Root Dip Treatments Affect Fungal Growth in vitro and Survival of Loblolly Pine (Pinus taeda)

Tom E. Starkey and David B. South

Research Fellow and Professor, Southern Forest Nursery Cooperative, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL

Abstract

Hydrogels and clay slurries are the materials most commonly applied to roots of pines in the Southern United States. Most nursery managers believe such applications offer a form of "insurance" against excessive exposure during planting. The objective of this study was to examine the ability of root dip treatments to (1) support fungal growth and (2) protect roots from injury during exposure for 1, 2, or 4 h. Four treatments were tested: kaolin clay, two grades of polyacrylamide hydrogels, and a cornstarch-based hydrogel. In petri dish tests, kaolin clay was the only treatment that inhibited the growth of three soil-borne fungi (*Pythium*, *Fusarium, Rhizoctonia*). When applied to roots, however, the clay slurry did not effectively prevent permanent root damage during exposure and subsequent mortality. Gel treatment provided some protection when roots were exposed to air for 2 or 4 h. If a gel treatment reduces the need for replanting only 1 ha in 3,333, the benefit/cost ratio might equal 2 (assuming a cost of $500 ha^{-1} for replanting and a gel treatment cost of $250 per 3.3 million seedlings).

Introduction

During the 19th century, roots were often kept moist at the nursery during counting and sorting to improve the chance of seedling survival (Hodges 1883). The practice of "puddling" has been used for more than a century; this involves dipping roots into a mixture of clay and water (the consistency of paint) either at the nursery (Goff 1897) or at the planting site (Hodges 1883; Pinchot 1907). It is interesting to note what Toumey (1916) said about freshly lifted stock: "Puddling is not necessary and usually does more harm than good." We know that washing roots to remove soil can reduce seedling quality (Carey and others 2001), which might explain why Toumey believed puddling harmed seedling quality. In some cases, washing roots was recommended in cases where puddling resulted in problems with aeration due to mud adhering to roots (Goff 1897). Toumey did suggest, however, that roots be thoroughly puddled if roots became "over-dry" during storage. Some questioned this claim, so later he changed the recommendation to applying water but not puddling (Toumey and Korstian 1949). Even today, recommendations vary, depending on whom you ask.

Several materials have been added to roots before packing seedlings. Sphagnum moss was preferred during the 19th and the first half of the 20th century; as moss became harder to acquire, alternative treatments were investigated (Davey 1964; Fisher 1974). Slocum and Maki (1956; 1959) reported benefits of treating roots with clay when seedlings were exposed to an hour or two of drying. In 1960, Weyerhaeuser asked that their seedlings be treated with clay at the nursery (Bland 1964), and this practice was quickly adopted by the North Carolina Forest Service Nursery at Goldsboro, NC. Soon after, other researchers began to report on tests using clay slurries (Dierauf and Marler 1967; 1971), and the practice spread. Some preferred clay dipping to moss, believing it made it unnecessary to have water in planting buckets because clay "protects seedling roots both before and after planting" (Hamner and Broerman 1967).

A few years later, sodium alginate became popular as a gel treatment in Germany and was subsequently tested in other countries (Miller and Reines 1974; Dierauf and Garner 1975; Bacon and others 1979). When roots were treated with sodium alginate and then exposed in a greenhouse for up to 5 d, seedling survival and the relative water content of needles were improved (Miller and Reines 1974). During the 1980s, nursery managers began operational use of polyacrylamide gels. In some cases, use of gels increases survival compared with root treatment with a clay dip (Venator and Brissette 1983). Polyacrylamide gels likely are preferred over clay because they usually cost less, require less storage space, and are less messy (Bland 1964). A nursery that produces 25 million seedlings may only need a pallet of product, while clay might require the delivery of 23 tonnes (25 tons) (Pryor 1988). Most managers agree with Alm and Stanton (1993), who believe that polymer gels “offer a form of insurance against survival loss resulting from seedlings being exposed to drying during the planting process.”

Despite this “insurance” aspect, there are no economic studies to support the use of either gels or clays in the production of loblolly pine (*Pinus taeda* L.). Therefore, these trials were initiated to examine the effects of three root dip treatments on their ability to (1) support fungal growth and (2) protect roots from injury during exposure.

Materials and Methods

Study I: Fungal Growth. This study was designed to address concerns that root treatments may support the growth of soil-borne fungi. In some cases, this might be detrimental to seedling survival. Treatments included kaolin clay, two grades of polyacrylamide hydrogels [PAM gels A and B (Soil Moist®, JRM Chemicals, Cleveland, OH)] and a cornstarch-based hydrogel, CSB gel (Zeba®, Absorbent Technologies, Beaverton, OR). Samples of the kaolin clay and PAM gels were obtained from the nursery; the CSB Gel was provided by the manufacturer. The particle size for each material was determined by passing the material through 250-µ and 500-µ sieves. The particle size and rate of material used for each treatment are provided in table 1. Companies offer different gel formulations based on particle size (Venator and Brissette 1983). Particle size can affect physical properties such as water-holding capacity and ability to go into suspension. The fungi used were pathogenic isolates of *Pythium* sp., *Fusarium* sp. and *Rhizoctonia* sp.

A 3-mm (0.12-in) plug of the fungus was placed on the center of a water-agar petri plate [85 mm diameter (3.3-in)] that had been augmented with either clay, PAM gel “A” or “B”, or CSB gel at a rate comparable to nursery use. Water agar is a basic medium made with distilled water that supports minimal fungal growth. Control plates held water agar without any gel or clay amendments. Each treatment was replicated 12 times. The radial growth of each fungus was recorded daily. Differences in fungal growth on the various amended media demonstrate the ability of the gel or clay to support fungal growth, relative to that of unamended media.

Study II: Seedling Survival Following Exposure. Each treatment (table 1) was mixed in a separate bucket with 7.5 L (2 gal) of tap water. The clay had to be stirred continuously during treatment, since the clay never dissolved. Both PAM gels dissolved with less than 1 min of stirring; gel “A” dissolved faster than gel “B”. The CSB gel, however, was very difficult to mix. When it was placed in the water, it immediately clumped and required considerable stirring and agitation to break up the clumps. Once this was done, it was similar in appearance to the PAM gels.

The amount of gel sprayed operationally on roots of machine-lifted loblolly pine is approximately 3.6 g (0.13 oz) per seedling. Dipping roots of 20 seedlings 5 times removed about 72 g (2.5 oz) of gel solution, or about 3.6 g (0.13 oz) of gel per seedling. All root gel or clay treatments were hand-dipped five times before exposure.

Seedlings were treated with one of four root treatments (table 1); the roots of control seedlings were dipped into water. The seedlings (20 per experimental unit) were laid on an expanded metal bench in the greenhouse for 0, 1, 2, or 4 h. Greenhouse temperatures during exposure ranged from 28 to 37 °C (82.4 to 98.6 °F); relative humidity ranged from 16 to 38 percent. The average solar radiation measured within the greenhouse was 22,700 lumen m^{-2} (2,100 lux).

After exposure, seedlings were transplanted at the Southern Forest Nursery Cooperative’s seedling testing facility. This facility consists of six pits [23 m (75 ft) × 23 m (75 ft) × 1 m (3 ft)] containing 100 percent sand. Twenty treatments (5 root × 4 exposure treatments) were replicated 12 times in a randomized complete block design with 5 seedlings per experimental unit. The sand in the pits was irrigated for 4 h before planting. In order to obtain a separation among treatments, irrigation was withheld after

Table 1. Percentage of material passing through a 500-µ and a 250-µ sieve and rate of material used expressed as total mass of material per liter (L) of water.

Particle size	Material (%)			
	Clay	PAM gel “A”	PAM gel “B”	CSB gel
>500µ	3.4	60.0	3.0	0
500–250µ	16.2	22.8	54.2	34
<250µ	80.4	17.2	42.8	66
Mass (g)	300	2.2	3.3	1.8

transplanting. Rainfall for the test period from February 7 to May 7 totaled 15.9 cm (6.3 in): 5.0 (2 in), 7.1 (2.8 in), 3.8 (1.5 in), and 0.0 cm (0 in) for February, March, April, and May, respectively). At the end of the study period (May 7, 2007), seedling survival was recorded.

Study III: Root Growth Potential. The gel and clay treatments for this study were the same as above (table 1). After root treatments had been applied, the seedlings were exposed for 1, 2, or 4 h. Greenhouse temperatures ranged from 29 to 33 °C (84.2 to 91.4 °F); relative humidity ranged from 18 to 42 percent. The average solar radiation within the greenhouse during the study was 20,500 lumen m^{-2} (1,900 lux).

The trial used two seedlings per experimental unit, with 18 replications (a total of 36 seedlings per treatment-exposure); 15 experimental units were contained in one aquarium (5 treatments × 3 exposure times). Seedling roots were suspended in aerated water, and water level in each aquarium was adjusted daily. After 4 wk, the numbers of new white root tips on each seedling were counted.

Data from each study were analyzed by analysis of variance (ANOVA) for a randomized complete block design. When the F-test for treatment was significant (α=0.05), treatment means were separated using Duncan's New Multiple Range Test. The SPSS® software (SPSS Inc, Chicago, IL, spss.com) was used for all data analysis.

Results

Study I: fungal growth. Particle size varied considerably among the gel treatments. PAM gel "A" had a greater percentage of large particles, while the CSB gel had a greater percentage of fine material (table 1). The water agar control was the baseline for each fungus tested. Therefore, any growth less than that observed in control plates indicated an inhibitory effect on the fungus (table 2), whereas more growth than in the controls indicated that the fungus was able to use the amendment as a food source. *Rhizoctonia* grew the fastest, with one or more treatments reaching the edge of the petri plate before day 6.

In all cases, clay inhibited fungal growth. All of the gel treatments inhibited growth of *Pythium* sp., but the clay treatment had the greatest effect. There was more plate-to-plate variation with the *Pythium* sp. than the other fungi. The growth of *Fusarium* sp. on the CSB gel was greater than for the control plates; clay was the only inhibitory treatment. Growth of *Rhizoctonia* sp. was increased by all the gels.

Study II: seedling survival following exposure. Treatments significant affected seedling survival, but there were no differences among treatments with 0 or 1 h of exposure (table 3). The root gels increased survival after 2 or 4 h of exposure. Clay or water dips, however, did not protect the roots exposed to these longer times of desiccation. This is very evident at 4 h of exposure, where the gel treatments increased survival by 40 percentage points or more.

Table 2. Fungal growth (mm) on amended or unamended water agar medium.

Amendment	*Pythium* (Day 6)	*Fusarium* (Day 6)	*Rhizoctonia* (Day 4)
Clay	10d	51c	58c
PAM gel "A"	26c	60b	75a
PAM gel "B"	31c	60b	74a
CSB gel	42b	63a	76a
Control	69a	61b	70b
$lsd_{(0.05)}$	6.5	1.6	2.8

[1] Means in columns followed by the same letter are not significantly different (=0.05; Duncan's new multiple range test).

Table 3. Loblolly pine survival (percent) after 3 mo, as affected by root dip treatment and length of exposure.

Dip treatment	Length of exposure (h)			
	0	1	2	4
PAM gel "B"	94.5a	86.8a	87.0b[1]	60.0b
PAM gel "A"	82.6a	88.9a	93.5b	56.1b
CSB gel	79.2a	76.2a	85.9b	52.8b
Clay	91.2a	87.9a	52.9a	12.1a
Water	97.8a	85.7a	77.2ab	12.1a

[1] Means in columns followed by the same letter are not significantly different (=0.05; Duncan's new multiple range test).

Study III: root growth potential. The root growth potential (RGP) study showed similar trends as the survival study. In the water-only treatment, 1 h of exposure reduced RGP by half, compared with the clay or CBS gel. In both the 2- and 4-h desiccation treatments, the RGP was reduced to fewer than 4 roots in both the clay and water treatments (table 4). Even when placed in water, the desiccated roots were not able to recover and produce new root tips. The gels provided some protection during the extended desiccation periods.

Discussion

When seedlings are handled carefully, not exposed to drying conditions, and not stored, outplanting survival can be greater than 80 percent (Venator and Brissette 1983). Under ideal conditions, roots would never be exposed to 2–4 hours of desiccation and would always be planted in moist soil. However, nursery managers typically have no control of seedling care after stock is shipped from the nursery. Every nursery manager has a file full of examples of seedlings transported incorrectly, stored in the sun at the planting site, and handled incorrectly by the planting crew.

Many studies have exposed roots after treatment with clay or gels (Slocum and Maki 1956; Williston 1967; Miller and Reines 1974; Dierauf and Gardner 1975; Alm and Stanton 1993). In this study, we decided to subject treated seedlings to various times of desiccation and then transplant them into moist sand to allow seedlings to become established.

Results from the survival and RGP studies agreed, but the RGP test detected treatment differences after just 1 h of desiccation. Our data agree with those of others who found that gels provided an increase in survival (Echols and others 1990; Alm and Stanton 1993). Although clay was not effective in preventing permanent root damage to the seedlings in our study, clay did improve seedling survival in a previous study (Slocum and Maki 1959).

During the 1980s, there were concerns that fermentation of wood fiber mulches or starch gels would result in deterioration of seedlings stored in the shade (Barnard and others 1981). The concern was that the wood fibers (or starch) were providing a substrate for pathogenic microbes. Therefore, some nursery managers have expressed a concern that root gels, especially the starch-based gels, could support the growth of soil-borne fungi. In order for disease to develop, three factors must occur. First, the environment must be conducive to disease development (this generally means optimal moisture and temperature). Second, the host must be susceptible. In some cases, the host may be too old to be susceptible. Third, you must have a virulent pathogen.

Of the four root dips tested, kaolin clay was the only treatment that did not support, but in fact inhibited, the growth of the three soil-borne fungi tested. The other root dips tested stimulated fungal growth, especially of *Fusarium sp.* and *Rhizoctonia sp*. Since these are common nursery fungi, they could utilize the polyacrylamide hydrogels or the cornstarch-based hydrogel as a food source. Thus, the gels might have negative ramifications during seedling storage, especially the CSB gel in the presence of *Fusarium sp*.

In many cases, a researcher wants to see significant differences among treatments before making a recommendation. In fact, many researchers do not even consider the benefit/cost ratio of a treatment if the treatment is significant at α=0.15. In many outplanting trials, researchers cannot declare a 10 percent or more increase in seedling survival as statistically significant, due to trials with low statistical power. For example, in one root-treatment trial in Louisiana, a 50 percent increase in survival was not statistically significant (Venator and Brissette 1982). Therefore, some might say that a treatment that is not "statistically signifi-

Table 4. Average number of white root tips at 4 wk, as affected by root dip treatment and length of exposure.

Treatment	Length of exposure		
	1 h	2 h	4 h
PAM gel "B"	32.1ab[1]	29.3b	19.9a
PAM gel "A"	41.3a	16.8c	22.6a
CBS gel	45.3a	39.3a	14.9a
Clay	43.1a	1.2d	0.0b
Water	22.0b	3.4d	0.0b

[1] Means in columns followed by the same letter are not significantly different (=0.05; Duncan's new multiple range test).

cant" but consistently increases survival by 5 percent is not worth the cost, even though it costs only pennies per acre.

Nursery managers have a different view. They may want to know if an inexpensive treatment provides some "insurance" against adverse conditions (Alm and Stanton 1993). At one site in Texas (Kroll and others 1984), treating loblolly pine with a gel increased survival from 19.6 to 50.8 percent and survival of slash pine was increased from 16.9 to 20.8 percent. In the loblolly pine case, the savings might be \$500 ha^{-1} (cost of replanting) and the cost of the gel treatment might be 7.5 cents ha^{-1}. This equals a cost benefit ratio of 6,666 (i.e., \$500/\$0.075). If preventing a replant was very rare (say 1 ha out of 6,666), the cost of the treatment (\$500 per 6.6 million seedlings) would equal the benefit (e.g., \$500). If the gel treatment reduced replanting by only 1 ha in 3,333, the benefit/cost ratio might equal 2 (e.g., \$500/\$250). As a comparison, Echols and others (1990) reported an increase in survival in 1 out of 3 sites. Therefore, some nursery managers believe the use of gels makes sense both economically and from a "marketing" perspective.

Management Implications and Conclusions

When freshly lifted seedlings were exposed for 1 h, some protection (as measured by RGP) was provided by both the kaolin clay and the PAM gel root dip treatments. When seedlings were exposed 2 h or more, only the gel root dip treatments increased seedling survival and RGP. Thus, continued use of gel root dip treatments by nursery managers as "insurance" against poor handling after seedlings leave the nursery is worth the cost of the materials. Kaolin clay inhibited all three soil-borne fungi, whereas gel-based root dips increased growth of *Rhizoctonia* sp. In all cases, treating loblolly pine roots with root gels kept short roots alive so they could elongate when placed into a favorable environment.

Results from these studies are applicable only when seedlings are transplanted within a few days of treatment. Additional research is required to determine if gels affect fungal growth during long-term, cool storage (e.g., 1 degree above freezing) of seedlings.

Address correspondence to: T.E. Starkey, School of Forestry and Wildlife Sciences, 602 Duncan Drive, Auburn University, AL, 36849-5418; e-mail: starkte@auburn.edu; Tel. 334-844-8069. Fax. 334-844-1084.

Acknowledgments

This research was funded by the Forest Service State and Private Forestry under terms of the Forest and Rangeland Renewable Resources Research Act of 1978.

References

Alm, A.; Stanton, J. 1993. Polymer root dip increases survival of stressed bareroot seedlings. Northern Journal of Applied Forestry. 10: 90–92.

Barnard, E.L.; Rowan, S.J.; and Lantz, C.W. 1981. Wood fiber mulch and gelatinized starch should be carefully evaluated as seedling packing media. In: Lantz, C.W. (technical coordinator). Southern Pine Nursery Handbook. Atlanta, GA: Forest Service, State and Private Forestry: 10.13–10.17.

Bacon, G.J.; Hawkins, P.J.; and Jermyn, D. 1979. Comparison of clay slurry and Agricol root dips applied to 1-0 slash pine seedlings. Tree Planters' Notes. 30(1): 34–35.

Bland, W.A. 1964. New packaging method for forest tree planting stock–North Carolina's "mudding." In: Hitt, R.G. (technical coordinator). Region 8 Forest Nurserymen's Conference; 1964 August 19–20; Morganton, NC. Atlanta, GA: Forest Service, State and Private Forestry: 50–52.

Carey, W.A.; South, D.B; Williford, M.; and Britt, J. 2001. Washing roots reduces vigor of loblolly pine seedlings. Southern Journal of Applied Forestry. 25: 25–30.

Davey, C.B. 1964. Current studies on clay slurry root dips. In: Hitt, R.G. (technical coordinator). Region 8 Forest Nurserymen's Conference: 1964 August 19–20; Morganton, NC. Atlanta, GA: Forest Service, State and Private Forestry: 53–59

Dierauf, T.A.; Garner, J.W. 1975. A test of Agricol as a root dip. Occasional Report 47.Charlottesville, VA: Virginia Division of Forestry. 5 p.

Dierauf, T.A.; Marler, R.L. 1967. Clay dipped vs. bare rooted seedling survival. Occasional Report 27. Charlottesville, VA: Virginia Division of Forestry. 5 p.

Dierauf, T.A.; Marler, R.L. 1971. Effect of exposure, clay treatment, and storage on survival and growth of loblolly pine seedlings. Occasional Report 34. Charlottesville, VA: Virginia Division of Forestry. 10 p.

Echols, R.J.; Meier, C.E.; Ezell A.W.; and 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(2): 13–32.

Fisher, D. 1974. Packing material for seedlings—are there suitable alternatives to moss? In: McConnell, J.L., technical coordinator. Southeastern Nurserymen's Conference; 1964 August 6–8; Gainesville, FL. Atlanta, GA: Forest Service, State and Private Forestry: 135–137.

Goff, E.S. 1897. Principles of plant culture. Madison, WI. Published by the author. 276 p.

Hamner, J.C.; Broerman, F.S. 1967. A comparison of three packaging methods for slash pine. Tree Planters' Notes. 18(4): 3–4.

Hodges, L.B. 1883. The Forest Tree Planters' Manual. 3rd ed. St. Paul: MN. H.M. Smyth Printing Co. 116 p.

Kroll, J.G.; Deauman, W.C.; Foster, C.D.; Kulhavey, D.L.; and Tracey, D. 1985. Survival of pines on droughty soils: two year results. In: Shoulders, E., editor. Third Biennial Southern Silviculture Research Conference; 1984 November 7–8; Atlanta, GA. General Technical Report SO-054. New Orleans: Forest Service, Southern Forest Experiment Station: 128–131.

Miller, A.E.; Reines, M. 1974. Survival and water relations in loblolly pine seedlings after root immersion in alginate solution. Forest Science. 20: 192–194.

Pinchot, G. 1907. How to pack and ship young forest trees. Circular 55. Washington, DC: Forest Service. 2 p.

Pryor, W.L. 1988. Kaolin clay dipping at Union Camp's Bellville Forest Tree Nursery. In: Hagwood, R., chairman. Southern Forest Nursery Association; 1988 July 25–28; Charleston, SC. Atlanta, GA: Forest Service, State and Private Forestry. 19–24.

Slocum, G.K.; Maki, T.E. 1956. Exposure of loblolly pine planting stock. Journal of Forestry. 54: 313–315.

Slocum, G.K.; Maki, T.E. 1959. Exposure of longleaf pine planting stock. Journal of Forestry. 57: 825–827.

Toumey, J.W. 1916. Seedling and Planting in the Practice of Forestry. 1st ed. New York, NY: John Wiley and Sons.

Toumey, J.W.; Korstian, C.F. 1949. Seedling and Planting in the Practice of Forestry. 3rd ed. New York, NY: John Wiley and Sons.

Venator, C.R.; Brissette, J.C. 1983. The effectiveness of superabsorbent materials for maintaining southern pine seedlings during cold storage. In: Brissette, J.; Lantz, C., comprs. Southern Nursery Conferences; 1982 July 12–15; Savannah, GA. Technical Publication R8-TP4. Atlanta, GA: Forest Service, State and Private Forestry: 240–245.

Williston, H.L. 1967. Clay slurry root dip impairs survival of loblolly pine seedlings in Mississippi. Tree Planters' Notes. 18(4): 28–30.