Cost of inoculation seedlings with *Pisolithus tinctorius* spores

David B South

Emeritus Professor, School of Forestry and Wildlife Science, Auburn University, AL

✉ southdb@auburn.edu

Abstract

Although the production of commercial products of vegetative Pt (*Pisolithus tinctorius* (Pers.) Coker & Couch) inoculums has ceased in North America due to a lack of demand by consumers, the number of products that contain Pt spores has increased. The quality, quantity and price of these products vary considerably. The cost of inoculating 1,000 tree seedlings with Pt basidiospores can vary from $0.45 to more than $30. The cost of treating with Pt spores is lowest when seedlings are inoculated in a container nursery using rates that are less than 0.4 mg per seedling. However, with some products the cost to treat 1,000 bareroot seedlings is greater than $500 when spores are applied in the planting hole. Three decades ago, 1 g of Pt spores could be purchased for $0.13 and now the price of 1 g can exceed $14. Although many research papers provide data on the biological response to inoculating seedlings with spores, few document the cost of inoculation. Commercial products that are marketed toward homeowners containing both ectomycorrhizal and endomycorrhizal spores are more expensive than products that contain only ectomycorrhizal spores. In situations where survival and growth of seedlings are not increased, the benefit/cost ratio will typically be less than one.

Keywords

Bareroot nursery; Container nursery; Basidiospores; Ectomycorrhiza; Symbiont

ARTICLE INFO

Citation:
South BD (2018) Cost of inoculation seedlings with *Pisolithus tinctorius* spores. Reforesta 5: 33-53.
DOI: https://dx.doi.org/10.21750/REFOR.5.06.52

Editor: Vladan Ivetić, Serbia
Received: 2018-06-16
Accepted: 2018-06-28
Published: 2018-07-10

CrossMark

Contents

1 Introduction

At some nurseries Pt (*Pisolithus tinctorius* (Pers.) Coker & Couch) mycorrhiza will form naturally on pine roots (figure 1). In some situations, adding this fungal symbiont to the growing media will increase the growth of pine seedlings in bareroot and container nurseries. In the past, some nursery managers collected fruiting bodies and then applied Pt spores to seedbeds in hopes of increasing the amount of mycorrhiza. For example, more than three decades ago nursery managers in South Africa (Strubbings 1958; Young 1981) and Oklahoma (Mexal 1980) applied Pt spores to nursery soil.

Pt research prior to 1970 was limited (Marx and Bryan 1969; Zak and Bryan 1963) but since that time, interest in conducting research with this symbiont has increased exponentially (figure 2). Positive results from nursery trials during the 20th century likely explain why the number of commercial products using Pt spores has increased over time. Although several nursery manuals mention the use of mycorrhizal products, most nursery managers today do not purchase or apply commercial products that contain Pt spores. This raises the question: why has the number of commercial Pt products increased, yet the use in forest tree nurseries decreased? This paper summarizes research regarding applying Pt spores in tree nurseries and provides some reasons why nursery managers are reluctant to purchase and use commercially available products.

Figure 1. The amount of *Pisolithus tinctorius* on short roots in October can affect both the color and mass of *Pinus taeda* seedlings that had roots cut in August (South et al. 1989). Green and yellow seedlings had *Pisolithus tinctorius* on about 67 percent and 1 percent of the seedlings, respectively. The percentage of short roots that were mycorrhizal were 51 percent and 31 percent for green and yellow seedlings, respectively. Root of yellow seedlings were dominated by *Telephora terrestris* mycorrhiza and green seedlings had about 20 percent more dry mass than yellow seedlings. The locations of *Pisolithus tinctorius* basidiocarps are indicated with white flags. (Photo by David South, 1983).

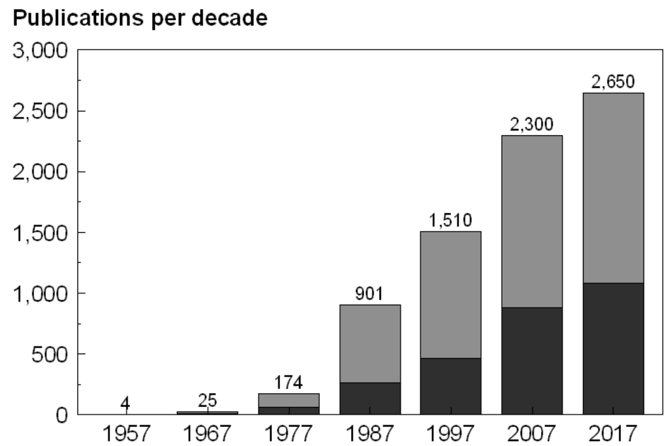

Figure 2. The trend in research papers that include the words *Pisolithus tinctorius*. Black bars represent papers that also include the word "spore".

2 Spores in bareroot seedbeds

When ectomycorrhizal inocula are absent from seedbeds and surrounding areas (Hatch 1936; McComb and Griffith 1946; Wakeley 1954), adding Pt spores to seedbeds at time of sowing can increase seedling growth. At one bareroot nursery with low levels of native mycorrhiza, applying Pt spores at time of sowing increased seedling mass by 12 percent to 26 percent (Marx et al. 1979). In contrast, when seedlings have ectomycorrhiza on 35 to 49 percent of the short roots, then adding Pt spores might increase the amount by 8 percentage points (table 1), but this might have no effect on seedling mass (Marx et al. 1989b). Thus, a growth response may be achieved when spore and mycelia counts are low in the rhizosphere, but benefits may not occur at nurseries with naturally occurring ectomycorrhiza (Cram et al. 1999; Castellano and Trappe 1991). It is not uncommon for harvested seedlings to naturally have more than 30 percent ectomycorrhiza (i.e. percent of short roots that have a Hartig net).

Inoculations with Pt spores provide little benefit at nurseries that have adequate soil inocula (Marx et al. 1978) and high soil fertility (Cram et al. 1999; Cram and Dumroese 2012; Cordell et al. 1974) or when Pt spores have been stored improperly (Alvarez and Trappe 1983a, Marx et al. 1986). Although some report that fertilization will reduce the percentage of ectomycorrhizal short roots, nitrogen applications totaling 300 kg/ha/yr or more do not inhibit Pt ectomycorrhiza of loblolly pine (*Pinus taeda* L.) (Marx 1990; Marx et al. 1978; Marx et al. 1979). In addition, soil phosphorus levels up to 150 ppm do not prevent the formation of ectomycorrhizae in pine seedbeds (Marx et al. 1989a; figure 3). In contrast, certain fungicides will inhibit the formation of Pt ectomycorrhiza.

Table 1. A list of trials with *Pisolithus tinctorius* spores in bareroot pine seedbeds that were not treated with the fungicide triadimefon. Testing of spore inocula in bareroot seedbeds in the southern United States declined after these tests were published, in part, because the use of triadimefon fungicide increased. Within a row, an asterisk (*) indicates applying spores increased the level of ectomycorrhiza (α =0.05).

Year	**Location**	**Ectomycorrhizal short roots (%)**		**Spores per seedling (mg)**	**Reference**
		Control	Spores		
1972	Athens, GA	44	57	87	Marx and Bryan 1975
1973	Morgan, GA	>53	>53	7	Marx et al. 1976
1973	Andrews, FL	66	75	6	Marx et al. 1976
1973	Morganton, NC	50	68*	7	Marx et al. 1976
1975	Ft. Towson, OK	48	47	0.3	Marx et al. 1978
1975	Ft. Towson, OK	46	55	0.3	Marx et al. 1978
1976	Ft. Towson, OK	??	??	2.3	Marx et al. 1979
1978	Medford, OR	??	??	3	Castellano and Trappe 1991
1979	Lee, FL	??	??	1	Leach and Gresham 1983
1980	Sebastopol, CA	97	100	4?	Alvarez and Trappe 1983a
1980	Athens, GA	35	45	1	Marx et al. 1984
1983	Athens, GA	60	??	15	Marx and Bell 1985
1983	GA?	72	76	21	Rowan 1984
1986	Trenton, SC	49	53*	2.4	Marx et al. 1989b
1986	Blenheim, SC	42	52	2.4	Marx et al. 1989b
1986	Vallonia, IN	35	41	2.4	Marx et al. 1989b
1986	Lee, FL	16	16	2.4	Marx et al. 1989b
1986	Athens, GA	33	57*	2.4	Marx et al. 1989b
1987	Trenton, SC	50	54	2.2	Marx and Cordell 1990a
1987	Trenton, SC	29	34	5	Marx and Cordell 1990b

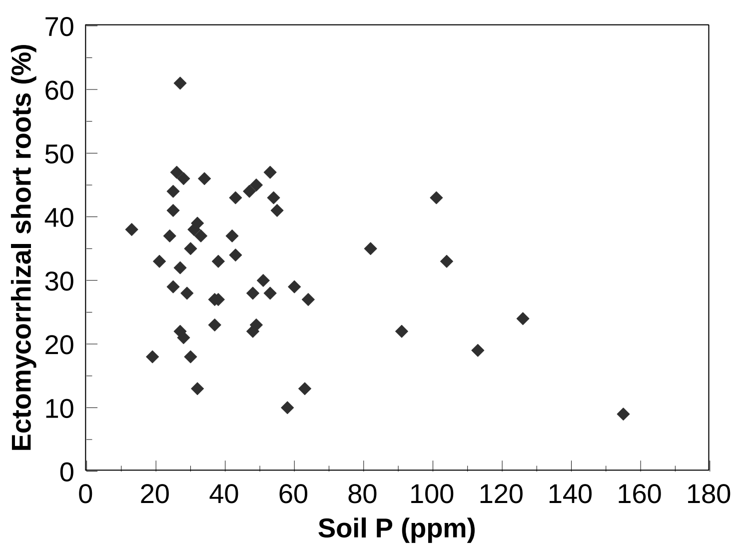

Figure 3. Operational data (control plots) from bareroot conifer seedlings growing in different nursery soils (n=41) indicate an unpredictable relationship between soil phosphorus at sowing and frequency of ectomycorrhizal short roots at time of harvest (adapted from Marx et al. 1984a). When soil phosphorus was less than 150 ppm (double acid extraction), the ectomycorrhizal short roots averaged 32 percent (n = 40).

In various inoculation trials, the fungicide triadimefon was not used because it can inhibit the development of Pt ectomycorrhiza (Marx et al. 1986; Marx et al. 1989a, Rowan 1984). When certain other fungicides are used, the application of Pt spores may increase ectomycorrhizal short roots by as much as 10 percentage points. However, when triadimefon is applied to seedlings, the formation of Pt ectomycorrhiza is suppressed and other ectomycorrhiza short roots are reduced by 9 percentage points or more (Kelley 1987; Marx et al. 1986; Rowan 1984). Managers in the southern United States routinely apply triadimefon to control fusiform rust caused by *Cronarium quercuum* (Berk.) Miyabe ex Shirai F. sp. f*usiforme* Burdsall and Snow (Carey and Kelley 1993; South et al. 2016). This is because managers would rather produce seedlings with only 20 percent ectomycorrhizal short roots than to spend time culling diseased seedlings. In fact, sometimes outplanting survival is increased when seedlings are treated with triadimefon (Rowan 1984). Observations suggest that use of triadimefon has resulted in a decline in the production of Pt basidiocarps in bareroot nurseries. In the past, the occurrence of Pt basidiocarps was common at some bareroot nurseries (South et al. 1989). Despite the negative effects on Pt ectomycorrhiza, the benefits of rust fungicides to pine seedlings outweigh such concerns (Cram and Dumroese 2012; Rowan 1984).

3 Spores in containers

The growth response from applying Pt spores to container media depends on the level of native basidiospores in the surrounding environment. When there are no airborne ectomycorrhizal spores, untreated pine seedlings will likely be nonmycorrhizal and stunted, especially when the growing media has no inoculum. However, to produce conifer seedlings without ectomycorrhiza requires the potting media to be free of live fungal spores. For this reason, mycorrhizal researchers may sterilize media using fumigation (Mitchell et al. 1984; Ruehle 1980) or heat (Ambriz et al. 2010; Báez-Pérez et al. 2017; Lu et al. 1998; Molina, R. 1979). In addition, some researchers use special procedures to keep airborne spores away from germinating seedlings (Hatch 1936; Marx and Bryan 1969; Stottlemyer et al. 2008). For example, Marx (1976) steamed media three times and then kept the containers in an electronically air-filtered plant growth room. As a result, non-inoculated seedlings were stunted, had no mycorrhiza and were about 36 percent smaller five months after sowing. In greenhouses located in regions with a lack of spore contamination, seedlings growing in twice sterilized sand may be 26 percent smaller than seedlings treated with *Pisolithus* spores (Lu et al. 1998).

At many locations, airborne basidiospores will naturally inoculate container media and seedlings will not be stunted (Cram and Dumroese 2012). As a result, artificially inoculating containers with Pt spores may result in no increase in height growth (Trofymow and van den Driessche 1991; Whitesell et al. 1992; table 2). For example, in one test non-inoculated seedlings had ectomycorrhiza on 21 percent of the short roots (at age 16 weeks) and seedling mass (3.3 mg) was the same as for seedlings treated with Pt spores (Marx et al. 1984b). Likewise, container-grown oak seedlings (in unsterilized peat:vermiculite media) were not affected by adding Pt spores (Boling et al. 2006).

Table 2. Examples of the effect of adding spores to height of container-grown pine seedlings in Spain and in the United States. *Pinus taeda* and *Pinus ponderosa* seedlings were grown in the USA while other species were grown in Spain. In Spain, adding Pt spores to container media may increase seedling height (>2 cm) in greenhouses about 30 percent of the time while in the USA an increase in height growth is rare.

Species	None	Spores	Difference	Spores per seedling	Reference
	cm	cm	cm	mg	
***Pinus halepensis* Mill.**	6.7	9.4	2.7	0.9	Dominguez-Núñez et al. 2013
***Pinus halepensis* Mill.**	12	18	6.0	1.4	Querejeta et al. 1998
***Pinus halepensis* Mill.**	7.7	12.2	4.5	3.6	Torres and Honrubia 1994
***Pinus halepensis* Mill.**	7.8	6.6	-1.2	3.6	Torres and Honrubia 1994
***Pinus halepensis* Mill.**	17	18.5	1.5	1,364	González-Ochoa et al. 2003
***Pinus halepensis* Mill.**	8.1	7.8	-0.3	1,364	González-Ochoa et al. 2003
***Pinus pinaster* Ait.**	19.4	15.9	-3.5	1,364	González-Ochoa et al. 2003
***Pinus pinaster* Ait.**	9.0	9.5	0.5	1,364	González-Ochoa et al. 2003
***Pinus pinaster* Ait.**	33.5	34.5	1.0	0.9	Rincón et al. 2007
***Pinus pinea* L.**	38.1	30.9	-7.2	1	Rincón et al. 2001
Pinus ponderosa	9.0	8.0	-1.0	?	Harrington et al. 2001
***Pinus taeda* L.**	17	17	0	0.84	Ruehle 1980
***Pinus taeda* L.**	22.8	15.5	-7.3	1	Marx et al. 1984b
***Pinus taeda* L.**	18.3	18.4	0.1	8	Marx et al. 1984b
***Pinus taeda* L.**	20.1	18.8	-1.3	1	Marx et al. 1984b
***Pinus taeda* L.**	17	16.4	-0.6	1.8	Ruehle and Marx 1977
***Pinus taeda* L.**	27	27	0	4.4	Marx and Bell 1985
***Pinus taeda* L.**	28	28	0	2.2	Marx and Cordell 1990
***Pinus taeda* L.**	21.1	21.2	0.1	10.9	Hua et al. 1991
***Pinus taeda* L.**	10.0	10.4	0.4	10.9	Hua et al. 1991
***Pinus taeda* L.**	14.4	9.5	-4.9	1.6	Marx and Barnett 1974

4 Spores per seedling

The cost of using commercial Pt products depends on the number of spores applied per seedling. Most researchers apply high rates of spores that are not economical for operational nurseries. For example, some researchers applied more than 50 mg per seedling (Ambriz et al. 2010; Beckjord et al. 1986; González-Ochoa et al. 2003; Jorgensen 2014; Marx and Bryan 1975). Since it might cost $30 to apply just 1 g of Pt spores (figure 4), applying 50 mg of a commercial product would cost $1.50 per seedling. To reduce the cost, the rate per seedling was lowered, in some cases, by 98 percent. The rate selected for encapsulated seed was 1.1 million spores per seedling (Marx et al. 1984b) and a spore pellet contained approximately 3 million spores (Marx and Bell 1985; Walker and McLaughlin 1991). Although 27 research studies (out of 36 examined) used rates of at least 1 mg per seedling, most commercial products labels suggest applying lower rates.

While one nursery guide recommends 0.5 mg per seedling (Barnett and McGilvray 1997), nursery managers decrease costs by applying lower rates. In one trial, applying 0.15 mg of spores per seedling resulted in 67 percent of the pine seedlings with some Pt mycorrhiza (Preve et al. 1984) and 0.1 mg per seedling proved effective in two trials (figure 4). In 2017, one manager applied 0.03 mg per seedling to his entire seedling crop (Free 2017).

Commercial products vary widely in the amount of spores they contain. One product contains 30,000 Pt spores per kg and the label lists 99.9 percent inert ingredients. When this product is used as a side dressing, the recommended rate averages 7.5 to 15 spores m^{-2}. At 250 seedlings m^{-2}, this is equivalent to as few as one spore per 33 seedlings. This rate would not cause a measurable growth benefit.

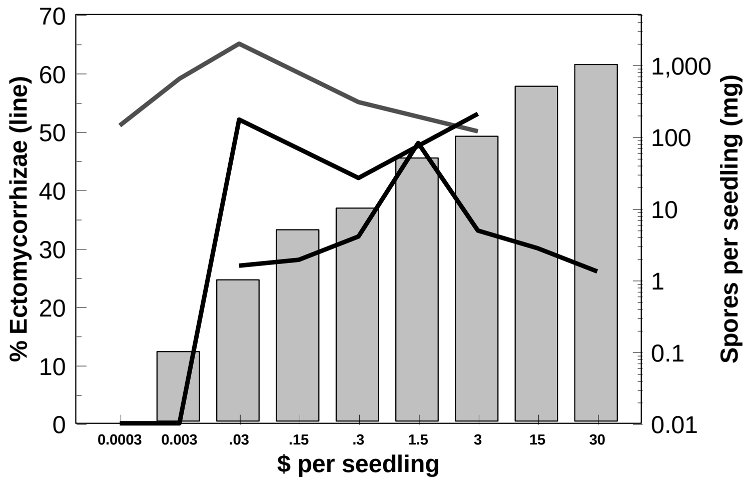

Figure 4. The cost of inoculation with *Pisolithus tinctorius* spores is directly related to the amount of spores applied per container (bars). When 1 g costs $30, then applying 1 mg of spores will cost $0.03 per seedling. The upper, middle and lower lines for percent ectomycorrhizal short roots are from Pera et al. (1994), Rincon et al. (2001) and Marx (1976), respectively.

5 Spores in planting hole

Adding Pt spores in the planting hole is both ineffective and costly. It is costly because often more spores are applied in comparison to typical rates used by nursery managers (Repáč 2011). It is ineffective because successful invasion by native mycorrhizal mycelia (colonizing seedling roots) can be a major problem in obtaining a desired response (Garcia-Barreda et al. 2015). For example, applying Pt spores to planting holes in non-fumigated forest soils failed to increase growth (Beckjord and McIntosh 1984; Bryson 1980; Davis and Jacobs 2004; Pilz and Znerold 1986; Wood 1985). At one location, applying too many spores to roots at time of transplanting reduced the survival of conifers (Alverez and Trappe 1983b). Thus far, research trials have not detected a significant growth benefit from adding Pt spores either in the planting hole (Beckjord et al. 1984) or when potting nursery stock (Weissenhorn 2002). Simply adding soil to the planting hole (Amaranthus and Perry 1987; Colinas et al. 1994; Helm and Carling 1993; Querejeta et al. 1998; Roldán et al. 1996) may have a higher probability of a positive seedling growth response.

Several Pt products that are marketed as a benefit to transplanting also include fertilizers. When a fertilizer tablet is applied in the planting hole, it will sometimes increase seedling growth (Hatchell and Marx 1987; Walker 2002). When mycorrhizal spores are added to a fertilizer tablet, the cost per ha might increase by 300 percent or more. If extra growth does occur, it is usually not known if the addition of spores played any role in increasing height growth.

Several products (targeted for use in planting holes) include additional compounds such as kelp, seaweed, humic acids, bone meal and vitamins. One information sheet says “if you think one, or two, aren’t important, forget them and focus on the rest!” In fact, some do focus on the positive effects from using hydroscopic gels (Crous 2017; Sarvaš et al. 2007; Starkey et al. 2014) or applying more chemical fertilizers in the nursery (Davis et al. 2011; Marx 1990; South and Donald 2002). In fact, the British Standards Institute (Anonymous 2014) says “there is little literature to support the value of adding commercial mycorrhizal cocktails to the backfill soil used for young tree planting.” Their recommendation agrees with Wilde (1944) who said “the question of mycorrhiza in the routine practice of silviculture has been unduly exaggerated”.

6 Cost of inocula in 1990

The use of ectomycorrhizal inoculants in tree nurseries increased during the second half of the 20th Century and at least ten companies were producing ectomycorrhizal inocula (Rossi et al. 2007). In North America, about five out of 78 container nurseries had an active inoculation program (Castellano and Molina 1990). The company Mycorr Tech, Inc. (Worthington, PA) sold vegetative inoculums at a price of \$10 per liter and more than 6 million seedlings were treated using this product (Cordell et al. 1989). When mixed in the seedbed at a rate of 1.08 liter m^{-2} (and with 250 seedlings m^{-2}), this added about 4.3 cents to the cost of producing a seedling (Marx et al. 1984). This treatment increased the number of Pt short roots by about 4 percent (figure 5). Without any Pt inoculation, pine seedlings typically have about 30 percent mycorrhizal short roots, primarily from species other than *Pisolithus tinctorius* (Marx et al. 1984a).

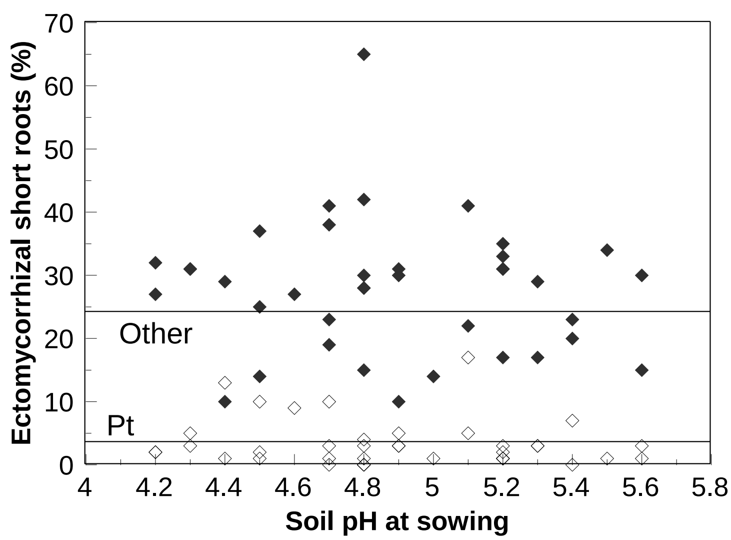

Figure 5. A commercial vegetative inoculum (Abbot Laboratories - 1.08 l m^{-2}) of *Pisolithus tinctorius* (Pt) was applied to 37 pine seedbeds (Marx et al. 1984a) at a cost of about 4.3 cents per plantable seedling. Seedlings lifted from this treatment had, on average, 3.5 percent of short roots infected with Pt (□) while 24 percent were infected with other species (■). There was no strong relationship between soil pH and frequency of mycorrhizal roots. On average, non-inoculated pine seedlings (not shown) had an average of 31 percent mycorrhizal short roots (range = 9 percent to 61 percent).

For some oak species (Pope 1988), applying 2.2 liters m^{-2} might cost 28.8 cents per seedling, doubling the price of seedlings. As a result, methods were developed to apply lower rates. The cost for pine seedlings was reduced by 63 percent when the rate was lowered to 0.4 liter m^{-2} (Marx and Cordell 1988). When applying vegetative inocula it might take 6 weeks to form mycorrhizae compared to 15 weeks when applying Pt spores (Marx et al. 1979).

Applying spores to either bareroot seedbeds or to growth media (in containers) was a less expensive method of inoculation (Castellano and Molina 1990; Lu et al. 1998). To make spore application easier, methods were developed to encapsulate seed with spores (Marx et al. 1984b) and to make spore pellets. Spore-encapsulated seeds were marketed by SouthPine Inc. (Birmingham, AL) and by International Forest Seed Co. (Odenville, AL). In comparison to the cost of vegetative inoculum, the cost of using encapsulated seeds was up to 78 percent less expensive (table 3; Marx and Cordell 1988). In one trial, adding spores to encapsulated seed reduced seedling size because seedling emergence was reduced by about 3 weeks (Marx et al. 1984b). In 1995; a MycorTree™ Pt spore spray kit was marketed by Plant Health Care Inc. (Pittsburg, PA) and contained approximately 250,000 spores per mg of product (Martin et al. 2003).

Pt spores were not commercially available in 1978, but a decade later double-sifted spores could be purchased for \$132 kg^{-1} (table 3). To supply companies with enough Pt spores, some mushroom collectors were paid \$33 kg^{-1} for Pt sporocarps (figure 6). When collected at the right time, one large sporocarp might weight 200 g and would be worth more than \$6 when harvested and \$26 after spores were sifted. Estimates of the mass of Pt spores vary from 1.1 to 1.2 billion (10^9) spores per g (Alvarez and Trappe 1983a, Marx 1976).

Table 3. The cost of *Pisolithus tinctorius* (Pt) spore inocula during the 1980's. Rates listed are for spores (S) or product (P) and cost estimates (1987) assume a seedbed density of 269 seedlings m^{-2} (Cordell et al. 1989).

Inoculum type	Stock	Application method	Rate per seedling (mg)	Cost per kg	Cost per thousand seedlings
Spores in hydromulch	Bareroot	At sowing	2.1 – S	$132	$0.28
Double-sifted spores	Bareroot	At sowing	2.6 – S	$165	$0.43
Spores + humate	Container	In mix	156 – P	$9.74	$1.52
Spore spray	Container	After sowing	1 – S	--	$2.00
Spore encapsulated seed	Container	At sowing	1 - S	--	$2.22
Spore pellet (2.75 mg)	Bareroot	Before sowing	36 – P	$76.40	$2.75
Spores + Endo spores	Bareroot	Transplanting	425 – P	$120	$51.00

7 Cost of inocula in 2017

Significant changes have occurred in the cost and availability of Pt spores over the past three decades. In 1993 ectomycorrhizal spores could be purchased from Forest Mycorrhizal Application (Grants Pass, OR) at a cost equivalent to 1.5 cents per seedling (Landis 1993) at a price of about \$0.20 per gram. Today, the price has increased and a gram of Pt spores is sometimes sold in products for \$15 or more. A price offered on the internet was \$40 per gram. As a comparison, a bareroot loblolly pine seedling in 1978 could be purchased for 1.2 cents and now one seedling will cost about 6 cents.

Figure 6. Puffball fruiting body of *Pisolithus tinctorius* may be found on areas with little or no ground vegetation. Some people collect these in the fall and sell the sporocarps to others for perhaps $33 per kg. (Photo by Michelle Cram, 2010).

There are currently numerous commercial products that include Pt spores and a few organizations provide "pure-culture" inocula (Wilkinson and Janos 2014). There is a wide range in the value of these products as one gram of product may contain more than 5 million spores while others contain less than 60 spores per gram (Wiseman et al. 2009). Most of these products are marketed for use in landscape areas, horticultural nurseries and homeowners. For this reason, most of the products that contain Pt spores also contain more expensive endomycorrhizal spores (table 4). This increases the range of tree species that may be added to the label. However, a few mycorrhizal products that are marketed as "general purpose" contain no ectomycorrhizal spores so, as with most products, it is important to read and understand the label.

The commercial price of endomycorrhizal spores is greater than Pt spores. This is because it is much easier to harvest a billion Pt spores, and because Pt spores are not as effective on a per spore basis (Báez-Pérez et al. 2017). In some cases, a penny will purchase either 58 endomycorrhizal spores (Cram and Fraedrich 2015) or 540 Pt spores. Therefore, adding endomycorrhizal spores to a commercial product increases the overall cost without providing any additional benefit to pine seedlings. In one example, 83 percent of the price per kg of a product was due to endomycorrhizal spores. A kg of a product that contains 132,000 endomycorrhizal spores might cost $13.55 while adding 110 million ectomycorrhizal spores might increase the price by $2.84. Products that contain only endomycorrhizal spores are occasionally used in nurseries at a cost of almost $20,000 per ha with little or no beneficial results to seedlings (Cram and Fraedrich 2015; Kűlling 2008; Wiseman et al. 2009).

In 2013-2014, five container nurseries were applying Pt spores to seedlings on an operational base. These nurseries were located in Alabama (Westervelt), Florida (IFCO), Georgia (IFCO), Louisiana (IFCO) and Nebraska (USFS Bessey Nursery). The cost of applying commercial products at these nurseries ranged from $1 to $3.45 per thousand. One liter of product was used to treat 130,000 to 250,000 seedlings. Since triadimefon was used at the southern nurseries, spores were applied (as a drench about the end of August) after the fungicide applications ceased. This fungicide was not used at the Bessey Nursery and, therefore, spores were applied at time of sowing. Managers indicate the treatments increased the production of Pt ectomycorrhiza but there was no noticeable effect on the overall amount of ectomycorrhiza. The additional cost did not produce a documented effect on seedling sales (especially in years when demand for seedlings was greater than the supply). As a result, only two nurseries applied Pt spores to 9 million container-grown pine seedlings in 2017.

Table 4. A partial list of commercial products that contain spores of *Pisolithus tinctorius* (Pt). The cost per thousand seedlings is determined using the cost per billion Pt spores and an arbitrary rate of Pt spores. Some products are applied in the nursery (N), the gels are applied to roots (R) and some products are applied in the planting hole (H). Many products contain more than one species (#) of ectomycorrhizal spores (ECT) and several species of endomycorrhizal spores (END) and some products also include fertilizers (FERT). In some products, Pt spores represent 45 percent of all ectomycorrhizal spores.

Site	Type	ECT #	END #	FERT	Pt spores/g	% Pt	Cost per kg of product	Cost per billion Pt spores	Pt spores per seedling	Cost per thousand seedlings
N	Drench	2	0	No	12,502,000	86%	$176	$14	32,000	$0.45
N	Liquid	7	0	No	8,000,000	84%	$243	$30	32,000	$0.97
N	Soluble	5	4	No	440,000	83%	$52	$118	7,040	$0.83
N	Soluble	10	9	No	1,100,000	68%	$176	$160	17,600	$2.82
N	Powder	7	4	No	220,000	75%	$27	$123	108,900	$13.37
N	Liquid	1	0	No	30	100%	$18	$600,000	30	$18.00
N	Powder	7	9	No	187,875	85%	$188	$1,001	35,320	$35.34
N	Kelp	11	9	No	1,251,000	56%	$194	$155	236,439	$36.67
R	Root gel	2	0	Yes	4,167,400	86%	$61	$15	175,000	$2.56
R	Root gel	5	9	No	100,000	92%	$44	$440	270,000	$118.80
H	Humic	2	7	No	625,110	86%	$8.30	$13	18,753,300	$249.00
H	Granular	7	4	Yes	220,000	91%	$40.5	$184	770,000	$141.75
H	Tablet	5	9	No	9,000,000	90%	$682	$76	9,000,000	$682.00

8 Economic benefit

The economic benefit of commercial products "should be calculated prior to the decision to use mycorrhizal products" (Vosátka et al. 2008). Simply saying a particular treatment is "worthwhile" is insufficient. This is because some products may prove to be ineffective (Anonymous 2014; Cram and Fraedrick 2015; DeMuro et al. 1990; Maltz and Treseder 2015; Marx et al. 1984a, Repáč 2011; Repáč et al. 2014; Vosátka et al. 2008). Profits can't be made when products do not work (Dettweiler-Robinson et al. 2013). Even when a treatment increases growth (Holuša et al. 2009), the treatment must cost less than the potential economic gains (Marx et al. 2002).

The total cost of applying Pt spores is easy to determine by adding the cost of application to the cost of materials. Once the total cost is determined, then estimates can be made of how much of a gain in crop value must be achieved in order to "break even." When the price per g is $30, and 1 mg of spores is applied per seedling, then the cost per seedling is $0.03. In order for some bareroot nurseries to "break even," they would have to raise the price of pine seedlings by 50 percent without losing any customers.

Applying Pt spores in containers might increase early height growth of pine seedlings about a quarter of the time (table 2). However, most managers in the southern United States top-prune seedlings to keep seedlings from growing too tall. Without the use of Pt spores, the median height growth of container-grown pines is 32 cm (South et al. 2016). Therefore, increasing height by an additional 1 or 2 cm may not be economical. In contrast, increasing seedling production is economical at nurseries where the costs do not exceed revenue from additional seedling sales (table 5). In the past, Pt spores were sometimes applied as a marketing tool (Landis 1993) in hopes this would attract customers away from other nurseries (thereby increasing seedling sales).

Table 5. Examples of the economics of applying spores of *Pisolithus tinctorius* (Pt). A profit results (examples 1 and 2) when the use of spores increases seedling production at a cost that is exceeded by the sale of extra seedlings. A financial loss occurs in cases where treatment costs exceed the gain in revenue (example 3) or when the treatment failed to work as expected (example 4).

Example	Treatment	Cost per g	Cost/ha	Seedling value / ha (5 cents each)	Profit-(Loss)
0	No spores	$0	$0	$88,000	--
1	Pt spores	$0	$0	$104,000	$16,000
2	Pt spores	$3	$12,000	$104,000	$4,000
3	Pt spores	$15	$60,000	$104,000	($44,000)
4	Pt spores	$3	$12,000	$88,000	($12,000)

Some suggest the use of nursery fertilizers can be reduced by applying mycorrhizal inocula (Dixon et al. 1981; Marx and Artman 1978; Sousa et al. 2012; Väre 1990). However, nursery managers know that fertilization is a relatively inexpensive way to achieve the target seedling in a limited amount of time. Applying a commercial Pt product is a more expensive method of increasing growth (figure 7). In one example (Khasa et al. 2001), spending 5 cents per seedling on vegetative Pt inocula (Coredell et al. 1989) reduced fertilizer cost by less than 0.1 cent per seedling (Clements and Dominy 1990; South and Zwolinski 1996). Likewise, when using fertilizers, applying Pt spores to containers appears to be an unreliable method of increasing height growth (table 2). Managers prefer to apply fertilizer to produce 30 cm tall seedlings (3.5 mm in diameter) than to spend more money to inoculate with Pt and produce smaller seedlings (23 cm height; 2.5 mm diameter) by using one-fourth the amount of fertilizer (Ruehle and Wells 1984).

Most researchers who publish papers do not include either the cost of Pt application or the effect of the treatment on the number of plantable seedlings produced. Therefore, researchers may test rates of Pt spores that are far from economical. As a result, nursery managers may need to collect their own data to determine any economic benefit of using lower dosages of Pt spores. A sound calculation should include (1) cost of spores, (2) cost of applying the spores, (3) effect on seedling production, and (4), probability of achieving an increase in production. The hardest part to determine is the probability of achieving an increase in production since it requires multiple tests conducted over several years. For example, in 21 tests with loblolly pine over a four-year period, a commercial (vegetative) Pt product caused a statistically significant (α =0.05) increase in plantable seedlings in only one test (Marx et al. 1984a). Likewise, when applying Pt spores at three *Eucalyptus* nurseries over a two-year period (Brundrett et al. 2005), inoculation resulted in Pt ectomycorrhiza in 2 out of 8 attempts. Variable results such as these and the discrepancy found between laboratory and nursery studies help to explain why some managers do not inoculate seedlings with Pt spores (Araujo et al. 2018).

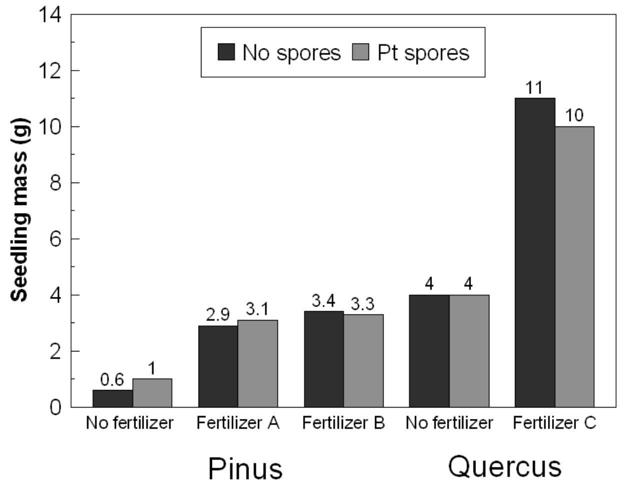

Figure 7. Although the cost of applying commercial products containing *Pisolithus tinctorius* (at one million spores per seedling) could exceed \$15 per thousand seedlings, the cost of applying chemical fertilizers can cost less than \$0.40 per thousand seedlings. In trials with *Pinus pinaster* (Rincón et al. 2007) and *Quercus rubra* (Beckjord et al. 1985), fertilized seedlings were more than twice as large as seedlings treated with only Pt spores.

9 Effect of Pt spores on outplanting performance

There are two schools of thought regarding the use of Pt spores in pine nurseries. One school "A" believes the application of spores in commercial products will increase the survival potential on adverse sites, while those from school "B" say the benefit/cost ratio of applying Pt spores will likely be less than 1 for many outplanting sites. Although several studies show that use of vegetative inoculants can sometimes increase survival of loblolly pine (Trofymow and van den Driessche 1991), only a few commercial products have been tested. Treating pines with Pt spores (at time of sowing) increased survival in just one of 14 outplanting trials (figure 8) and in New Mexico, treating seedlings 6 weeks after sowing increased survival of one out of seven species (Harrington et al. 2001). Spores did not increase survival of container-grown longleaf pine (*Pinus palustris* Mill.) but height growth was increased by 19 cm (4 years after planting) (Cram et al. 1999).

Some say that 50 to 60 percent infection with Pt is needed in order for a Pt treatment to achieve a significant increase in survival and growth (Marx et al. 1984b, Ruehle 1980; Ruehle et al. 1981). These Pt infection rates (short roots) may be difficult to achieve using Pt spores, especially at nurseries that use Pt inhibiting fungicides (Marx 1987). Even when alternative fungicides are applied to Pt spore treated seedlings, the Pt index of bareroot loblolly pine seedlings may range from 33 percent to 46 percent (Marx et al. 1989b). Therefore, to obtain a Pt index of 50 percent may require the culling of some (otherwise plantable) seedlings (Cram et al. 1999).

When discussing seedling quality, mycorrhizae are often not mentioned (Duryea 1984; Grossnickle 2012; Grossnickle and South 2017; Lavender, 1984; Puttonen 1996; Ritchie 1984; South 1993; Wilson and Jacobs 2005). This is because pine and oak seedlings typically have ectomycorrhiza and, at most operational nurseries, the percentage of short roots with mycorrhiza is not a reliable predictor of field performance. In fact, well fertilized container-grown seedlings (without Pt mycorrhiza) have outperformed smaller seedlings with plenty of Pt ectomycorriza (Barnett 1983; Echols et al. 1990). This type of response likely explains why some researchers (DeMuro et al. 1990; Repac et al. 2011; South and Skinner 1998) and most silviculturists do not count the number mycorrhizal short roots on nursery stock.

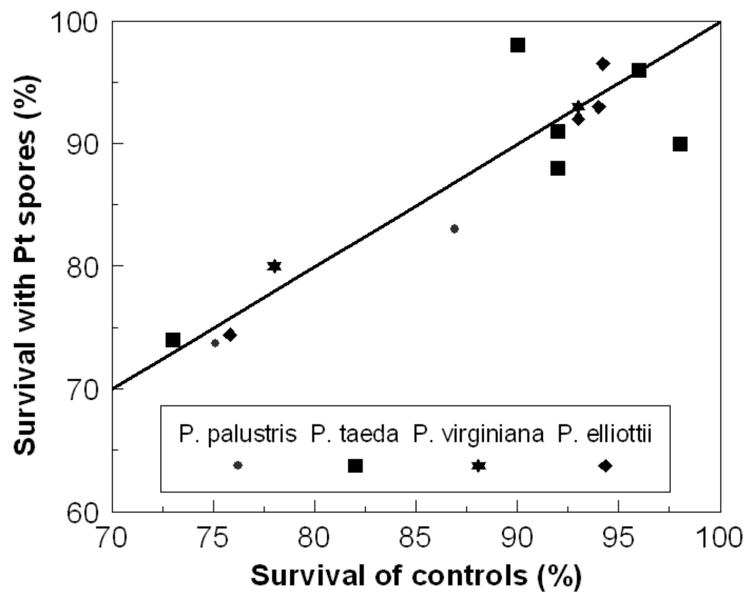

Figure 8. Applying *Pisolithus tinctorius* spores before or just after sowing in the nursery typically does not increase seedling survival in the field. Only one of the paired tests was associated with a significant increase in survival (i.e. 8 percent above the diagonal line). Data are from loblolly pine (Pinus taeda), slash pine (*Pinus elliottii*), Virginia pine (*Pinus virginiana*) (Marx et al. 1977), slash pine (Rowan 1984), loblolly pine (Leach and Gresham 1983) and longleaf pine (*Pinus palustrius)* (Cram et al 1999).

10 The decline effect

"Many scientifically discovered effects published in the literature seem to diminish with time" (Schooler 2011). When studies are repeated, the magnitude of the treatment response may be less than expected, based on the initial published results. This phenomenon was discovered in research into parapsychology but the phenomenon also occurs in biology. For example, South (1998) reported on 30 top-pruning studies installed from 1949 to 1979. As it turned out, the initial 1949 study reported a 55 percent increase in survival, while none of the subsequent studies reported an increase of 37 percent or more.

This phenomenon may also exist with Pt spore research. In places like India, South Africa, Iowa and Hawaii, the amount of airborne spores was initially low and adding spores to nursery soil and media was beneficial (McComb 1943; Thapar and Paliwal 1982). However, the area of exotic conifer plantations increased over time and since the atmosphere now contains more mycorrhizal spores, artificial inoculation may no longer be necessary (Whitesell et al. 1992). A higher level of airborne spores is one explanation for the "decline effect." In addition, a decline phenomenon may also exist in regions with naturally high levels of airborne spores. In 1973; applying Pt spores to pine seedbeds at the Edwards Nursery in North Carolina increased total ectomycorhiza by 18 percentage points but since that time, an increase that large has only been documented once (table 1). An alternative explanation for "decline effect" exists because rates used operationally in 2017 were much lower than the 7 mg per seedling rate used in 1973.

11 Final thoughts

Although the production of commercial products containing Pt spores has increased globally, their use in North American tree nurseries has declined. At some nurseries, the decline occurred because the cost was greater than the benefits. High quality, ectomycorrhizal seedlings can be produced at most nurseries that are located in regions with sufficient levels of airborne spores. Although some researchers recommended combining high spore rates with reduced rates of fertilizers and restricting use of certain fungicides, most managers find profits are greater when these suggestions are ignored.

12 Acknowledgments

The author thanks Scott Enebak (Auburn University), Michelle Cram (U.S. Forest Service, Wayne Bell (International Forest Company), Steve Grossnickle (NurseryToForest Solutions), Pedro Vilar-Salvador (University of Alcalá),), John Mexal New Mexico State University), Peter Harington (Scion), Ken McQuage (International Forest Company), John Blake (retired), for providing helpful comments on the manuscript.

13 References

Alvarez IF, Trappe JM (1983a) Effects of application rate and cold soaking pretreatment of *Pisolithus* spores on effectiveness as nursery inoculums on western conifers. Can J Forest Res 13(3): 533-537.

Alvarez IF, Trappe JM (1983b) Dusting roots of *Abies concolor* and other conifers with *Pisolithus tinctorius* spores at outplanting time proves ineffective. Can J Forest Res 13(5): 1021-1023.

Amaranthus MP, Perry DA (1987) Effect of soil transfer on ectomycorrhiza formation and the growth and survival of conifer seedlings on old, nonreforested clearcuts. Can J Forest Res 17(8): 944-951.

Ambriz E, Báez-Pérez A, Sánchez-Yáñez JM, Moutoglis P, Villegas J (2010) Fraxinus-Glomus-Pisolithus symbiosis: plant growth and soil aggregation effects. Pedobiologia 53(6): 369-373.

Anonymous (2014) Trees: from nursery to independence in the landscape - recommendations. London, UK: British Standards Institution. 8545:2014. 90 p.

Araujo GC, Sousa NR, Ramos MA, Vega AL, Castro PML (2018) Performance of *Quercus suber* L. at nursery state – application of two bio-inoculants under two distinct environments. Ann Forest Sci 75(1): 29.

Báez-Pérez, A.L.; Lindig-Cisneros, R.; Villegas, J. 2017. Survival and growth of nursery inoculated *Fraxinus* *uhdei* in acrisol gullies. Madera Bosques 23(3): 7-14.

Barnett JP (1983) Relating field performance of containerized longleaf and shortleaf pine seedlings to mycorrhizal inoculation and initial size. In: Thielges BA (ed.) Proceedings, 7th North American Forest Biology Workshop. University of Kentucky, Lexington, KY: 358-367.

Barnett JP, McGilvray JM (1997) Practical guidelines for producing longleaf pine seedlings in containers. Gen. Tech. Rep. SRS-14. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: 28 p.

Beckjord PR, McIntosh MS (1984) Growth and fungal persistence by *Quercus rubra* inoculated with ectomycorrhizal fungi and planted on a clear-cutting and strip mine. Can J Bot 62(8): 1571-1574.

Beckjord PR, McIntosh MS, Hacskaylo E, Melhuish JH (1984) Inoculation of loblolly pine seedlings at planting with basidiospores of ectomycorrhizal fungi in chip form. Res. Note NE-324. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 4 p.

Beckjord PR, Melhuish JH, McIntosh MS (1985) Effects of nitrogen and phosphorus fertilization on growth and formation of ectomycorrhizae of *Quercus alba* and *Q. rubra* seedlings by *Pisolithus* and *Scleroderma auranteum*. Can J Bot 63(10): 1677-1680.

Beckjord PR, Melhuish JH, Hacskaylo E (1986) Ectomycorrhiza formation on sawtooth oak by inoculation with basidioispore chips of *Pisolithus tinctorius* and *Scleroderma citrinum*. Journal of Environmental Horticulture 4(4): 127-129.

Boling BC, Naab FU, Smith D, Duggan JL, McDaniel FD (2006) Leaf elemental analysis in mycorrhizal post oak seedlings. Nucl Instrum Meth B 251(1): 182-190.

Brundrett N, Malajczuk G, Mingdqin X, Daping X, Snellling S, Dell B (2005) Nursery inoculation of *Eucalyptus* seedlings in Western Australia and southern China using spores and mycelia inoculums of diverse ectomycorrhizal fungi from different climatic regions. Forest Ecol Manag 209(3): 193-205.

Bryson HL (1980) *Pisolithus tinctorius* mycobiont inoculations as a factor in performance of containerized and bare-root shortleaf pine seedlings on lignite minesoils in Panola County, Texas. Austin, TX: Stephen F. Austin. State University. 418 p. Ph.D. Dissertation.

Carey WA, Kelley WD (1993) Seedling production trends and fusiform rust control practices at southern nurseries, 1981-1991. South J Appl For 17(4): 207-211.

Castellano MA, Molina R (1990) Mycorrhiza. In: Landis TD, Tinus RW, McDonald SE, Barnett JP (eds.) The container tree nursery manual, Volume 5. Agricultural Handbook 674. Washington, DC: U.S. Department of Agriculture, Forest Service: 103-167.

Castellano MA, Trappe JM (1991) Fails to improve plantation performance of inoculated conifers in southwestern Oregon. New Forest 5(4): 349-358.

Clements SE, Dominy SWJ (1990) Costs of growing containerized seedlings using different schedules at Kingsclear, New Brunswick. North J Appl For 7(2): 73-76.

Colinas C, Perry D, Molina R, Amaranthus M (1994) Survival and growth of *Pseudotsuga menziesii* seedlings inoculated with biocide-treated soils at planting in a degraded clearcut. Can J Forest Res 24(8): 1741-1749.

Cordell CE, Omdal DW, Marx DH (1989) Operational, ectomycorrhizal fungus inoculations in forest tree nurseries: 1989. In: Landis TD (tech coord.) Proceedings: Intermountain Forest Nursery Association - 1989. Gen. Tech. Rep. RM-184. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station: 86-92.

Cordell CE, Marx DH, Bryan C (1974) Mycorrhizae in forest nurseries. In: Southeastern Nurserymen's Conferences – 1974. Atlanta, GA: USDA Forest Service, Southeastern Area, State and Private Forestry: 175-180.

Cram MM, Dumroese RK (2012) Mycorrhizae in forest tree nurseries. In: Cram MM, Frank MS, Mallams KM (eds.) Forest Nursery Pests. Ag. Handbook 680. Washington, DC: U.S. Department of Agriculture, Forest Service: 20-23.

Cram MM, Fraedrich SW (2015) Inoculation of fumigated nursery beds and containers with arbuscular mycorrhizal products for eastern redcedar production. Tree Planters' Notes 58(3): 33-39.

Cram MM, Mexal JG, Souther R (1999) Successful reforestation of South Carolina Sandhills is not influenced by seedling inoculation with *Pisolithus tinctorius* in the nursery. South J Appl For 23(1): 46-52.
Crous JW (2017) Use of hydrogels in the planting of industrial wood plantations. Southern Forests: A Journal of Forest Science 79(3): 197-213.
Davis AS, Jacobs DF (2004) First-year survival of northern red oak seedlings planted on former surface coal mines in Indiana. In: Barnhisel RI (ed.) Proceedings, American Society of Mining and Reclamation, Lexington, KY: American Society for Surface Mining and Reclamation. 25: 480-503.
Davis AS, Ross-Davis AL, Dumroese KR (2011) Nursery culture impacts cold hardiness in longleaf pine (*Pinus palustris*) seedlings. Restor Ecol 19(6): 717-719.
DeMuro G, Jencks EM, Hindal DF (1990) Survival and growth of bigtooth aspen on acidic surface-mine soils as influenced by *Pisolithus tinctorius* and nitrogen and phosphorus fertilization. Proceedings of the Mining and Reclamation Conference and Exhibition. American Society of Mining and Reclamation 2: 573-578.
Dettweiler-Robinson E, Bakker JD, Evans JR, Newsome H, Davies GM, Wirth TA, Pyke DA, Easterly RT, Salstrom D, Dunwiddie P (2013) Outplanting Wyoming big sagebrush following wildfire: stock performance and economics. Rangeland Ecology & Management 66(6): 657-666.
Dixon RK, Wright GM, Garrett HE, Cox GS (1981) Container- and nursery-grown black oak seedlings inoculated with *Pisolithus tinctorius*: growth hand ectomycorrhizal development during seedling production period. Can J Forest Res 11(3): 487-491.
Dominguez-Núñez JA, Muñóx D, de la Cruz A, Saiz de Omeñaca JA (2013) Effects of *Pseudomonas fluorescens* on the water paramerters of mycorrhizal and non-mycorrhizal seedlings of *Pinus halepensis*. Agronomy 3(3): 571-582.
Duryea ML (1984) Nursery cultural practices: impacts on seedling quality. In: Duryea ML, Landis TD (ed.) Forest Nursery Manual: production of bareroot seedlings. Martinus Nijhoff / Dr. W. Junk Publishers, The Hague: 143-164. Chapter 15.
Echols RJ, Meier CE, Ezell AW, McKinley CR (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(2) 13-21.
Free G (2017) Personal communication. Nursery Manager, Westervelt, Eutaw, AL.
Garcia-Barreda S, Molina-Grau S, Reyna S (2015) Reducing the infectivity and richness of ectomycorhial fungi in a calcareous *Quercus ilex* forest through soil preparations for truffle plantation establishment: a bioassay study. Fungal Biol-UK 119(11): 1137-1143.
González-Ochoa AI, de las Heras J, Torres P, Sánchez-Gómex E (2003) Mycorrhization of *Pinus halelpensis* Mill. and *Pinus pinaster* Aiton seedlings in two commercial nurseries. Ann Forest Sci 60(1): 43-48.
Grossnickle SC (2012) Why seedlings survive: importance of plant attributes. New Forest 43:711-738.
Grossnickle SC, South DB (2017) Seedling quality of southern pines: influence of plant attributes. Tree Planters' Notes 60(2): 29-40.
Hamm PB, Campbell SJ, Hansen EM (1990) Growing healthy seedlings: identification and management of pests in Northwest forest nurseries. Special publication 19. Corvallis, OR. Forest Research Laboratory, Oregon State University. 110 p.
Harrington JT, Wagner AM, Dreesen DR (2001) The influence of *Pisolithus tinctorius* inoculation on greenhouse growth and first-year transplant survival of conifer seedlings. In: Vincent R, Barnhisel RI (eds.) Proceedings, Land reclamation – a different approach. Lexington, KY: American Society for Surface Mining and Reclamation 18: 255-264.
Hatch AB (1936) The role of mycorrhizae in afforestation. J Forest 34(1): 22-29.
Hatchell GE, Marx DH (1985) Response of longleaf, sand, and loblolly pines to *Pisolithus* ectomycorrhizae and fertilizer on sandhills site in South Carolina. Forest Sci 33 (2): 301-315.
Helm DJ, Carling DE (1993) Use of soil transfer for reforestation on abandoned mined lands in Alaska. Mycorrhiza 3(3): 97-106.
Holusa J, Peskova V, Vostra L, Pernek M (2009) Impact of mycorrhizal inoculation on spruce seedling: comparisons of a 5-year experiment in forests infested by honey fungus. Period Biol 111(4):

413-417.

Hua X, Cordell CE, Stambaugh WJ (1991) Synthesis of *Pisolithus tinctorius* ectomycorrhizae and growth responses on some commercially important Chinese tree species. Forest Ecol Manag 42(4): 283-292.

Jorgensen JM (2014) The effects of different concentrations of phosphate on ectomycorrhiza formation by *Pisolithus tinctorius*. Chattanooga, TN: University of Tennessee at Chattanooga. 67 p. MS thesis.

Khasa PD, Sigler L, Chakravarty P, Dancik BP, Erickson L, McCurdy D (2001) Effect of fertilization on growth and ectomycorrhizal development of container-grown and bare-root nursery conifer seedlings. New Forest 22(3): 179-197.

Kelley WD (1987) Effect of triadimefon on development of mycorrhizae from natural inoculums in loblolly pine nursery beds. South J Appl For 11(1): 49-52.

Kulling W (2008) Real case applications of commercial mycorrhiza products in the Netherlands" "Prove us that mycorrhiza works and we will use it." In: Feldmann F, Kapulnik Y, Baar J (eds.) Mycorrhiza Works. Braunschweig, Germany. 17-24.

Landis TD (1993) A practical look at mycorrhizal fungi in nurseries: part two. Forest Nursery Notes (July): 12-18.

Lavender DP (1984) Plant physiology and nursery environment: interactions affecting seedling growth. In: Duryea ML, Landis TD (eds.) Forest Nursery Manual: production of bareroot seedlings. Martinus Nijhoff / Dr. W. Junk Publishers, The Hague: 133–141. Chapter 14.

Leach GN, Gresham HH (1983) Early field performance of loblolly pine seedlings with *Pisolithus tinctorius* ectomycorrhizae on two lower Coastal Plain sites. South J Appl For 7(3): 149-153.

Lu X, Malajczak N, Dell B (1998) Mycorrhiza formation and growth of *Eucalyptus globulus* seedlings inoculated with spores of various ectomycorrhizal fungi. Mycorrhiza 8(2): 81-86.

Maltz MR, Treseder KK (2015) Sources of inocula influence mycorrhizal colonization of plants in restoration projects: a meta-analysis. Restor Ecol 23(5): 625-634.

Martin TP, Harris JR, Eaton GK, Miller OK (2003) The efficacy of ectomycorrhizal colonization of pin and scarlet oak in nursery production. Journal of Environmental Horticulture 21(1): 45-50.

Marx DH (1976) Synthesis of ectomycorrhizae on loblolly pine seedlings with basidiospores of *Pisolithus tinctorius*. Forest Sci 22(1): 13-20.

Marx DH (1987) Triadimefon and *Pisolithus* ectomycorrhizae affect second-year field performance of loblolly pine. Res. Note. SE-349. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station. 6 p.

Marx DH (1990) Soil pH and nitrogen influence *Pisolithus* ectomycorrhizal development and growth of loblolly pine seedlings. Forest Sci 36(2):224-245.

Marx DH, Barnett JP (1974) Mycorrhizae and containerized forest tree seedlings. In: Tinus RW, Stein WI, Balmer WE (eds.) Proceedings, North American Containerized forest tree seedling symposium. Great Plains Agricultural Council. Publication 68: 85-92.

Marx DH, Bell W (1985) Formation of *Pisolithus* ectomycorrhizae on loblolly pine seedlings with spore pellet inoculums applied at different times. Res. Pap. SE-249. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station. 6 p.

Marx DH, Bryan WC (1969) Studies on ectomycorrhizae of pine in an electronically air-filtered, air-conditioned, plant-growth room. Can J Bot 47(12): 1903-1909.

Marx DH, Bryan WC (1975) Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. Forest Sci 21(3): 245-254.

Marx DH, Bryan WC, Cordell CE (1976) Growth and ectomycorrhizal development of pine seedlings in nursery soils infested with the fungal symbiont *Pisolithus tinctorius*. Forest Sci 22(1): 91-100.

Marx DH, Bryan WC, Cordell CE (1977) Survival and growth of pine seedlings with *Pisolithus* ectomycorrhae after two years on reforestation sites in North Carolina and Florida. Forest Sci 23(3): 363-373.

Marx DH, Artman JD (1978) Growth and ectomycorrhizal development of loblolly pine seedlings in nursery soil infested with *Pisolithus tinctorius* and *Thelephora terrestris* in Virginia. Res. Note. SE-256. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest

Experiment Station. 6 p.
Marx DH, Cordell CE (1988) Bed density and *Pisolithus ectomycorrhizae* affect morphology of loblolly pine seedlings. In: Hagwood R (ed.) Proceedings, southern forest nursery association—1988. Atlanta, GA: U.S. Department of Agriculture, Forest Service, State and Private Forestry: 70-79.
Marx DH, Cordell CE (1990a) Development of *Pisolithus tinctorius* ectomycorrhizae on loblolly pine from spores sprayed at different times and rates. Res. Note SE-356. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station. 6 p.
Marx DH, Cordell CE (1990b) Inoculation of fall- and spring-sown longleaf pine seedlings with *Pisolithus tinctorius*. Res. Note SE-358. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station. 6 p.
Marx DH, Cordell CE, France RC (1986) Effects of triadimefon on growth and ectomycorrhizal development of loblolly and slash pines in nurseries. Phytopathology 76(8): 824-831.
Marx DH, Cordell CE, Kenney DS, Mexal JG, Artman JD, Riffle JW, Molina RJ (1984a) Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorhizae on bare-root tree seedlings. Forest Sci 30(3): Monograph 25.
Marx DH, Cordell CE, Kormanik P (1989a) Mycorrhizae: benefits and practical application in forest tree nurseries. In: Cordell CE, Anderson RL, Hoffard WH, Landis TD, Smith RS, Toko HV (tech. cords.) Forest Nursery Pests. Ag. Handbook 680. Washington, DC: U.S. Department of Agriculture, Forest Service. 18-21.
Marx DH, Cordell CE, Maul SB, Ruehle JL (1989b) Ectomycorrhizal development on pine by *Pisolithus tinctorius* in bare-root and container seedling nurseries. New Forest 3(1): 57-66.
Marx DH, Jarl K, Ruehle JL, Bell W (1984b) Development of *Pisolithus tinctorius* ectomycorhizae on pine seedlings using basidiospore-encapsulated seeds. Forest Sci 30(4): 897-907.
Marx DH, Marrs LF, Cordell CE (2002) Practical use of the mycorrhiza fungal technology in forestry, reclamation, arboriculture, agriculture, and horticulture. Dendrobiology 47(1): 27-40.
Marx DH, Mexal JG, Morris WG (1979) Inoculation of nursery seedbeds with *Pisolithus tinctorius* spores mixed with hydromulch increases ectomycorrhizae and growth of loblolly pines. South J Appl For 3(4): 175-178.
Marx DH, Morris WG, Mexal JG (1978) Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated and nonfumigated nursery soil infested with different fungal symbionts. Forest Sci 24(2): 193-203.
McComb AL (1943) Mycorrhizae and phosphorus nutrition of pine seedlings in a prairie soil nursery. Iowa Agricultural Experiment Station Research Bulletin 314: 582-603.
McComb AL, Griffith JE (1946) Growth stimulation and phosphorus absorption of mycorrhizal and nonmycorrhizal northern white pine and Douglas fir seedlings in relation to fertilizer treatment. Plant Physiol 21(1): 11-17.
Mexal JG (1980) Aspects of mycorrhizal inoculation in relation to reforestation. New Zealand Journal of Forestry Sci 10(1): 208-217.
Mitchell RJ, Cox GS, Dixon RK, Garrett HE, Sander IL (1984) Inoculation of three species with eleven isolates of ectomycorrhizal fungi. II. Foliar nutrient content and isolate effectiveness. Forest Sci 30(3): 563-572.
Molina R (1979) Ectomycorrhizal inoculation of containerized Douglas-fir and lodgepole pine seedlings with six isolates of *Pisolithus tinctorius.* Forest Sci 25(4): 585-590.
Pera J, Parlade J, Alvarez LF (1994) Eficacia del tipo de inoculo de *Pisolithus tinctorius* en la formacion de mixorrizas en *Pinus pinaster* y *Pseudotsuga menziesii*. Investigación agrarian. Sistemas y recursos forestales 3(1): 19-29.
Pilz D, Znerold RM (1986) Comparison of survival enhancement techniques for outplanting on a harsh site in the western Oregon Cacades. Tree Planters' Notes 37(4): 24-28.
Pope PE (1988) *Pisolithus tinctorius* increases the size of nursery grown red oak seedlings. New Forest 2(1): 5-16.
Preve RE, Burger JA, Kreh RE (1984) Influence of mine spoil type, fertilizer, and mycorrhizae on pines seeded in greenhouse trays. J Environ Qual 13(3): 387-391.
Puttonen P (1996) Looking for the "silver bullet" – can one test do it all? New Forest 13 (1): 9-27.

Querejeta JI, Roldan A, Albaladejo J, Castillo V (1998) The role of mycorrhizae, site preparation, and organic amendment in the afforestation of a semi-arid Mediterranean site with *Pinus halepensis.* Forest Sci 43(2): 203-211.

Repáč I (2011) Ectomycorrhizal inoculums and inoculation techniques. In: Ray M, Varma A (eds.) Diversity and biotechnology of ectomycorrhizae, Soil Biology vol 25. Springer, Berlin Heidelberg, 43-63. Chapter 3.

Repáč I, Balanda M, Vencurik J, Kmet J, Krajmerova D, Paule L (2014) Effects of substrate and ectomycorrhizal inoculation on the development of two-years-old container-grown Norway spruce (*Picea abies* Karst.) seedlings. iForest 8(4): 487-496.

Repáč I, Tućeková A, Sarvašová I, Vencurik J (2011) Survival and growth of outplanted seedlings of selected tree species on the High Tatra Mts. windthrow area after the first growing season. J Forest Sci 57(8): 349-358.

Rincón A, Alvarez IF, Pera J (2001) Inoculation of containerized *Pinus pinea* L. seedlings with seven ectomycorrhizal fungi. Mycorrhiza 11(6): 265-271.

Rincón A, Alvarez IF, Pera J (2007) Influence of the fertilization method in controlled ectomycorrhizal inoculation of two Mediterranean pines. Ann Forest Sci 64(5): 577-583.

Ritchie GA (1984) Assessing seedling quality. In: Duryea ML, Landis TD (ed.) Forest Nursery Manual: production of bareroot seedlings. Martinus Nijhoff / Dr. W. Junk Publishers, The Hague: 243-266. Chapter 23.

Roldán A, Querejeta I, Albaladejo J, Castillo V (1996) Survival and growth of Pinus halepensis Miller seedlings in a semi-arid environment after forest soil transfer, terracing and organic amendments. Ann Forest Sci 53(6): 1099-1112.

Rossi MJ, Furigo A, Oliveira VL (2007) Inoculant production of ectomycorrhizal fungi by solid and submerged fermentation. Food Technol Biotech 45(3): 277-296.

Ruehle JL (1980) Inoculation of containerized loblolly pine seedlings with basidiospores of *Pisolithus tinctorius*. Res. Pap. SE-291. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station. 4 p.

Ruehle JL, Marx DH (1977) Developing ectomycorrhizae on containerized pine seedlings. Res. Pap. SE-242. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station. 8 p.

Ruehle JL, Marx DH, Barnett JP, Pawuk WH (1981) Survival and growth of container-grown and bare-root shortleaf pine seedlings with *Pisolithus* and *Thelephora* ectomycorrhizae. South J Appl For 5(1): 20-24.

Ruehle JL, Marx DH, Barnett JP, Pawuk WH (1981) Development of Pi*solithus tinctorius* ectomycorrhizae on container-grown pine seedlings as affected by fertility. Forest Sci 30(4): 1010-1016.

Sarvaš M, Pavlenda P, Takáčová E (2007) Effect of hydrogel application on survival and growth of pine seedlings in reclamations. J Forest Sci 53(5): 204-209.

Schooler J (2011) Unpublished results hide the decline effect: some effects diminish when tests are repeated. Nature 470: 437.

Sousa NR, Franco AR, Oliveira RS, Castro PML (2012) Ectomycorrhizal fungi as an alternative to the use of chemical fertilizers in nursery production of *Pinus pinaster*. J Environ Manage 95(supplement): S296-S274.

South DB (1993) Rationale for growing southern pine seedlings at low seedbed densities. New Forest 7(1): 63-92.

South DB (1998) Needle-clipping longleaf pine and top-pruning loblolly pine in bare-root nurseries. South J Appl For 22(4): 235-240.

South DB, Donald DGM (2002) Effect of nursery conditioning treatments and fall fertilization on survival and early growth of *Pinus taeda* seedlings in Alabama, U.S.A. Can J Forest Res 32(7): 1171-1179.

South DB, Kelley WD, Chapman W (1989) "Fall mosaic" of loblolly pine in a forest tree nursery. Tree Planters' Notes 40(1): 19-22.

South DB, Skinner MF (1998) Nursery stock and field fertilizer application affect early performance of *Pinus radiata* on a phosphorus-deficient site in Northland. NZ J Forestry Sci 28(3): 361-372.

South DB, Starkey TE, Enebak SA (2016) Forest nursery practices in the southern United States. Reforesta 1(1): 106-146.

South DB, Zwolinski JB (1996) Chemicals used in southern forest nurseries. South J Appl For 20(3): 127-135.

Starkey TE, Enebak SA, South DB, Cross RE (2012) Particle size and composition of polymer root gels affect loblolly pine seedling survival. Native Plants 13(1): 19-26.

Stottlemyer AD, Wang GG, Wells CE, Stottlemyer DW, Waldrop TA (2008) Reducing airborne ectomycorrhizal fungi and growing non-mycorrhizal loblolly pine (*Pinus taeda* L.) seedlings in a greenhouse. Mycorrhiza 18(5): 269-275.

Strubbings JA (1958) Raising and use of large close-rooted transplants for commercial afforestation in southern Rhodesia. South African Forestry Journal 32(1): 36-55.

Thapar HS, Paliwal DP (1982) Studies on pine mycorrhiza in nursery seedlings. Indian Forester 109(1): 51-57,

Torres P, Honrubia M (1994) Inoculation of containerized *Pinus halepensis* (Miller) seedlings with basidiospores of *Pisolithus arhizus* (Pers) Rauschert, *Rhizopogon roseolus* (Corda) Th M Fr and *Suillus collinitus* (Fr) O Kuntze. Annuals of Forest Science 51(5): 521-528.

Trofymow JA, van den Driessche R (1991)Mycorrhizas. In: van den Driessche R (ed.) Mineral Nutrition of Conifer Seedlings. Boston, MA: CRC Press. 183-227. Chapter 8.

Väre H (1990) Effect of soil fertility on root colonization and plant growth of *Pinus sylvestris* nursery seedlings inoculated with different ectomycorrhizal fungi. Scand J Forest Res 5(1): 493-499.

Vosátka M, Látr A, Albrechtová J (2008) How to apply mycorrhizal inocula in a large-scale and what outcome can be expected in respect of plant growth and cultivation costs. In: Feldmann F, Kapulnik Y, Baar J (eds.) Mycorrhiza Works. Braunschweig, Germany. 323-339. P11631.typo3server.info/fileadmin/download/MycorrhizaWorks.pdf

Wakeley PC (1954) Planting the southern pines. Agricultural Monograph 18. Washington, DC: U.S. Department of Agriculture, Forest Service. 233 p.

Walker RF (2002) Fertilization and liming effects on the growth and nutrition of bareroot Jeffrey pine outplanted on an eastern Sierra Nevada surface mine. West J Appl For 17(1): 23-30.

Walker RF, McLaughlin SB (1991) Growth and root system development of white oak and loblolly pine as affected by simulated acidic precipitation and ectomycorrhizal inoculation. Forest Ecol Manag 46(1): 123-133.

Weissenhorn I (2002) Mycorrhiza and salt tolerance of trees. Final Report of Partner 9. EU-project MYCOREM. 36 p. www.servaplant.nl/new/wp-content/uploads/2015/10/MycoremReport.pdf

Wilde SA (1944) Mycorrhizae and silviculture. J Forest 42(4): 290-291.

Wilkinson KM (2009) Beneficial microorganisms. In: Dumroese RK, Luna R, Landis TD (eds.) Nursery Manual for native plants: a guide for tribal nurseries. Agricultural Handbook 730. Washington, DC: U.S. Department of Agriculture, Forest Service: 247-261. Chapter 14.

Wilkinson KM, Janos DP (2014) Beneficial microorganisms. In: Wilkinson KM, Landis TD, Haase DL, Daley BF, Dumroese RK (2014) Tropical Nursery Manual. Agricultural Handbook 732. Washington, DC: U.S. Department of Agriculture, Forest Service: 253-271. Chapter 13.

Wilson BC, Jacobs DF (2006) Quality assessment of temperate zone deciduous hardwood seedlings. New Forest 31(3): 417-433.

Wiseman PE, Colvin KH, Wells CE (2009) Performance of mycorrhizal products marketed for woody landscape plants. Journal of Environmental Horticulture 27(1): 41-50.

Whitesell CD, DeBell DS, Schubert TH, Strand RF, Crabb TB (1992) Short-rotation management of Eucalyptus: guidelines for plantations in Hawaii. Gen. Tech. Rep. PSW-GTR-137. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 30 p.

Wood GA (1985) Two year survival and growth of loblolly pine seedlings from two Texas seed sources on lignite minesoils. Austin, TX: Stephen F. Austin. State University. 113 p. MS. Thesis.

Young C (1981) Open-root nursery techniques and planting methods. South African Forestry Journal 117(1): 68-70.

Zak B, Bryan WC (1963) Isolation of fungal symbionts from pine mycorrhizae. Forest Sci 9(3): 270-278.