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ROOT DIP TREATMENTS AFFECT FUNGAL GROWTH AND SURVIVAL OF LOBLOLLY PINE (*PINUS TAEDA*) SEEDLINGS FOLLOWING EXPOSURE

by
Tom Starkey and David B. South

INTRODUCTION

During the 19th Century, the practice of wetting roots at the nursery (during counting and sorting) was employed to improve the chance of seedling survival. Damp moss was often used in shipping containers as a means of retaining moisture (Toumey 1916). Likewise, the practice of "puddling" involved dipping roots into a mixture of clay and water (the consistency of paint) at the planting site (Toumey 1916; Slocum and Maki 1956). Both practices (dipping roots in the field and soaking roots with water at the nursery) are techniques that have been employed for more than a century. It is interesting to note that, for freshly lifted stock, Toumey (1916) said "Puddling is not necessary and usually does more harm than good." We know that washing roots (to remove soil) can reduce seedling quality (Carey et al. 2001) and this might explain why Toumey believed puddling harmed seedling quality. However, if roots became "over-dry" during storage, he suggested roots be thoroughly puddled. Some questioned this claim and so later he changed the recommendation to applying water but not puddling (Toumey and Korstian 1949). Even today, recommendations will vary depending upon who you ask.

A number of different materials have been added to roots before packing seedlings. Sphagnum moss was the preferred treatment during the first half of the 20th Century but moss later became harder to acquire so alterative 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 at the Goldsboro Nursery. 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 over moss since they believed it was unnecessary to have water in planting buckets since 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 et al. 1979). When roots were treated with sodium alginate and then exposed in a greenhouse for up to 5 days, seedling survival and the relative water content of needles were improved (Miller and Reines 1974). Although polyacrylamide gels might increase survival when compared with clay dip treatments (Venator and Brissette 1983), gels are likely preferred over clay because they usually cost less, require less storage space, and are not as messy (Bland 1964). A nursery that produces 25 million seedlings may only need a pallet of product while clay might require the delivery of 25 tons (Pryor 1988). Most managers agree with Alm and Stanton (1993) who believe that 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 that involve the production of loblolly pine (*Pinus taeda* L.). Therefore, these trials were initiated to examine the effects of three root dip treatments on (1) their ability to support fungal growth and (2) their ability to protect roots from injury during exposure.

METHODOLOGY

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 hydro gels (PAM Gel) (Soil Moist®, JRM Chemicals) and a corn-starch based hydro gel (CSB Gel) (Zeba®, Absorbent Technologies). Samples of the kaolin clay and PAM Gels were obtained from the nursery while the CSB Gel was provided by the manufacturer. The genera of fungi used were *Pythium sp.*, *Fusarium* sp. and *Rhizoctonia sp*. Operational nursery treatments were used at recommended rates (Table 1).

Table 1. Percentage of material passing through a 500µ and 250µ sieve and rate of material used per liter of water.

	Clay	**PAM Gel "A"**	**PAM Gel "B"**	**CSB Gel**
Greater than 500µ	3.4%	60.0%	3.0%	0%
500µ to 250µ	16.2%	22.8%	54.2%	34%
Less than 250µ	80.4%	17.2%	42.8%	66%
Grams/liter	300	2.2	3.3	1.8

A 3-mm plug of the fungus was placed on the center of a water-agar petri plate that had been augmented with either Clay, PAM Gel "A", PAM Gel "B" or CSB Gel at a rate comparable to nursery use (Table 1). Water-agar is a basic media made with distilled water which supports minimal fungal growth. The control treatment involved a water-agar without any root gel/clay amendments. Each treatment was replicated 12 times and the radial growth of each fungus was recorded daily. Differences in fungal growth on the various amended media will determine the gels' or clay's ability to support fungal growth over non-amended media.

Study II: Seedling Survival Following Exposure

Each treatment (Table 1) was mixed in a separate bucket with 7.5 liters of tap water. The clay had to be continuously stirred during treatment since the clay never went into solution. Both PAM Gels "A" and "B" entered into solution with less than one minute of stirring. PAM Gel "A" entered into solution faster than PAM Gel "B". The CSB Gel, however, was very difficult to mix. When it was placed in the water, it immediately clumped together 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 was measured and was approximately 3.6 grams per seedling. It was determined that dipping roots of 20 seedlings five times removed about 72 g of gel solution (about 3.6 grams of gel/seedling). All root gel/clay treatments were hand-dipped five times prior to exposure.

Groups of 20 seedlings were treated with the root treatments (Table 1) plus a control that involved dipping roots into water. The seedlings were laid on an expanded metal bench in the greenhouse for either 0, 60, 120, or 240 minutes. Greenhouse temperatures during exposure ranged from 28° to 37°C and relative humidity ranged from 16% to 38%. The average solar radiation as measured in close proximity to the greenhouse was 4,271 watt-hours/m^2.

After exposure, seedlings were transplanted at the Nursery Cooperative's seedling testing facility. This facility consists of six pits (35' x 32' x 3') containing 100% sand. Twenty treatments (5 root treatments x 4 exposure treatments) were replicated 12 times using a randomized complete block design with 5 seedlings per experimental unit. The sand in the pits was irrigated for four hours prior to planting. In order to obtain a separation among treatments irrigation was withheld after transplanting. Rainfall for the test period from February 7th to May 7th totaled 15.9 cm (5.0, 7.1, 3.8 and 0.0 cm for Feb, Mar., Apr. 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 applying root treatments, the seedlings were exposed for either 60, 120 and 240 minutes. Greenhouse temperatures ranged from 29° to 33°C and relative humidity ranged from 18 to 42%. The solar radiation averaged 4,140 watt-hours/m^2.

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

RESULTS

Study I: Fungal Growth

There was considerable variation on the particle size of 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). Since the water-agar control was the baseline for each fungus tested, any growth less than that observed in control plates indicated an inhibitory effect on the fungus (Table 2). Treatments that grew more (relative to the control) indicate that the fungus was able to use the amendment as a food source. *Rhizoctonia* grew the fastest of all fungi, with at least one of the root treatments reaching the edge of the petri plate (85mm) after day 4. *Fusarium* and *Pythium* grew slower, with at least one of the root treatments reaching the plate limit after day 6.

Table 2. Fungal growth (mm) on amended or non-amended water-agar media.

	Pythium - Day 6	Fusarium - Day 6	Rhizoctonia - Day 4
Clay	10 d	51 c	58 c
PAM Gel "A"	26 c	60 b	75 a
PAM Gel "B"	31 c	60 b	74 a
CSB Gel	42 b	63 a	76 a
Control	69 a	61 b	70 b
lsd	*6.5*	*1.6*	*2.8*

[1] Letters are from Duncan's Multiple Range test (0.05 level).

All of the gel treatments had an inhibitory effect on the growth of *Pythium* sp., but the clay treatment had the greatest effect. There was also 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, but clay was the only inhibitory treatment. Growth of *Rhizoctonia* sp. was increased by the CSB Gel and both of the PAM Gels. In all cases, clay produced an inhibitory effect on fungal growth.

Study II: Seedling Survival Following Exposure

Treatments had a significant effect on seedling survival, but there were no differences among treatments with 0 or 1 hour of exposure (Table 3). It appears that, for short desiccation times (i.e. < 1 hr), injury to roots is minimal if seedlings are planted into moist soil. The seedlings at 0 and 60 minutes may not have had a sufficient amount of root death to impact survival. Once planted, the moist soil may have allowed roots to rehydrate. However, the application of the root gels increased survival after 2 or 4 hrs of exposure. Clay or water dips did not provide protection to the roots exposed to these longer times of desiccation. This is very evident at 4 hrs of exposure where the gel treatments increased survival by 40 percentage points or more.

Table 3. Loblolly pine survival (at three months) as affected by root dip treatment and length of exposure.

	0 min	60 min	120 min	240 min
PAM Gel "B"	94.5	86.8	87.0 b[1]	60.0 b
PAM Gel "A"	82.6	88.9	93.5 b	56.1 b
CSB Gel	79.2	76.2	85.9 b	52.8 b
Clay	91.2	87.9	52.9 a	12.1 a
Water	97.8	85.7	77.2 ab	12.1 a
lsd	*23.8*	*13.5*	*30.3*	*15.0*

[1] Letters are from Duncan's Multiple Range test (0.05 level).

Study III: Root Growth Potential
The RGP study showed similar trends as in the survival study. For the water-only treatment, one hour of exposure reduced RGP by half (when compared to the Clay or CBS Gel). In both the 120 and 240 minute periods, the RGP was reduced to less 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 degree of protection during the extended desiccation periods.

Table 4. Average number of white root tips (at four weeks) as affected by root dip treatment and length of exposure.

	60 min	120 min	240 min
PAM Gel "B"	32.1 ab[1]	29.3 b	19.9 a
PAM Gel "A"	41.3 a	16.8 c	22.6 a
CBS Gel	45.3 a	39.3 a	14.9 a
Clay	43.1 a	1.2 d	0.0 b
Water	22.0 b	3.4 d	0.0 b
lsd	*12.4*	*8.3*	*7.9*

[1] Letters are from Duncan's Multiple Range test (0.05 level).

DISCUSSION

A number of studies have exposed roots after treatment with clay or gels (Slocum and Maki 1956; Williston 1967; Miller and Reines 1974; Mullin 1978; Dierauf and Gardner 1978; Alm and Stanton 1993). When seedlings are handled carefully, not exposed to drying conditions, and not stored, outplanting survival can be greater than 80% (Venator and Brissette 1983). In the study at Auburn, we decided to subject treated seedlings to varying times of desiccation and then transplant them into moist sand to allow seedlings a chance to become established. 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. Our data agrees with others who found that gels provide some protection against desiccation of roots.

Results from the survival and RGP studies were in agreement, but the RGP test was able to detect treatment differences after just one hour of desiccation. The gels did provide some degree of protection to the roots of loblolly pine during the desiccation period. In our study, clay was not effective in preventing permanent root damage to the seedlings (Figure 1). However, in previous studies, clay improved seedling survival (Figure 2).

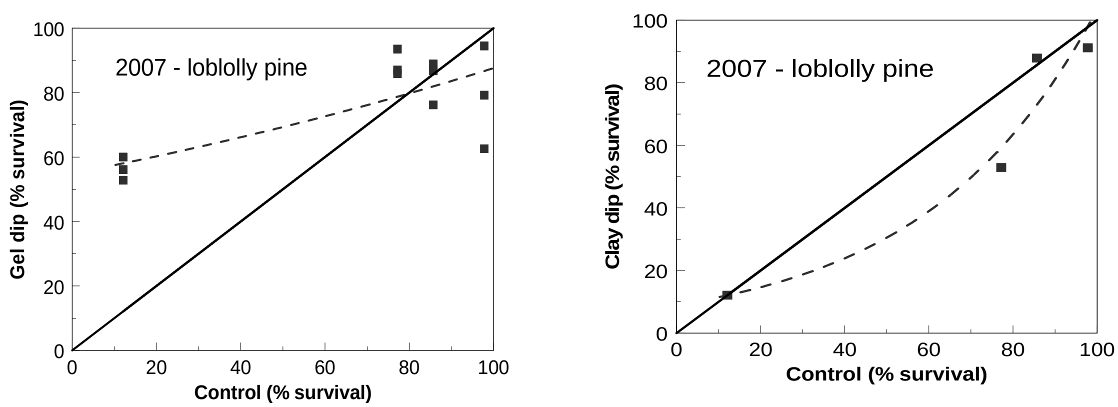

Figure 1. Effect of gel dipping (left) and clay dipping (right) on seedling survival. Points above solid line indicate an increase in survival (relative to the control) and points below the line indicate a decrease in survival.

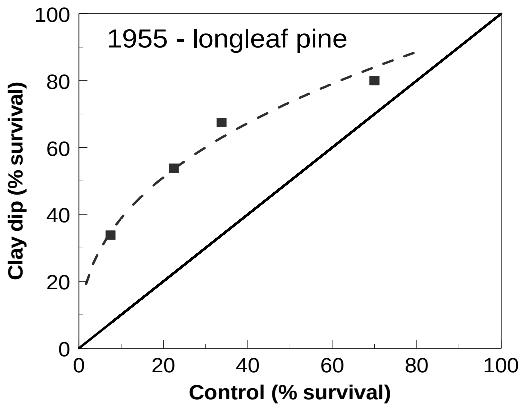

Figure 2. Effect of clay dipping on survival of longleaf pine (data from Slocum and Maki 1959). Points above solid line indicate an increase in survival (relative to the control) and points below the line indicate a decrease in survival.

There have been some concerns among nursery managers that root gels (especially the starch-based gels) added to seedling roots could support the growth of soil-borne fungi. For a disease to develop, three factors must occur. First, the environment must be conducive to disease development. (This generally means optimal moisture and temperature.) Second, you must have a susceptible host. In some cases, the host may be too old and therefore not susceptible. Third, you must have a virulent pathogen. 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 et al. 1981). The concern was that the wood fibers (or starch) were providing a substrate for pathogenic microbes. Of the four root dip treatments 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 had a stimulatory effect on fungal growth, especially *Fusarium sp.* and *Rhizoctonia sp.*. Since these fungi are common nursery fungi, it is possible they could utilize the polyacrylamide hydro gels and/or the corn-starch based hydro gel as a food source. Thus, it is possible that the gels might have negative ramifications during seedling storage, especially the CSB gel and *Fusarium sp.* combination.

In many cases, a researcher wants to see significant differences among treatments before they make a recommendation. In fact, many researchers do not even consider the benefit/cost ratio of a treatment if the treatment is significant at $\alpha = 0.15$. In many outplanting trials, researchers cannot declare a 10% 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% increase in survival was not statistically significant (Venator and Brissette 1982). Therefore, some might say a treatment is not "statistically significant" but consistently increases survival by 5% is not worth the cost (even though it costs only pennies per acre). On the other hand, 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 et al. 1984), treating loblolly pine with a gel increased survival by 30 percentage points (19.6 → 50.8%) and survival of slash pine was increased by 4% (16.9 → 20.8%). In the loblolly pine case, the savings might be $200/acre (cost of replanting) and the cost of the gel treatment might be 3 cents/acre. This equals a cost benefit ratio of 6,666 (i.e. $200/$0.03). If this increase in survival was very rare (say one acre out of 6,666), then the cost of the treatment would equal the benefit. If the gel-treatment reduced replanting only once in 3,333 acres, then the benefit/cost ratio would equal 2. 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 as well as from a "marketing" perspective.

MANAGEMENT IMPLICATIONS

When freshly lifted seedlings are exposed for one hour, some protection (as measured by RGP) was achieved by both the kaolin clay and PAM gel root dip treatments. However, when seedlings were exposed to two hours or more, only the gel root dip treatments increased seedling survival and RGP. Thus, continued use of 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-born fungi while 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 presented 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° C) of seedlings.

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