Effect of *Pisolithus tinctorius* Nursery Treatment on Long-Term Loblolly and Longleaf Pine Survival and Growth in the South Carolina Sandhills

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Abstract

The long-term effects of artificial inoculation of southern pines with *Pisolithus tinctorius* (Pt) in the nursery were tested in a demonstration project established on the U.S. Department of Agriculture, Forest Service-Savannah River Site, South Carolina. Loblolly (*Pinus taeda* L.) and longleaf pine (*Pinus palustris* Mill.) bareroot seedlings were produced from 1987 to 1991 with either a vegetative Pt inoculum or no inoculum (NI) at Taylor Nursery, Trenton, SC. At the Savannah River Site, two sites were planted per year for a total of 10 demonstration plantings. In 1991, a containerized longleaf pine treatment was added with and without Pt spores. Survival and growth of the seedlings were monitored at planting, after 4 years, and when sites were 15 to 19 years old. The Pt inoculation of longleaf pine produced a negative effect in the survival of bareroot seedlings in two out of ten plantings after 15 years. The addition of the Pt to loblolly seedlings in the nursery increased diameter at planting for four sites; however, this 8- to 16-percent increase in size did not affect tree size or survival over time. The only positive long-term effect with artificial inoculation with Pt was an increase in overall pine survival for site 2. The containerized longleaf pine treatment, added to the last two sites planted, increased seedlings survival over the bareroot longleaf pine. The addition of Pt in containers had no effect after 15 years on longleaf pine growth or survival. Artificial inoculation of southern pines with Pt did not provide a positive effect to warrant its use for reforestation of the sandhills in South Carolina. This paper was presented at the Joint Annual Meeting of the Southern Forest Nursery Association and the Northeast Forest and Conservation Nursery Association (Pensacola, FL, July 17–19, 2018).

Introduction

In the eastern United States, interest in producing seedlings with *Pisolithus tinctorius* (Pers.) Coker and Couch (Pt) ectomycorrhizae (figure 1) was initiated following observations of *P. tinctorius* associated with increased tree survival and growth on mine wastes (Lampky and Peterson 1963, Marx 1975). Controlled studies in the Southeast showed Pt pine seedlings on coal spoils grew better than pine seedlings with the

Figure 1. *Pisolithus tinctorius* ectomycorrhizae. (Photo by Michelle Cram, 2010)

more common ectomycorrhizal species, *Thelephora terrestris* Ehrh. Ex Fr. (Tt) (Berry 1982, Marx 1975, Marx and Artman 1979). Extreme conditions of mine spoils include high temperatures, low pH, and low organic matter. Mycorrhizal species adapted to these extreme conditions, such as Pt, can influence tree survival and growth (Danielson 1985). The increase in survival and growth of seedlings with Pt ectomycorrhizae in high heat (up to 40 ºC) in comparison to seedlings with Tt is particularly interesting to Southern reforestation, as soil temperatures can go above 40 ºC (Marx and Bryan 1971, Marx and Bryan 1975).

Several studies in bareroot nurseries found that loblolly pine (*Pinus taeda* L.) seedlings grown with mycelial Pt inoculum were often larger than seedlings grown in non-inoculated soils (Marx and Bryan 1975, Marx et al. 1976, Marx et al. 1978, Marx et al. 1979). Extensive testing of Pt inoculations in 33 nurseries on 11 different pine species found considerable variation in inoculum effectiveness, and seedling response; however, loblolly pine was often larger with the inoculant from the Institute for Mycorrhizal and Development (Marx et al. 1984). Outplanting on reforestation sites also had mixed results for both longleaf (*Pinus palustris* Mill.) and loblolly pine (Kais et al. 1981, Ruehle 1982). A high level of Pt colonization was found to be necessary for any chance of a positive growth response or survival effect on general reforestation sites (Kais et al. 1981, Marx et al. 1982, Marx et al. 1988) although high Pt root colonization does not always result in a positive effect (Berry and Marx 1980, Echols et al. 1990, Leach and Gresham 1983).

The Savannah River Site (SRS), a National Environmental Research Park located near Aiken, SC, consists predominately of upper coastal plain and sandhill physiographic provinces. This site is known to have periods of severe drought in 2 out of 10 years (Rogers 1990). An earlier study on the SRS by Hatchell and Marx (1987), found bareroot longleaf pine had better survival and growth than non-inoculated seedlings after 7 years. Loblolly pine with Pt ectomycorrhizae also had improved survival and growth, but only in the first year (Hatchell and Marx 1987). Based on these and other positive results with Pt seedlings in reforestation, the U.S. Department of Agriculture (USDA), Forest Service began a demonstration project on the SRS to operationally test the use of Pt-inoculated seedlings on 10 sites with deep sandy soils over a 5-year period. The purpose of this demonstration project was to determine if Pt ectomycorrhizae on longleaf and loblolly pine could improve tree survival and growth. The demonstration plantings (figure 2) were measured yearly over 6 years and the 4th year (5th year for site 5) data was reported by Cram et al. (1999). In 2007, a final measurement was taken of all 10 demonstration plantings at ages 15 to 19, and the survival and volume of longleaf and loblolly were compared (Cram et al. 2010). The purpose of this paper is to present the effects of the nursery-applied Pt mycorrhizae treatment over the long term.

Methods

Bareroot (1+0) loblolly pine and longleaf pine seedlings for the 10 demonstration plantings on the SRS were produced at the South Carolina Forestry

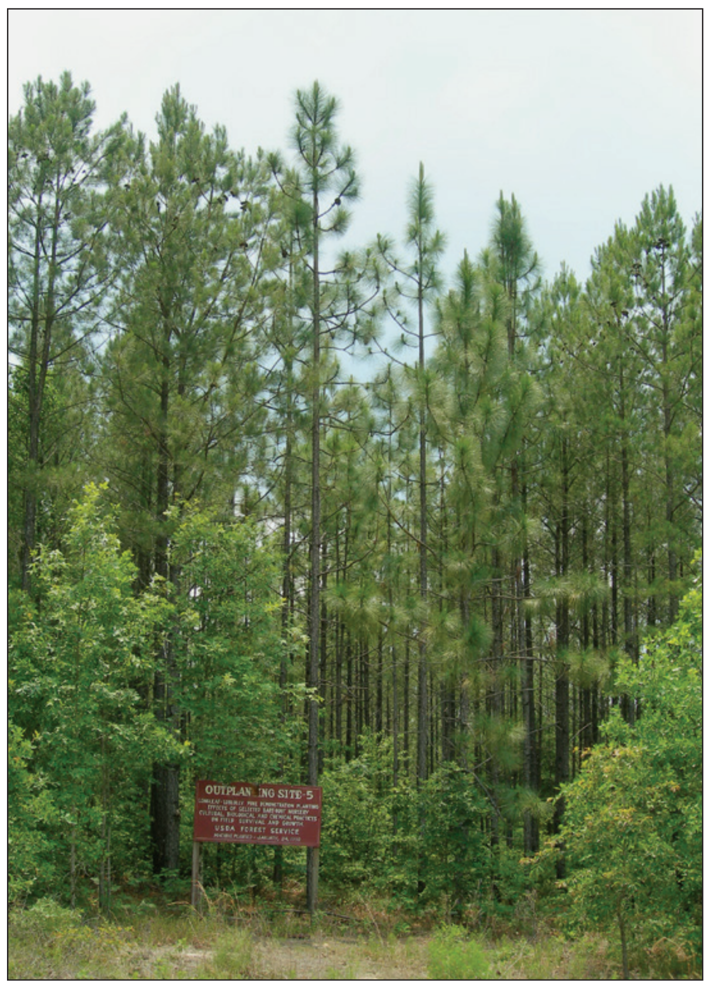

Figure 2. Demonstration site 5 on the Savannah River Site. (Photo by Michelle Cram, July 2007)

Commission's Taylor Nursery in Trenton, SC, to be outplanted from 1988 to 1992. The last year of seedling production also included container (98.32 cm^3) longleaf pine seedlings produced at the South Carolina State Creech Seed and Orchard-Container facility in Wedgefield, SC. Loblolly pine seed was sourced from genetically improved lots from the coast of South Carolina. The longleaf pine seed was from South Carolina and Georgia. The following method descriptions are taken from a more detailed description of the seedling production, inoculation, and planting documented in Cram et al. (1999).

Seedling Production and Inoculation

Bareroot seedling production and inoculation were applied in the same manner every year. Spring fumigation with 98-percent methyl bromide (394 kg/ha) was used prior to inoculation and sowing. The Pt inoculum was from a Georgia isolate that originated from loblolly pine and was produced as a vegetative mycelial product from Sylvan Spawn Labs in 1987 and from Mycorr Tech, Inc. in 1988–1991. Vegetative Pt inoculum was applied to loblolly and longleaf seedbeds at a rate of 0.28 l/m^2 prior to sowing. Pt inoculum for containerized longleaf pine in 1991 came from Pt fruiting bodies collected the previous year at Taylor Nursery from inoculated beds. The spores were applied at a rate of 0.5 g/1000 to emerging seedlings. Control beds or containers (1991 longleaf only) were not inoculated (NI), allowing for naturally occurring nursery mycorrhizae (predominately *T. terrestris*) to eventually develop.

All bareroot seedlings were laterally root pruned in early August and again in October. Before lifting, the Pt index was determined for each seedbed (Marx et al. 1984). The index was calculated by percentage of seedlings with Pt (average percent feeder root with Pt divided by average percent of feeder root with total ectomycorrhizae). Only beds with a Pt index of 50 or greater were used for Pt plots in the demonstration plantings. The Pt index was also assessed for treated container longleaf pine seedlings. Minimum culling standards were 0.32-cm root-collar diameter (RCD) and 15-cm root length for loblolly pine seedlings, and 1.0-cm RCD and 15-cm root length for longleaf pine. Seedlings were refrigerated at 4.4 to 7.2 °C after lifting and stored for less than 5 days before outplanting to the demonstration sites.

Demonstration Sites

Demonstrations sites were limited to sites that had been clearcut the year before and had deep, sandy soils with little slope. Two sites were selected each year for a total of 10. The soil series and site preparation of each site by year planted are listed in table 1. The experimental design for each site was a randomized complete block with species by inoculation treatment replicated 8 times. All seedlings were machine planted in treatment plots, each consisting of 3 rows of 50 seedlings spaced at 1.8 by 3.0 m. In the first 8

Table 1. Planting dates, soil series, and site preparation for loblolly and longleaf pine planting sites in South Carolina sandhills.

Site	Planting date	Soil series[1]	Sand depth (ft)	Site preparation
1	January 1988	Blanton sand	3.94	Chopped, burned, and hexazinone (1.5 lb/ac)
2	January 1988	Troup sand	4.43	Chopped, burned, and hexazinone (2.5 lb/ac)
3	January 1989	Lakeland sand	6.56	Chopped, burned, and hexazinone (2.5 lb/ac)
4	January 1989	**Wagram sand** Blanton sand	1.80 3.94	Chopped and burned
5	January 1990	Blanton sand	3.94	Sheared and raked
6	January 1990	Blanton sand	3.94	Chopped, burned, and hexazinone (2.5 lb/ac)
7	January 1991	Lakeland sand	6.56	Chopped, burned, and hexazinone (2 lb/ac)
8	January 1991	**Fuquay sand** Dothan sand	1.80 0.59	Sheared, raked, and hexazinone (2.5 lb/ac)
9	January 1992	Blanton sand **Lakeland sand**	3.94 6.56	Burned and partially raked
10	January 1992	Troup sand	4.43	Raked

[1] Sites with two soil series – Bold letters indicate the predominate soil type (Rogers 1990)

Table 3. Effect of *Pisolithus tinctorius* nursery treatment on loblolly pine growth over time.[1]

Site	Treat[2]	Diameter (cm)[3]			Height (m)		Survival (%)	
		0 yr	4 yr	16–19 yr	4 yr	16–19 yr	4 yr	16–19 yr
1	NI	0.36	3.7	14.5	3.29	14.32	95	92
	Pt	0.33	3.7	15.0	3.31	14.87	97	91
2	NI	0.41b	4.5	14.8	3.61	13.53	95b	81
	Pt	0.46a	4.5	15.1	3.76	13.62	99a	91
3	NI	0.33b	2.7	15.7	2.58	13.62	96	44
	Pt	0.36a	2.7	15.3	2.57	12.92	92	49
4	NI	0.36b	3.8	17.5	3.08	15.82	90	81
	Pt	0.40a	3.7	17.6	3.02	16.00	90	86
5	NI	0.37b	5.0b	15.3	3.82	13.62	90	78
	Pt	0.44a	5.2a	15.6	3.95	13.75	90	73
6	NI	0.43	3.2	14.5	2.88	13.44	90	81
	Pt	0.48	3.4	15.2	3.04	13.23	89	82
7	NI	0.43	1.7b	12.6	2.09b	10.48	91	84
	Pt	0.43	2.0a	13.2	2.23a	11.03	89	85
8	NI	0.42	3.2	15.9	2.86	13.47	93	60
	Pt	0.45	3.4	15.4	2.93	13.75	93	66

[1] Treatments within a site followed by a different letter are significantly different at the 0.05 level. Data in years 0–4 taken from Cram et al. (1999); data in years 15–19 associated with Cram et al. (2010).

[2] Treatments = *Pisolithus tinctorius* (Pt) and not inoculated (NI).

[3] Diameters measured at the root collar year 0; all other diameters measured at breast height (DBH).

sites, the treatments consisted of longleaf and loblolly pine with artificial Pt inoculation or an NI control. Sites 9 and 10, had an additional pine species treatment of containerized longleaf pine. Although the first 8 sites were designed to include postplant herbicide with sulfometuron-methyl as an additional treatment in the study, only sites 3 and 4 received the treatment, which was applied in March 1989.

Data were collected on the middle row of each treatment plot at each site. Seedlings were measured at planting for RCD (excluding sites 9 and 10), and during the dormant season on the 4th year after outplanting (5th year on site 5) for diameter at breast height (DBH), total height, and survival. In 2007, a final measurement of all 10 sites (15–19 years since planting) was conducted prior to a planned thinning. None of the herbicide plots were included in the 2007 measurement. Final measurements consisted of DBH of all trees in the center row of treatment plots and height of every fifth live tree without a broken top. Trees with broken tops were skipped and the next live unbroken tree was measured for height instead.

Statistical Analysis

Data taken at planting and in the 4th year after outplanting were analyzed as described in Cram et al. (1999). Direct comparison of pine species was not done due to the grass stage of longleaf pine; therefore, the analysis of variance was within site and species. The plots designated for herbicide treatment that did not receive any application (sites 1, 2, 5, 6, 7, and 8) were treated as within-block replication. A 2 by 2 factorial analysis was used on data from sites 3 and 4 that received a postplant herbicide application. An analysis of covariance was also used to determine if initial RCD affected subsequent growth. On sites 9 and 10, longleaf pine was analyzed as a 2 by 2 factorial with contrasts due to the addition of the longleaf container treatment.

The data collected in 2007 were analyzed as described in Cram et al. (2010). A linear, mixed-model approach was used to analyze the DBH, height, and survival for each site. Significant differences were at the critical level $\alpha = 0.05$. The blocks were treated as random effects, while the mycorrhizae and tree species were treated as fixed effects. The container longleaf pine was included as a species treatment for sites 9 and 10 and a Bonferroni correction was used to adjust each pairwise comparison to test at the 0.05/3 = 0.0167 level.

Results

Loblolly Pine

Pt inoculation of loblolly pine in the nursery resulted in 8- to 16-percent larger initial RCD at 4 out of 8 sites (table 2). This larger RCD at planting only persisted on one site after 4 years. Of those without an initial treatment difference in RCD, only inoculated loblolly pine on site 7 developed larger RCD after 4 years. After 16 to 19 years, there were no treatment effects on height, diameter, or survival for loblolly pine on any site (table 2).

Longleaf Pine

Pt inoculation of longleaf pine resulted in smaller RCD at planting on two sites (table 3). After 4 years, the RCD and height of inoculated longleaf pine was significantly less than the NI seedlings at sites 1 and 6. Four-year survival of inoculated longleaf pine was lower than NI seedlings on sites 6, 7, and 8 but higher on site 2. After 16 to 19 years, there were no significant treatment effects on any site (table 3).

Container and Bareroot Comparison

Seedling diameter of bareroot and container longleaf and loblolly pine on sites 9 and 10 were not affected by Pt inoculation in the nursery after 4 or 15 years (table 4). In the 4th year, height was greater for Pt containerized longleaf in site 9 and NI bareroot longleaf in site 10. No height differences were associated with the mycorrhizae treatments by the 15th year. In the 4th year, survival was significantly less for Pt bareroot longleaf than the NI bareroot at both sites.

Table 3. Effect of *Pisolithus tinctorius* nursery treatment on longleaf pine growth over time.[1]

Site	Treat[2]	Diameter (cm)[3]			Height (m)		Survival (%)	
		0 yr	4 yr	16–19 yr	4 yr	16–19 yr	4 yr	16–19 yr
1	NI	1.02a	6.0a	13.3	1.99a	14.36	90	87
	Pt	0.92b	5.8b	13.0	1.71b	13.66	91	84
2	NI	1.00	5.6	13.3	1.50	13.41	81b	77
	Pt	1.01	5.9	13.2	1.64	13.13	92a	88
3	NI	1.09a	4.7	12.6	1.17	12.19	88	76
	Pt	1.03b	4.5	11.7	1.10	12.01	88	84
4	NI	1.15	5.3	14.5	1.42	14.51	84	79
	Pt	1.19	5.4	13.9	1.47	14.26	89	79
5	NI	1.08	3.4	12.8	2.27	12.86	82	70
	Pt	1.01	3.3	12.5	2.23	12.13	81	74
6	NI	1.13	6.0a	13.3	1.72a	12.71	76a	72
	Pt	1.10	5.7b	13.2	1.55b	12.89	70b	64
7	NI	1.02	4.6	11.6	0.91	11.00	72a	71
	Pt	1.04	4.7	11.5	0.89	10.73	62b	61
8	NI	1.08	4.8	12.8	1.08	11.83	68a	49
	Pt	1.19	4.9	13.3	1.03	11.73	48b	42

[1] Treatments within a site followed by a different letter are significantly different at the 0.05 level.
Data in years 0–4 taken from Cram et al. (1999); data in years 16–19 associated with Cram et al. (2010).

[2] Treatments = *Pisolithus tinctorius* (Pt) and not inoculated (NI).

[3] Diameters measured at the root collar year 0; all other diameters measured at breast height (DBH).
Significant difference at the 0.05 level between the Pt and NI treatments within a site.

Table 4. Effect of *Pisolithus tinctorius* nursery treatment on growth over time for site 9 and 10.[1]

Site	Species (culture)	Treat[2]	Diameter (cm)[3]		Height (m)		Survival (%)	
			4 yr	15 yr	4 yr	15 yr	4 yr	15 yr
9	Longleaf (container)	NI	4.0	11.1	0.60b	10.37	90	87a
		Pt	4.4	12.0	0.79a	11.00	85	83a
	Longleaf (bareroot)	NI	3.7	11.6	0.54	10.83	65a	63b
		Pt	3.5	12.3	0.54	10.64	45b	43c
	Loblolly (bareroot)	NI	1.3	14.0	1.74	11.68	68	67b
		Pt	1.5	14.3	1.89	11.89	75	73ab
10	Longleaf (container)	NI	4.1	12.4	0.77	11.02	83	75a
		Pt	4.4	12.0	0.93	10.86	80	74a
	Longleaf (bareroot)	NI	4.1	12.8	0.78a	11.58	57a	51b
		Pt	3.9	12.8	0.62b	10.84	37b	35c
	Loblolly (bareroot)	NI	2.2	14.9	2.35	12.65	87	79a
		Pt	1.8	14.3	2.14	12.20	85	79a

[1] In the 4th year data (Cram et al. 1999) treatments within a site and species (culture) followed by a different letter are significantly different at the 0.05 level; in year 15 (Cram et al. 2010) treatments within a site followed by a different letter were significantly different at the Bonferroni adjusted 0.05/15 = 0.0033 level.
[2] Treatments = *Pisolithus tinctorius* (Pt) and not inoculated (NI).

After 15 years, survival of bareroot longleaf pine was significantly lower than container longleaf pine seedlings at both sites regardless of mycorrhizae treatment. Furthermore, inoculated bareroot longleaf pine had significantly lower survival than the NI bareroot longleaf pine (table 4).

Overall, data from the 10 demonstration sites showed only one site with a positive Pt treatment effect (tables 2 and 3). Although analysis of mycorrhizal treatment by individual pine species showed no overall survival effect, survival on site 2 was increased 10 percent (P = 0.016) with Pt inoculation (Cram et al. 2010).

Discussion

Success of nursery treatments for improving reforestation are rarely monitored long term. The 10 demonstration plantings on the SRS were unique in that Pt treatments in the nursery were subsequently monitored for 15 to 19 years after outplanting (Cram et al. 1999). Hatchell and Marx (1987) tested Pt as a nursery treatment to improve longleaf pine seedling establishment on the sandhills of South Carolina over a 7-year period. In a similar study, Marx et al. (1988) monitored loblolly pine with a Pt index greater than 58 for 8 years. These previous studies indicate that it is possible for nursery-applied Pt to have significant positive effects on survival and growth of longleaf and loblolly pine over the long term. The long-term results from these 10 demonstration plantings, however, revealed only one positive outcome in overall survival with the Pt treatment. All other individual positive effects at planting or after 4 years did not persist.

Large-scale testing of a mycorrhizal treatment is particularly important because of the symbiotic interaction between fungus and plant. Mycorrhizal fungi require carbohydrates from the host (Corrêa et al. 2006, Cairney and Chambers 1997) and the host obtains benefits from the mycorrhizal fungus, such as increased uptake of nutrients and moisture, which offsets the loss of photosynthate (Dosskey et al. 1990). This balance can change under different environmental conditions of drought and nutrient availability (Cairney and Chambers 1997). Most of the sites selected for the 10 demonstration plantings were on deep, sandy soils and expected to be drought prone (Rogers 1990); however, the monthly precipitation on the SRS during and following planting on the 10 sites did not indicate the occurrence of drought, and thus, could not be correlated with seedling performance (Cram et al. 1999). Soil depth was found to significantly affect height growth of both pine species, but there was no interaction with the mycorrhizae treatment (Cram et al. 2010). One factor that was thought to affect survival of bareroot longleaf pine was the less-intensive site preparation, especially on sites 9 and 10, which can affect proper planting depth (Boyer 1988). Container longleaf pine are less affected by negative environmental conditions than bareroot stock (Boyer 1988, South et al. 2005); therefore, the relatively high survival rates of container longleaf on sites 9 and 10 were not surprising. Survival results indicate that bareroot longleaf seedlings were under greater stress than container seedlings, and the presence of Pt on these stressed seedlings had a negative effect. The Pt treatment appeared to act as a carbon sink for stressed seedlings, with little or no positive counterbalance, resulting in a significant loss.

Many other studies have demonstrated negative effects from artificial mycorrhizal inoculation when there is no counterbalance to the carbohydrate usage (Castellano and Trappe 1991, Corrêa et al. 2006, Dosskey et al. 1990, Echols et al. 1990). Individual isolates of Pt can have different levels of compatibility with host species, such that an isolate that performs well on one host could be less well-suited to another (Cairney and Chambers 1997, Marx 1981, Walker 2001). An example of this phenomenon is in a study by Marx (1981), where a Pt isolate (Georgia 227) colonized loblolly pine seedlings at high levels, but not longleaf pine. The commercially used Georgia Pt isolate had been tested for a wide range of host colonization (Marx 1981, Marx et al. 1984), but some species were not optimal hosts, as demonstrated by Castellano and Trappe (1991) with western conifers. The more negative than positive results with Pt inoculation of longleaf pine in our demonstration study might be the result of a less-than-optimal symbiotic relationship.

The results from the 10 demonstration sites show that, under operational conditions, the positive result of applying Pt to longleaf pine reported by Hatchell and Marx (1987) was not a typical outcome. Pt inoculation cannot be recommended for longleaf pine. Although Pt inoculation of loblolly pine had some early positive effects, these effects were lost after 15 or more years. The lack of a long-term effect with Pt inoculation of loblolly is similar to results obtained by other researchers (Echols et al. 1990, Leach and Gresham 1983). An earlier study of Pt-inoculated loblolly pine on the Savannah River Site also failed to show differences due to the natural colonization of the control seedlings by Pt within the first year of planting (Berry and Marx 1980). Mycorrhizal colonization of seedling roots were only examined prior to planting on the demonstration sites. Although we do not know the ectomycorrhizal species present on seedlings after planting, native mycorrhizae on a reforestation site would be expected to be present and colonizing new root tips (Miller at al. 1994, Pilz and Perry 1984, Tainter and Walstad 1977). The colonization of new roots by naturally occurring mycorrhizal fungi can occur within weeks of planting (Tainter and Walstad 1977), resulting in no growth differences between treatments over time.

The use of Pt inoculated loblolly and longleaf pine seedlings was found to be unnecessary for successful reforestation of the South Carolina sandhills (Cram et al 1999, Cram et al. 2010). In most cases, the presence of native mycorrhizal fungi in reforestation sites will make artificial inoculation of seedlings unlikely to provide enough positive effects to warrant the cost of the treatment. In harsh environments, especially where topsoil has been removed, the use of a particular mycorrhiza could make a sufficient difference to justify its use. This has recently been found to be true in other countries, such as China and Mexico, where Pt-inoculated seedlings performed well on abandoned mine sites (Gómez-Romeroa et al. 2015, Zong et al. 2015). In 1977, the Federal Surface Mining Control and Reclamation Act initiated changes in restoration of mined land that included replacing topsoil to cover the acid mining spoils. These changes created a less harsh environment for plants, and a quick return of mycorrhizae species diversity and population levels (Gould and Hendrix 1998), thus reducing the need for artificially inoculated seedlings. The use of Pt-inoculated seedlings in the United States is likely to be rarely justified as the cost outweigh the benefits.

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REFERENCES

Berry, C.R. 1982. Survival and growth of pine hybrid seedlings with Pisloithus ectomycorrhizae on coal spoils is Alabama and Tennessee. Journal of Environmental Quality. 11: 709–715.

Berry, C.R.; Marx, D.H. 1980. Significance of various soil amendments to borrow pit reclamation with loblolly pine and fescue. Reclamation Review. 3: 87–94.

Boyer, W.D. 1988. Effects of site preparation and release on the survival and growth of planted bare-root and container-grown longleaf pine. Georgia Forestry Commission, Georgia Forest Research Paper 76: 1–7.

Cairney, J.W.G; Chambers, S.M. 1997. Interactions between *Pisloithus tinctorius* and its hosts: a review of current knowledge. Mycorrhiza. 7: 117–131.

Castellano, M.A.; Trappe, J.M. 1991. *Pisolithus tinctorius* fails to improve plantation performance of inoculated conifers in southwestern Oregon. New Forests. 5: 349–358.

Corrêa, A.; Strasser, R.J.; Martins-Loução, M.A. 2006. Are mycorrhiza always beneficial? Plant and Soil. 279: 65–73.

Cram, M.M.; Mexal, J.G.; Souter, R. 1999. Successful reforestation of South Carolina sandhills is not influenced by seedling inoculation with *Pisolithus tinctorius* in the nursery. Southern Journal of Applied Forestry. 23: 46–52.

Cram, M.M.; Outcalt, K.W.; Zarnoch, S.J. 2010. Growth of longleaf and loblolly pine planted on South Carolina sandhill sites. Southern Journal of Applied Forestry. 34: 79–83.

Danielson, R.M. 1985. Mycorrhizae and reclamation of stressed terrestrial environments. In: Tate, R.L.; Klein, D.A., eds. Soil reclamation processes microbiological analyses and applications. New York: Marcel Dekker, Inc.: 173–201. Chapter 6.

Dosskey, M.G.; Linderman, R.G.; Boersma, L. 1990. Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. New Phytologist. 115: 269–274.

Echols, R.J.; Meier, C.E.; Ezell, A.W.; Mckinley, C.R. 1990. Dry site survival of bareroot and container seedlings of southern pines from different genetic sources given root dip and ectomycorrhizal treatments. Tree Planters' Notes. 41: 13–21.

Gómez-Romeroa, M.; Lindig-Cisnerosb,R; Saenz-Romeroc, C.; Villegasd, J. 2015. Effect of inoculation and fertilization with phosphorus in the survival and growth of *Pinus pseudostrobus*, in eroded acrisols. Ecological Engineering. 82: 400–403.

Gould, A.B.; Hendrix J.W. 1998. Relationship of mycorrhizal activity to time following reclamation of surface mine land in western Kentucky. II. Mycorrhizal fungal communities. Canadian Journal of Botany. 76: 204–212.

Hatchell, G.E.; Marx, D.H. 1987. Response of longleaf, sand, and loblolly pines to *Pisolithus ectomycorrhizae* and fertilizer on a sandhills site in South Carolina. Forest Science. 33: 301–315.

Kais, A.G.; Snow, G.A.; Marx, D.H. 1981. The effects of benomyl and *Pisolithus tinctorius* ectomycorrhizae on survival and growth of longleaf pine seedlings. Southern Journal of Applied Forestry. 5: 189-194.

Lampky, J.R.; Peterson, J.E. 1963. *Pisolithus tinctorius* associated with pines in Missouri. Mycologia. 55: 675–678.

Leach, G.N.; Gresham, H.H. 1983. Early field performance of loblolly pine seedlings with *Pisolithus tinctorius* ectomycorrhizae on two lower coastal plain sites. Southern Journal of Applied Forestry. 7: 149–153.

Marx, D.H. 1975. Mycorrhizae and establishment of trees on strip-mined land. Ohio Journal of Science. 75: 288–297.

Marx, D.H. 1981. Variability in ectomycorrhizal development and growth among isolates of *Pisolithus tinctorius* as affected by source, age, and reisolation. Canadian Journal of Forest Research. 11: 168–174.

Marx, D. H.; Artman, J.D. 1979. *Pisolithus tinctorius* ectomycorrhizae improve survival and growth of pine seedlings on acid coal spoils in Kentucky and Virginia. Reclamation Review. 2: 23–31.

Marx, D.H.; Bryan, W.C. 1971. Influence of ectomycorrhizae on survival and growth of aseptic seedlings of loblolly pine at high temperature. Forest Science. 17: 37–41.

Marx, D.H.; Bryan, W.C. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. Forest Science. 21: 245–254.

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

Marx, D.H.; Cordell, C.E.; Clark, A. III. 1988. Eight-year performance of loblolly pine with *Pisloithus ectomycorrhizae* on a good-quality forest site. Southern Journal of Applied Forestry. 12: 275–280.

Marx, D.H.; Cordell, C.E.; Kenney, D.S.; Mexal, J.G; Artman, J.D.; Riffle, J.W.; Molina, R.J. 1984. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on bare-root tree seedlings. Supplemental to Forest Science. Monograph 25. 101 p.

Marx, D.H.; Mexal, J.G; Morris, W.G. 1979. Inoculation of nursery seedbeds with *Pisolithus tinctorius* spores mixed with hydromulch increases ectomycorrhizae and growth of loblolly pines. Southern Journal of Applied Forestry. 3: 175–178.

Marx, D.H.; Morris, W.G.; Mexal, J.G. 1978. Growth and development of loblolly pine seedlings in fumigated and nonfumigated nursery soil infested with different fungal symbionts. Forest Science. 24: 193–203.

Marx, D.H.; Ruehle, J.L.; Kenney, D.S.; Cordell, C.E.; Riffle, J.W.; Molina, R.J.; Pawuk, W.H.; Navratil, S.; Tinus, R.W.; Goodwin, O.C. 1982. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on container tree seedlings. Forest Science. 28: 373–400.

Miller, S.L.; Torres, P.; McClean, T.M. 1994. Persistence of basidiospores and sclerotia of mycorrhizal fungi and Morchella in soil. Mycologia. 86: 89–95.

Pilz, D.P.; Perry, D.A. 1984. Impact of clearcutting and slash burning on ectomycorrhizal associations of Douglas-fir seedlings. Canadian Journal of Forest Research. 14: 94–100.

Rogers, V.A. 1990. Soil survey of Savannah River plant area, parts of Aiken, Barnwell, and Allendale Counties, South Carolina. U.S. Department of Agriculture, Soil Conservation Service. 127 p.

Ruehle, J.L. 1982. Field performance of container-grown loblolly pine seedlings with specific ectomycorrhizae on a reforestation site in South Carolina. Southern Journal of Applied Forestry. 6: 30–33.

Tainter, F.H.; Walstad, J.D. 1977, Colonization of outplanted loblolly pines by native ectomycorrhizal fungi. Forest Science. 23: 77–79.

South, D.B.; Harris, S.W.; Barnett, J.P.; Hainds, M.J.; Gjerstad, D.H. 2005. Effect of container type and seedling size on survival and early height growth of *Pinus palustris* seedlings in Alabama, U.S.A. Forest Ecology and Management. 204: 385–398.

Walker, R.F. 2001. Growth and nutritional responses of containerized sugar and Jeffrey pine seedlings to control release fertilization and induced mycorrhization. Forest Ecology and Management. 149: 163–179.

Zong, K.; Huang, J.; Nara, K.; Chen, Y.; Shen, Z.; Lian, C. 2015. Inoculation of ectomycorrhizal fungi contributes to the survival of tree seedlings in a copper mine tailing. Journal of Forest Research. 20: 493–500.