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Ectomycorrhiza Formation on Sawtooth Oak by Inoculation with Basidiospore Chips of *Pisolithus tinctorius* and *Scleroderma citrinum*

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Abstract
Basidiospores of the ectomycorrhiza-forming fungi *Pisolithus tinctorius* and *Scleroderma citrinum* incorporated into an organic hydrocolloid and stored up to five years can be used successfully in inoculations. Container-grown sawtooth oak seedlings were inoculated with basidiospores that were incorporated and stored in chips of compressed sand and peat moss. Basidiospore chips were manufactured each year after several collections of sporocarps from two locations and stored up to five years. This study showed that sufficient basidiospores remained viable in chip form for ectomycorrhiza formation of sawtooth oaks.

Index words: Ectomycorrhizae, sawtooth oak

Introduction
The concept and applicability of inoculating seedlings with ectomycorrhizal fungi has been documented (5) and the successful inoculations of loblolly pine (*Pinus taeda* L.) seedlings with basidiospores have been reported (4). Inoculating field-planted northern red oak (*Quercus rubra* L.) (1) and loblolly pine (2) with *Pisolithus tinctorius* (Pers.) Coker and Couch and *Scleroderma citrinum* (Pers.) in the form of basidiospore chips may be a useful alternative to inoculating seedlings with vegetative mycelium in greenhouses and nurserybeds before outplanting. Because of the complex host-fungus-site relationship, seedlings infected with pioneering mycorrhizal fungi might be more appropriate to special planting situations such as surface mines as opposed to fungi commonly found in nurseries that may be unsuitable for such use (3). Therefore, to determine if basidiospores of *Pisolithus tinctorius* and *Scleroderma citrinum* could be stored up to 5 years in chip form, this study was initiated to test the effectiveness of *Pisolithus tinctorius* and *Scleroderma citrinum* basidiospore chip inocula as ectomycorrhizal inoculum in greenhouse-grown sawtooth oak (*Quercus acutissima*, Carr.) seedlings.

Materials and Methods
Basidiospores of *Pisolithus tinctorius* and *Scleroderma citrinum* were collected weekly from sporocarps (puffballs) growing on unreclaimed coal surface mine spoils in western Maryland during the late summers of 1979, 1980, 1982, and 1983 and central Pennsylvania in 1980. Basidiospore chips were manufactured after each collection year (2). The basidiospore chips were prepared by gently and thoroughly blending the following materials in sequence: 50 ml autoclaved peatmoss (20
mesh); 25 ml basidiospores and debris; 50 ml J-tac (organic hydrocolloid); 1000 ml autoclaved sand; and 50 ml warm distilled water. This pasty blend was rolled out onto a flat metal surface to a thickness of 2 mm and then sliced into 450 squares, each 2 cm² (0.75 in²). The chips were air-dried under a supported plastic covering for 24 hours, lifted with a spatula, and stored in tight cardboard boxes at 3°C (38°F) until used. Control chips devoid of basidiospores and debris also were prepared. Sawtooth oak acorns were collected from one tree in the fall of 1983 and stratified for 12 months during which time they were rinsed 4 times with fungicide solutions. Sound acorns were rinsed with 10% sodium hypochlorite solution just before planting in October 1984.

The study consisted of 2 fungal species, 4 yearly collection dates from the Maryland source, 1 yearly collection date from the Pennsylvania source and 2 manufactured controls without spores from the years 1979 and 1983. There were thus 5 *Pisolithus tinctorius* treatments, 5 *Scleroderma citrinum* treatments, and 2 control treatments. For each treatment there were 7 plastic containers (Reps) with a volume of about 780 ml (47.6 in³). Enough steam sterilized river sand medium (pH = 5.2) to fill 7 containers was uniformly mixed with 12 crushed basidiospore chips (approx. 11 x 10⁶ spores per chip) for each treatment and two acorns were planted in each container. Thus, a total of 84 containers were inoculated, planted with acorns, and distributed in a completely randomized design on a single greenhouse bench. The bench had a wire mesh bottom. Containers were gently to avoid splashing. Air temperatures were held on or about 24°C (75.2°F) day and night and natural day length was supplemented with overhead incandescent lighting to provide a 15 hour photoperiod. Seedlings were harvested in March 1985 after 5 months of growth and root systems were separated, washed, and evaluated for ectomycorrhiza formation, fungus species identification and percent ectomycorrhiza short roots (PESR) (calculated by the number of ectomycorrhizal root tips divided by the sum of ectomycorrhizal and nonectomycorrhizal root tips per seedling).

### Results and Discussion

Data collected from the paired seedlings of each replicate container were averaged as the representative data for that container (Table 1). Twenty-seven of the 28 control seedlings lacked ectomycorrhizae. All seedlings inoculated with *Pisolithus tinctorius* were free of *Scleroderma citrinum* or other ectomycorrhizae, but 2 seedlings in one container from the *Scleroderma citrinum* 79Md treatment had trace contamination by *Pisolithus tinctorius* ectomycorrhizae. Ninety-two percent of living seedlings inoculated with *Pisolithus tinctorius* were ectomycorrhizal with *Pisolithus tinctorius* but the PESR was low; ranging from 4.5 to 12.5. The range of PESR of seedlings inoculated with *Pisolithus tinctorius* became narrower as newer basidiospore chips were utilized as inocula. Average PESR and associated standard deviations decreased as newer *Pisolithus tinctorius* chips were used but even though there was a trend in declining average PESR with the use of newer chips of *Pisolithus tinctorius*, there was no significant difference among the means.

Ninety-six percent of living seedlings inoculated with *Scleroderma citrinum* were ectomycorrhizal with *Scleroderma citrinum*.

### Table 1. Ectomycorrhiza formation of sawtooth oak from inoculations with stored basidiospores in chip form.

| Fungus species, collection year, and source of spores | Number of containers with ectomycorrhizal seedlings present | Percent surviving seedlings | Percent seedlings with ectomycorrhizae | Range | Average | S.D. ±
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<td>100</td>
<td>93</td>
<td>2.5-35.0</td>
<td>10.0</td>
<td>11.2</td>
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<td>93</td>
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<td>100</td>
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<td>1.0-22.5</td>
<td>9.5</td>
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<td>100</td>
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<td>100</td>
<td>15.0-70.9</td>
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<td>21.6</td>
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<td>7</td>
<td>0.0-0.5</td>
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<tr>
<td>Control—83</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0.0-0.9</td>
<td>0.0</td>
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²Spores were collected from 1979 through 1983 (except 1981) and chips were manufactured at those collection dates and stored until inoculation in October 1984.

³No spores collected in 1981. Control chips were manufactured in 1979 and 1983.

²Two seedlings in one container in Sc79Md were devoid of Sc ectomycorrhizae but had trace contamination by Pt ectomycorrhizae.

²One seedling in Control-79 had a PESR of 1.0 of Sc ectomycorrhizae. Pt = *Pisolithus tinctorius*, Sc = *Scleroderma citrinum*, Md = Maryland, Pa = Pennsylvania, PESR = Percent ectomycorrhizal short roots.
derma citrinum. The range of PESR (5.2-39.6) of seedlings inoculated with Scleroderma citrinum became broader as newer basidiospore chips were utilized as inocula. Average PESR and associated standard deviations increased as newer Scleroderma citrinum chips were used.

There was a trend in increasing average PESR with the use of newer chips of Scleroderma citrinum and only oaks inoculated with the 1979 Maryland source had a significantly lower average PESR than other sources of this fungus.

Basidiospore inoculum of Pisolithus tinctorius and Scleroderma citrinum in chip form were capable of synthesizing ectomycorrhizae after cold storage up to 5 years. Ectomycorrhizal formation fluctuated with duration of basidiospore chip storage. Viability of the spores may be a function of storage conditions and/or of specific collections. Average PESR from Pisolithus tinctorius inoculations decreased with shorter storage periods of the chips indicating that storage of Pisolithus tinctorius spores may influence internal spore physiology and subsequent germination. On the other hand, Scleroderma citrinum spores were less effective as inocula with increased storage time although they were two to three times more effective than Pisolithus tinctorius spores in average PESR. Average PESR increased as newer stored spores of Scleroderma citrinum were utilized. Cold storage may be beneficial for Pisolithus tinctorius spores and detrimental for Scleroderma citrinum spores in regards to viability and germination. No significant differences in ectomycorrhiza formation were apparent between the 1980 Maryland and the 1980 Pennsylvania spore sources as noted by a similar range and average infection.

Significance to the Nursery Industry

Pisolithus tinctorius and Scleroderma citrinum are among a number of vigorous pioneering mycorrhizal fungi that are commonly found on adverse sites. These are nurse-ectomycorrhizal fungi that assist trees in early establishment. Several years later, more sophisticated fungi inhabit the site and move on to the root system as the tree ages and the surrounding microclimate and edaphic conditions become less hostile. The use of basidiospore chips may be a viable alternative and less expensive than vegetative inoculum in seedling inoculation/production in greenhouses and nursery beds and in future regeneration programs with field inoculations. The inoculation of landscape trees with basidiospore chips at the time of planting or years after planting may also become a viable method of establishing a mycorrhizal root system, especially in soils that have some unfavorable characteristics such as at roadside construction and surface mines. Further studies are warranted to examine and monitor the biological contributions and economical conservation spore chip inoculum may have over other methods of inoculation, however, spore chips are storable and can be modified with numbers of spores, fungus species, or shape.

Literature Cited


