach 'Super Cell' tube containers (Ray Leach "Conetainer" Nursery, 1787 North Pine St., Canby, Oregon) containing the peat:perlite medium inoculated or not inoculated with isolate M3. Because of the risk of cross-contamination, seedlings of each experimental unit were grouped in a single block within the tube racks (7 rows of 7 tubes); however, environmental conditions for each block were identical. In a separate experiment, 1-month-old yellow birch seedlings were transplanted to 7.6 cm diameter pots containing the Weblite medium inoculated or not inoculated with isolate M1. Four weeks after transplanting the yellow birch seedlings were fertilized with 0.6 g/pot of 14N-6P-11.6K Osmocote and watered once with a complete minor element solution (2).

To test the effect that storage may have on viability of vegetative inoculum, *P. tinctorius* inoculum, isolate M3, was produced and prepared as previously described. About 2 liters of the inoculum was placed in a polyethylene bag, sealed and stored at 3°C. After 6 days, the inoculum was removed from storage and incorporated into a steamed 1 peat:1 perlite (by volume) medium using the same ingredients and following the same procedures previously described. Month-old Norway

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spruce and hemlock seedlings were transplanted into Leach 'Super Cell' tube containers containing the peat-perlite medium.

Seedlings were grown in the greenhouse under an extended 18 hr photoperiod (one 60 watt incandescent bulb/0.47 m^2) and prevailing greenhouse temperatures of 20 to 28°C during the day and 18 to 20°C at night and watered as needed with tap water. After 7 months, height and stem diameters were determined and the evaluations of mycorrhizal development were made by visual estimation. Statistical evaluations of growth data consisted of Student's "t" tests (13).

Results and Discussion

All 3 conifer species formed mycorrhizae with *Pisolithus tinctorius*. The large, swollen, yellow bifurcate mycorrhizae of Austrian pine (Fig. 1A) were conspicuous. The bifurcate mycorrhizae of Norway spruce were also prominant (Fig. 1B). The mycorrhizae of Canadian hemlock were not easily seen, but the characteristic brown mycelium of *P. tinctorius* was profuse on mycorrhizal root systems (Fig. 1C). From our experience, visible development of mycelium from inoculum never occurs in the absence of infection and mycorrhizal formation.

The percent of seedlings with mycorrhizae and percent of the root system that was mycorrhizal differed among species. Nearly all Norway spruce and Canadian hemlock seedlings were mycorrhizal while only 51 percent of the inoculated Austrian pine was mycorrhizal (Table 1). The percent of the root system that was mycorrhizal was similar for all three conifer species, ranging from 54 to 67.

Six of 20 yellow birch seedlings inoculated became mycorrhizal. On infected plants development of mycorrhizae

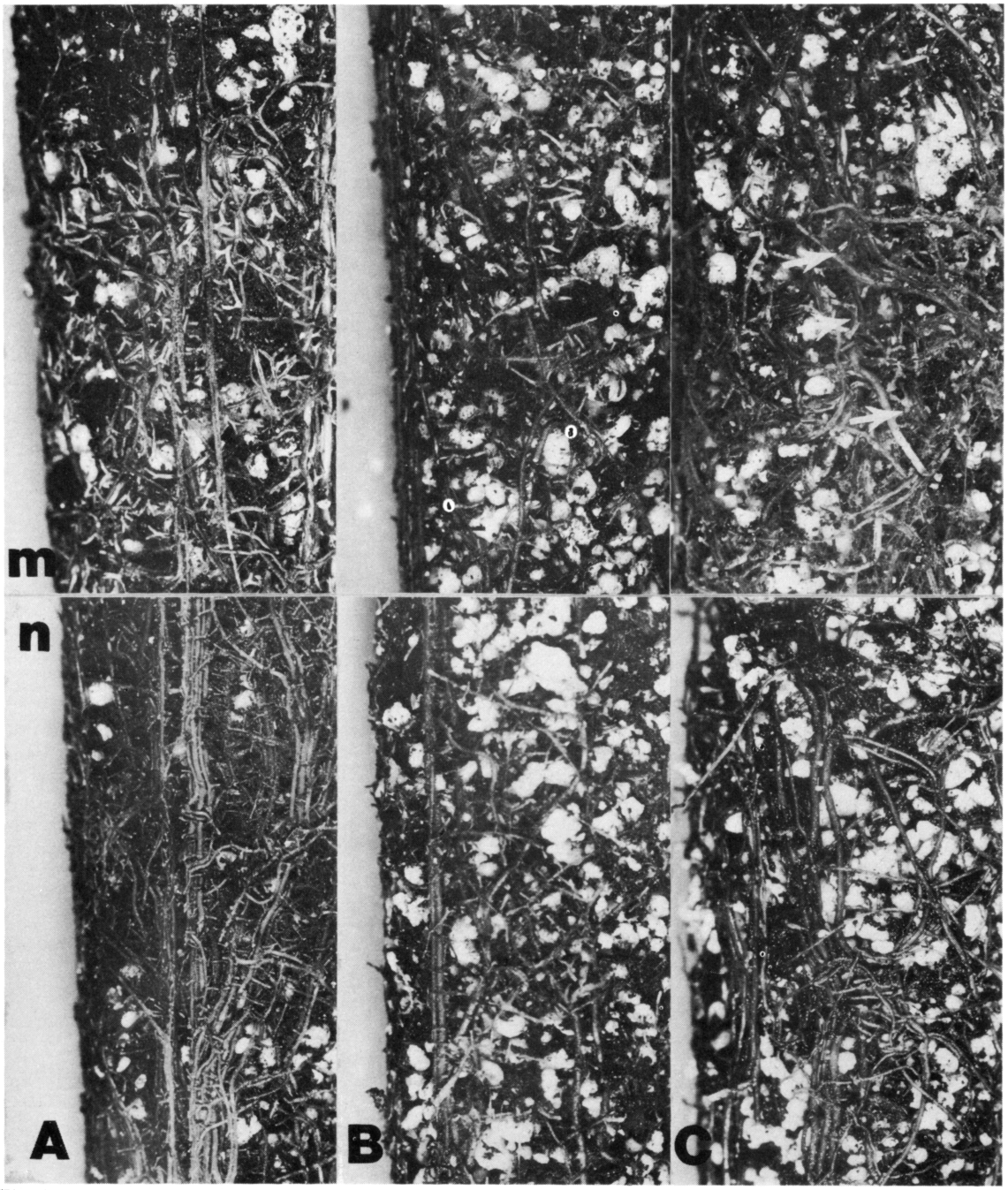

Fig. 1. Inoculated mycorrhizal (M, above) and noninoculated nonmycorrhizal (N, below) roots systems of Austrian pine (A), Norway spruce (B), and Canadian hemlock (C) seedlings. Intensive areas of mycelium of *Pisolithus tinctorius* on the mycorrhizal Canadian hemlock seedling are indicated by arrows.

Table 1. Effect of fresh and stored vegetative inoculum of *Pisolithus tinctorius* on mycorrhizal formation of 3 woody species after 7 months.

Species	Plants with mycorrhizae (%)[z]		Root system mycorrhizal (%)	
	Fresh inoculum	Stored inoculum	Fresh inoculum	Stored inoculum
Norway spruce	98	24	65	64
Canadian hemlock	100	4	54	40
Austrian pine	51	–	67	–

[z]Means of 49 seedlings.

Table 2. Growth of 3 woody species grown for 7 months with or without *Pisolithus tinctorius*.

Species	Height (cm)		Stem diameter (mm)	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Norway spruce	12.4	17.3*	2.4	2.9*
Canadian hemlock	15.5	15.1	2.1	1.9
Austrian pine	8.0	7.9	3.5*	3.1

*Significant at the 5% level. Means of 49 trees.

and mycelium was profuse, but unfortunately the percent of the root system that was mycorrhizal and growth measurements were not determined. Fruiting bodies of *Pisolithus tinctorius* were produced only on yellow birch seedlings 3 months after transplanting into the inoculated medium.

Infection by *Pisolithus tinctorius* significantly increased the height and stem diameters of only Norway spruce (Table 2). Heights of inoculated Canadian hemlock and Austrian pine seedlings were comparable to respective noninoculated seedlings, but inoculating Austrian pine seedlings with *P. tinctorius* significantly reduced stem diameters.

Storing *Pisolithus tinctorius* inoculum drastically reduced its capacity to synthesize mycorrhizae (Table 1). Using stored inoculum reduced the number of seedlings that were mycorrhizal for Norway spruce and Canadian hemlock by 74 and 96 percent, respectively. This result is consistent with a number of other experiments in which we inoculated pine and oak species with fresh and stored *P. tinctorius* inoculum. Until more information is available on storage conditions, especially moisture content, methods of drying, and storage temperature, we recommend using inoculum as soon as feasible after preparation.

Experimental proof for mycorrhizal production by *Pisolithus tinctorius* has been reported for European white birch, western hemlock, Austrian pine and noble fir (7), but this appears to be the first experimental synthesis of *P. tinctorius* mycorrhizae on Norway spruce, yellow birch and Canadian hemlock. Marx and Bryan (9) were unsuccessful in synthesizing *P. tinctorius* mycorrhizae on Norway spruce while experimental synthesis on Austrian pine was rated as poor. No quantitative measures of infection or number of seedlings infected was reported for Austrian pine. They used a different growing medium, fertilizer and isolate of *P. tinctorius* than those used in this study. We obtained good mycorrhizal development on all Norway spruce and Canadian hemlock seedlings. Although only one half of the inoculated Austrian pine seedlings were infected, the percent of the root system that was mycorrhizal was fairly good. The low percentage of yellow birch seedlings infected with *P. tinctorius* mycorrhizae in this study, may, in part, be attributed to isolate specificity, lower inoculum levels or the use of a different fertilizer on growing medium than that used for the 3 conifer species. Different isolates of *P. tinctorius* influenced growth of pin oak seedlings differently under a given set of cultural conditions (5). Successful mycorrhizal development and subsequent benefits of the association appear to be contingent on fungal isolate-host specificity, methods of handling of the mycorrhizal inoculum, and cultural conditions used during the production of seedlings.

Literature Cited

1. Harley. J. L. 1969. The biology of mycorrhizae. Leonard Hill, London.
2. Hoagland, D. R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. *Calif. Agr. Expt. Sta. Cir.* 347.
3. Kleinschmidt, G. D. and J. W. Gerdemann. 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhizae. *Phytopathology* 62:1447-1453.
4. Linderman, R. G. and G. A. Call. 1977. Enhanced rooting of woody plant cuttings by mycorrhizal fungi. *J. Amer. Soc. Hort. Sci.* 102: 529-632.
5. Maronek, D. M. and J. W. Hendrix. 1979. Growth acceleration of pin oak seedlings with a mycorrhizal fungus. *HortScience* 14:627-628.
6. _____and _____. 1980. Differential growth response to the mycorrhizal fungus *Glomus fasciculatus* of southern magnolia and 'Bar Harbor' junipers grown in containers in composted hardwood bark-shale. *J. Amer. Soc. Hort. Sci.* 105:206-208.
7. Marx, D. H. 1977. Tree host range and distribution of the ectomycorrhizal fungus *Pisolithus tinctorius. Can. J. Microbiol.* 23:217-223.
8. _____ and J. D. Artman. 1979. *Pisolithus tinctorius* ectomycorrhizal improve survival and growth of pine seedlings on acid spoils in Kentucky and Virginia. *Reclamation Rev.* 2:23-31.
9. _____ and W. C. Bryan. 1970. Pure culture synthesis of ectomycorrhizae by *Thelephora terrestris* and *Pisolithus tinctorius* on different conifer hosts. *Can. J. Bot.* 48:639-643.
10. _____and _____. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius. For. Sci.* 21:245-254.
11. _____, A. B. Hatch, and J. F. 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.
12. Meyer, F. H. 1974. Physiology of mycorrhizae. *Annu. Rev. Plant Physiol.* 25:567-586.
13. Steel, R. and J. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill, New York.
14. Slankis, V. 1974. Soil factors influencing formation of mycorrhizae. *Annu. Rev. Phytopathol.* 12:437-457.
15. Trappe, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. *Annu. Rev. Phytopathol.* 15:203-222.
16. U.S. Dept. of Agriculture. 1974. Seeds of woody plants in the United States. Forest Service Agr. Handb. 450, Washington, D.C.