RESEARCH REPORT 10-08

PARTICLE SIZE OF HYDROPHILLIC POLYMER ROOT GELS AFFECT SEEDLING SURVIVAL OF LOBLOLLY PINE (*PINUS TAEDA*): FROM PLANTING TO ESTABLISHMENT

by
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INTRODUCTION

A number of materials have been added to seedling roots before packing for protection. Sphagnum moss was the preferred treatment during the first half of the 20th Century but later moss 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, others 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 treatment in Germany and was subsequently tested in other countries (Miller and Reines 1974; Dierauf and Garner 1975; Bacon et al. 1979). Hydrophilic polymers, also known as hydrogels, include both starch-based and polyacrylamide gels. In addition to increasing survival when compared with clay dip treatments (Venator and Brissette 1983), gels are 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 one pallet of polyacrylamide product while clay might require the delivery 25 tons (Pryor 1988).

In 2004, Zeba®, a cornstarch based product, was introduced to the market as a competitor to the polyacrylamide gels. It is formulated from natural cornstarch by tweaking the molecular structure to increase the water-holding ability (http://www.Zeba.com/pdfs/9/_20049_FAQs-Consumer.pdf). In addition, the non-toxic, biodegradable, pH neutral properties of Zeba®

added to its marketing appeal. In forestry, Zeba® was marketed as for use as a soil amendment (http://Zeba.com/agriculture/agriculture12.htm) and as a root dip for use prior to planting (http://Zeba.com/nursery/nursery.htm)

Most seedling producers agree with Alm and Stanton (1993) 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 were no studies to support the economic use of either gels or clays involving the production of loblolly pine (*Pinus taeda*). In 2007, a study (Starkey and South 2009) was conducted 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 from lifting to the time of planting. 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.

Following this study, there were several unanswered questions associated with the root gels: (1) Could polyacrylamide gels be used in the planting hole to aid in establishment of the seedling? (2) Does the gel's particle size distribution make a difference in seedling survival? and (3) Does gel composition affect seedling survival?

METHODOLOGY

Study 1 – With or Without Gel Bags

A sample of a biodegradable paper tea bag, approximately 5.7 cm^2, filled with 6.7g of crosslinked polyacrylamide gels was provided by JRM Chemical, Inc (Figure 1). The directions for use indicate that one bag is to be placed on the side or base of the planting hole, backfilled and watered liberally (http://www.soilmoist.com/forms/JRM-Form1402.pdf).

The purpose of this study was to evaluate the effectiveness of this product to increase seedling survival during droughty conditions. In this study, 10 boxes (4'x 4'x 3') filled with course sand and protected from rainfall were used. Each box was considered a replication. Within each box, 20 loblolly pine seedlings from a drought-sensitive family were planted in ½ of each box with a gel bag added to the planting hole as describe by the product literature. A second group of 20 seedlings were planted in ½ of each box without a gel bag placed in the planting hole. Root gel had been applied to the root system of both groups of seedlings at lifting as a standard operating nursery procedure. The seedlings were watered to saturation the day of planting and one day following planting. All water was withheld for next 38 days after which seedling mortality was recorded and data analyzed.

Study 2 – Gel Bags – Whole or Broken

In a second study that used gel bags, 11 boxes were planted according to the following treatments:

1. Gel on roots @ lifting – No gel bag in planting hole
2. No Gel on roots @ lifting - No gel bag in planting hole (*designated as control*)
3. Gel on roots @ lifting plus whole gel bag in planting hole
4. Gel on roots @ lifting plus gel bag emptied into planting hole (no bag tissue in hole)
5. No Gel on roots @ lifting plus whole gel bag in planting hole
6. No Gel on roots @ lifting plus gel bag emptied into planting hole (no bag tissue in hole)

Six trees of each treatment were planted randomly in each box. Boxes were watered to saturation the day of planting and one day following planting. Water was then withheld for 84 days after which the number of dead seedlings for each treatment and each replication was recorded. Seedlings were removed from the soil and the dry weights of the roots for the six trees/treatment/replication were measured. Data were subjected to ANOVA and treatment effects were compared using contrast statements.

A sieve analysis was conducted to assess the particle size distribution for the polyacrylamide within the gel bags and a composite sample obtained from nine nurseries in the southeastern United States. A 12 g sample for each gel was placed in a series of soil sieves ranging from 3000 µm to 25 µm. The amount of gel that passed through each sieve was calculated.

Study 3 – Gel Particle Size and Composition

The particle size distributions of root gels were examined. Three different grades of crosslinked polyacrylamide gels were provided by JRM Chemical, Inc. Ohio (Soil Moist®). These were designated as: Soil Moist® Fines, Soil Moist® JRM-A, Soil Moist® Hydro. A sample of a corn starch based gel, Zeba® CSB, was also obtained from Absorbent Technologies, Inc. Oregon. A water control was included in the study.

A drought-sensitive loblolly pine family was hand-lifted for this study and no root gel was applied at the nursery on the seedlings prior to the study. A mixture of each gel product was prepared using 12.5 g/product/gal of water. Seedlings were dipped five times to give equivalent amount of gel on roots as the standard operation in the nursery. The experimental unit was eight seedlings for each treatment and there were eight replicates Boxes were watered to saturation after planting on each of the first two days of the study and this was followed by no additional irrigation. Beginning at week 8, and continuing weekly, the number of surviving seedlings was recorded for each treatment. After 98 days the number of dead seedlings was recorded. Seedlings were removed from the soil and the root morphology of surviving seedlings was analyzed using WinRhizo® software (Regents Instruments Inc, Quebec, Canada). Data were subjected to ANOVA and Regression analysis to determined treatment differences. Duncan's Multiple Range Test was used to separate treatment means ($\alpha = 0.05$).

To determine particle size distribution of the gels used, a sieve analysis was conducted. A 12 g sample for each gel was placed in a series of soil sieves ranging from 3000 µm to 25 µm. The amount of gel that passed through each sieve was calculated.

RESULTS

Study 1 – With or Without Gel Bags

The placement of a gel bag within a planting hole resulted in 28% mortality to loblolly pine seedlings. In contrast, loblolly pine seedlings that did not receive a gel bag at the time of planting had only 2% mortality (Prob > F = 0.0023). In addition, it was also observed that seedlings in which a gel bag was placed in the planting hole appeared to be disturbed. Seedlings were slightly pushed up causing many of the seedlings to appear slightly toppled (Figure 2).

Study 2 – Gel Bags – Whole or Broken

The seedlings with no gel on the roots from the nursery (control) (Trmt #2) had greater survival than the seedling with gel on the roots from the nursery (Trmt #1). The seedlings with no root gel (Trmt #2) also had greater survival than seedlings with root gel alone or root gel plus a gel bag, either broken or intact in the planting hole(Trmts #1, #3, and #4). There was no significant difference in seedling survival when gel was on the roots (Trmt #1) or the addition of either an intact gel bag or a broken gel bag in the planting hole (Trmts #3, and #4). Similarly, there was no difference in seedling survival when no gel was on the roots (Trmt #2) or the addition of either an intact gel bag or a broken gel bag in the planting hole (Trmts #5, and #6). The smallest root dry mass was for the treatments that did not have either an intact gel bag or a broken gel bag in the hole (Table 1 and Table 2).

Polyacrylamide gels from the tea bags had a relatively narrow size distribution compared to the composite gel samples collected from the nurseries. The distribution for particles from the gel bag was 800 µm (microns) to a 3000 µm with a peak at 1000 µm. The distribution of particles from the nursery composite sample was less than 50 µm to 1000 µm, with a peak at 200 µm.

Study 3 – Gel Particle Size and Composition

The use of Zeba® CSB at the time of planting loblolly pine resulted in significantly less seedling survival than either the Soil Moist® Fines or the Soil Moist® JRM-A gels. The survival of seedlings treated with Soil Moist® Fines and JRM-A gels was greater than seedlings that received the Soil Moist® Hydro (0.85 vs 0.69) at Prob>F = 0.0814. None of the root-dip gel treatments affected seedling growth as measured by root biomass dry-matter content. However, with respect to seedling root sizes, the control seedlings (which had no root gel) had smaller root diameters than seedlings treated with the Soil Moist® Fines, Hydro or Zeba® CSB. Seedlings treated with Soil Moist® – Fines had the largest root diameter than either of the other two Soil Moist® products tested. Loblolly pine treated with the three Soil Moist® gel products had an equal number of root tips as did the non-treated control seedlings. Seedlings treated with Zeba® CSB had fewer root tips compared to the control seedlings (Table 3).

Regression analysis indicated that seedlings treated with the Soil Moist® Fines had approximately 1% mortality per week between week 8 and week 14. Loblolly pine that received either the Soil Moist®– JRM-A or the Control treatments had 2% seedling mortality per week. In contrast, loblolly pine treated with Soil Moist® Hydro and Zeba® had the greatest seedling mortality with at 4% and 5% respectively from week 8 to week 14.

Analysis of particle sizes indicated that the Zeba® CSB, Soil Moist® Fines and Soil Moist® JRM–A had a similar shaped distributions with Zeba® CSB centered at 150 µm and Soil Moist® Fines and JRM-A centered at 300 µm. The particle distribution for the Soil Moist® Hydro had a larger range (25 µm – 2000 µm) than the other gels examine. Unlike the other four gels examined, there was no distinct single peak particle size with the Soil Moist® Hydro.

DISCUSSION

During the 19th Century, the practice of wetting roots at the nursery (during counting and sorting) was used to improve seedling survival after outplanting. For example, damp moss was often used in shipping containers as a means of retaining moisture (Toumey 1916) and 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). While both practices (dipping roots in the field and soaking roots with water at the nursery) 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." Nearly 80 years later, washing roots (to remove soil) has been shown to reduce seedling survival (Carey et al. 2001) and may be one reason why Toumey believed puddling harmed seedling quality.

Materials which have been added to roots in the seedling packing process include: sphagnum moss (Davey 1964; Fisher 1974) a clay slurry (Slocum and Maki 1956; 1959, Bland 1964), sodium alginate (Miller and Reines 1974; Dierauf and Garner 1975; Bacon et al. 1979) vermiculite, peat moss and sawdust (Sloan 1984) and hydromulch (Rowan 1982). In the late 1960's, hydrophilic gel use and popularity have increased due to their lower costs, ease in storage and were less messy (Bland 1964).

One would think that the use of hydrophilic gels gained in popularity due to the increase survival afforded the seedlings after planting. However, in a review of 24 studies using gel-based root dips in North America, Sloan (1984), reported 23 studies showed no increase in seedling survival when the seedlings were not exposed to dehydrating conditions before planting. In contrast, when were exposed to stress before planting, 10 studies reported an increase in seedling survival when root dips were used (Sloan 1984). Similar studies conducted by the Southern Forest Nursery Management Cooperative also reported an increase in seedling survival when hydrogels were used on stressed trees (Starkey and South 2009).

If a nursery manager could insure that "perfect" conditions would occur between seedling lifting, storing and planting, the value of root gels to increase seedling survival should be questioned. However, since many of these factors are out of the control of a nursery manager, and root gels have been shown to increase survival under these less than optimum conditions, many managers would agree with Alm and Stanton (1993) who stated that gels "offer a form of insurance against survival loss resulting from seedlings being exposed to drying during the planting process." In a cost/benefit analysis, (Starkey and South 2009) reported that the practice of using root gels to increase seedling survival makes sense both economically as well as from a "marketing" perspective.

The most adverse sites for planting southern pines are soils with a high sand content under droughty conditions. Sandy soils have a low water-holding capacity so the practice of placing a "tea-bag" of hydrogel in the planting hole to provide water in times of drought for seedling survival/establishment seems plausible. However, the results from the Gel Bag Study #1 were totally unexpected and go against what was assumed about water holding/releasing capacity of these gels. Loblolly pine seedlings in which a gel bag was placed in the planting hole at the time of planting had 26% less survival than the seedlings that did not receive a gel bag at the time of planting (controls). Also, the seedlings that received a gel bag appeared to be pushed up from their original planting depth, giving the appearance of toppling or frost heave. Similar results were reported by Sarvas et. al. (2007) who noted a 21% reduction in survival of Scots pine (*Pinus sylvestris*) compared to the controls when 7 g of polyacrylamide gel (200 - 800 µm) was placed in the planting hole. In a study that used 2-yr old blueberry (*Vaccinium ashei*) plants, 20 g of hydrogel incorporated into a loamy –sand soil planting hole decreased plant survival when compared to non-treated blueberry plants (Austin and Bondari, 1992). The authors speculated that non-buffered hydrogel "probably drew water from the blueberry plant roots" and they cautioned that use of hydrogels without organic matter "could create a potential lethal situation for young blueberry plants." Along those lines, Rietveld (1976) reported a reduction in germination of ponderosa pine (*Pinus ponderosa*) when 10 g of hydrogel was incorporated in a 5-inch-diameter germination area. The authors speculated that the hydrogel retained too much of the available moisture from the germinating seeds.

Depending upon the formulation, polyacrylamide gels have the capability of swelling and retaining 40 to 500 times their own weight in water (Sloan 1984). The cross linked polyacrylamide gel in the Soil Moist® tea bags can absorb 200 times its weight in tap water (http://www.soilmoist.com/forms/JRM-Form1402.pdf) and thus, the contents of one bag would be capable of absorbing 1.3 L of tap water. It was apparent that the loblolly pine seedlings planted with a gel bag were "pushed-up" from the actions of the expanding gel bags. In some cases the bag contained the expanding gel contents, in others the bag split open and the gel continued to absorb water and expand within the planting hole. A similar "pushed –up" affect was also noted by Sarvas et. al. (2007) when a quantity 7 g of polyacrylamide similar to the tea bag experiment was place in a planting hole of Scotch pine (*Pinus sylvestris*) in soils with low water holding capacity.

In the "Whole or Broken" Gel Bag study, one of the objectives was to determine if roots treated with hydrogels at the time of lifting/storing/shipping had any effect on seedling survival when a whole gel bag or the contents of the gel bag (less the paper bag) was placed in the planting hole. The loblolly pine seedlings with no root gel treatments at lifting had 20% more survival than those seedlings with root gels applied at lifting. When a gel bag (whole or broken) was added to seedlings treated with root gels decreased seedling survival by 22%. Gel bags placed in the hole (whole or broken) at the time of planting had no affect on survival of seedlings without root gel treatments at the time of lifting. Survival of non-treated seedlings (without root gels) may be attributed to the lack of stress from lifting to planting. The reduction in seedling survival when loblolly pine was treated with the root gels and planted with a gel bag could be a function of the gel particle size and the sandy soil in which they were planted. Austin and Bondari (1992) reported significant loss in survival of blueberries planted in a loamy sand soil when hydrophilic gels were used and Bhardwaj et al (2007) cautioned against the use of hydrogels in sandy soil unless the polymers were mixed first with a layer of sand. Simply put, the hydrogels placed on the seedling roots and into the planting hole, tied up the available moisture, making it unavailable for the seedling's use.

In addition to the possible particle size by seedling survival interaction, there is also a difference in hydrogel composition. The Zeba® CSB, a hydrogel based upon natural cornstarch with a particle size peak about 150 µm, had the lowest loblolly pine survival of all treatments tested. It cannot be determined if the poor performance of Zeba® was due to the composition or to an interaction of the composition and particle size. In contrast, the Soil Moist® products are a synthetic acrylic polyacrylamide with a potassium salt base, particle size peaks at or above 300 µm. There was a correlation of seedling survival when treated with the hydrogels and particle size. Generally, as particle size increased, seedling survival decreased which may be a function of the particle sizes' ability to hold water not making it available to the seedling. The two products that performed best, Soil Moist® Fines and JRM-A both had particle distribution peak at about 300 µm; the Soil Moist® Hydro, with a peak distribution between 500 and 700 µm, had less seedling survival.

This study was the first to examine the impacts that hydro gels in general and particle size in particular have on seedling survival. Venator and Brissette (1984) tested four grades of Terra-Sorb on loblolly pine, a starch based product. The Terra-Sorb 250 and Terra-Sorb 1000 had better survival than the Terra-Sorb 200 and Terra-Sorb 201. Unfortunately, the particle sizes were not reported. Sarvas et al (2007) examined three grades of Stockosorb on Scots pine in two different studies. In addition, we know of no study with root gels which have controlled rainfall or irrigation input throughout the study period.

While the environmental conditions in this study were severe, these conditions did provide differences. The stressful environment allowed us to evaluate the effect of particle size and the effect of hydrogel composition on seedling survival after outplanting.

MANAGEMENT IMPLICATIONS

The use of root dips at the time of lifting/packing/storing makes good economical and marketing success after seedlings leave the nursery. However, if the seedlings are not subjected to stress following lifting, these, and other studies have shown that root gels do not increase survival, and in some cases, seedling mortality can be increased. There are many sources of root gel compounds available to seedling nurseries. These root gels differ based upon composition (e.g. starch based vs. polyacrylamide). Also hydrogels with different grades can be purchased. Each gel grade (ex: fine, medium, course) has a range of particle sizes and peak distributions. Crosslinked polyacrylamide gels with a peak greater than 1000 µm caused significant reduction in seedling survival when planted in sandy soils. In this study, in sandy soils, the gel which provided the highest seedling survival was the gels with a peak distribution around 300 µm. Nurseries should evaluate their hydrogel to determine if the particle size distribution is close to the 300 µm peak.

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Figure 1. Intact gel bag and broken bag showing polyacrylamide gel.

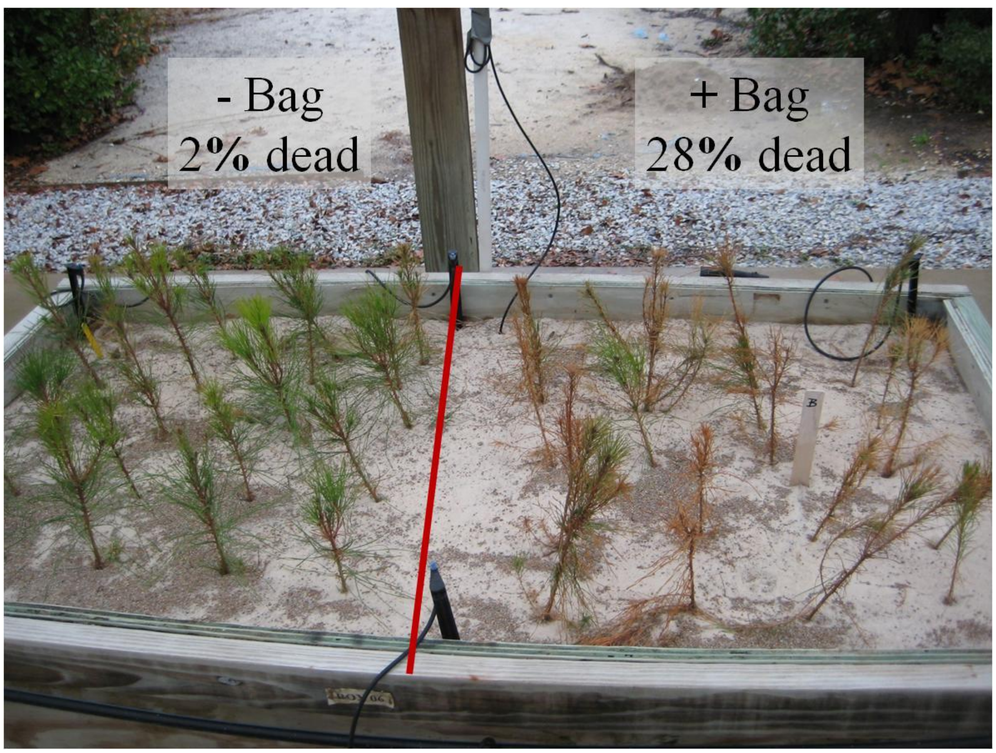

Figure 2. Example of stress box in which ½ seedling had a gel bag placed in the planting hole (right half). Note the disturbed and pushed up seedlings on the right.

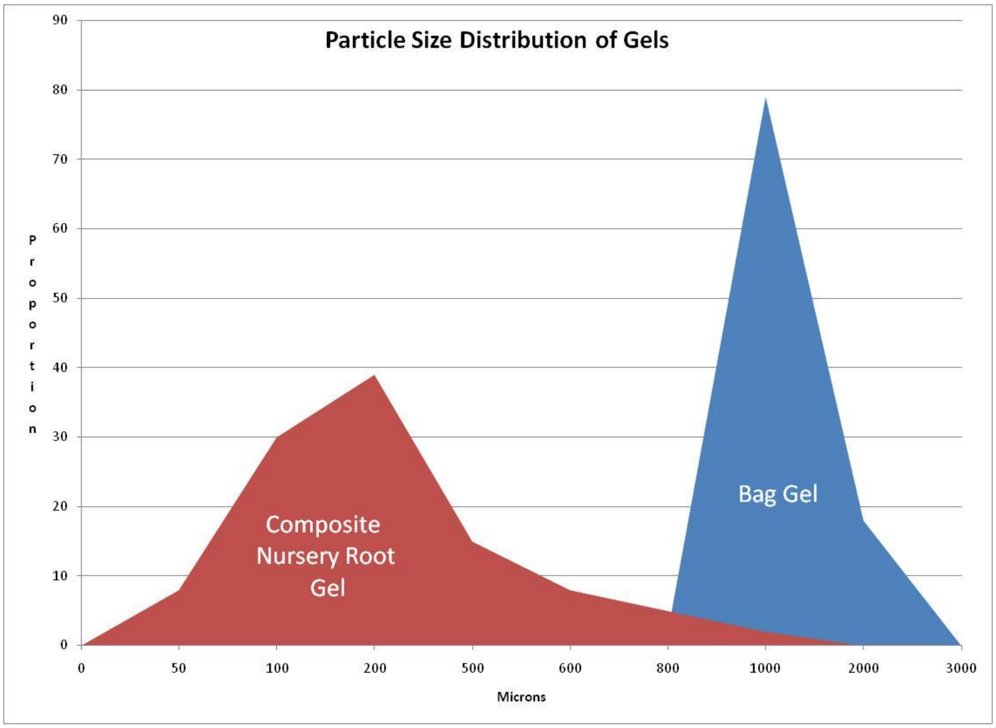

Figure 3. Comparison of polyacrylamide root gels used by nurseries and gel sample from gel bag.

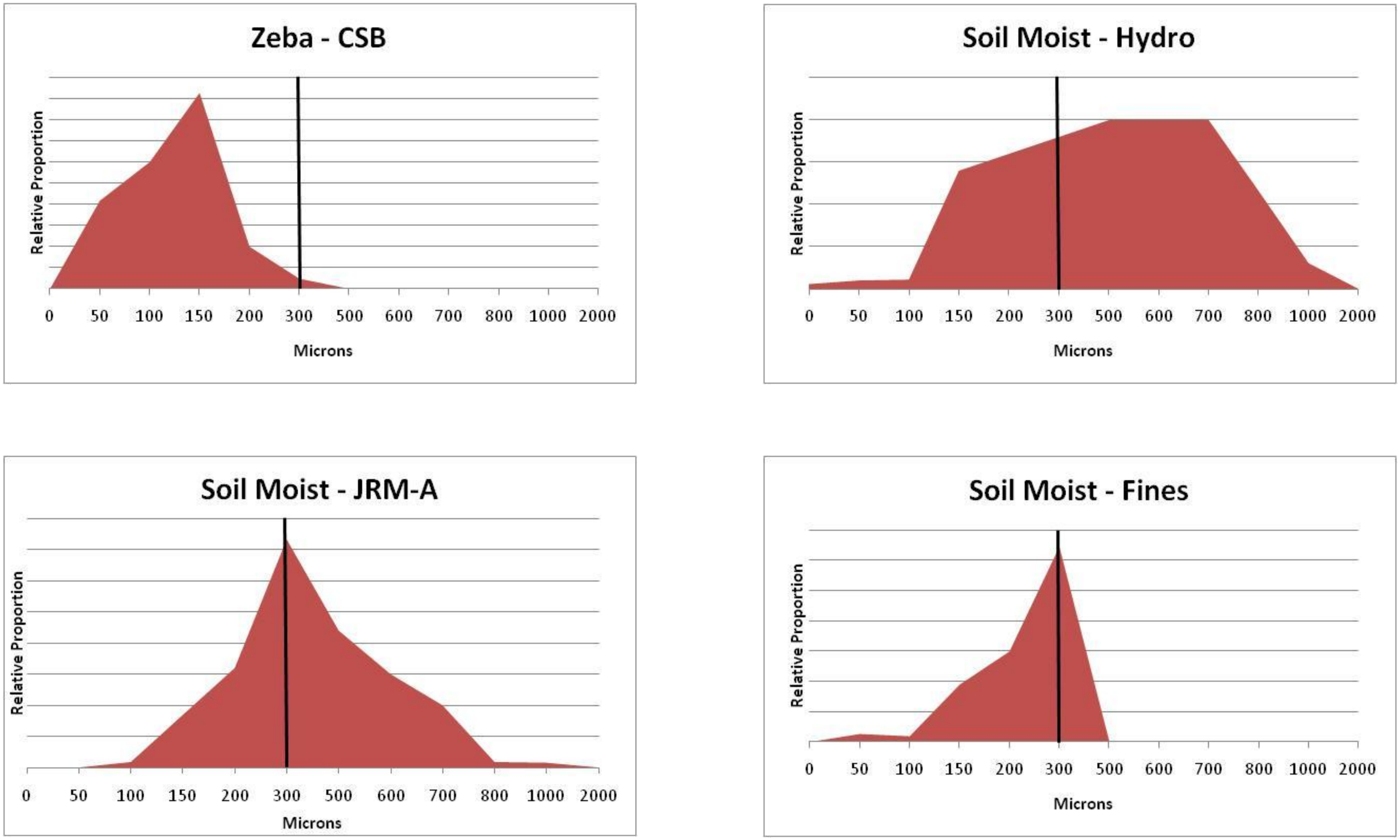

Figure 4. Comparison of sieve analysis for root gels. The black line at 300 microns is a reference mark for comparing particle size distribution.

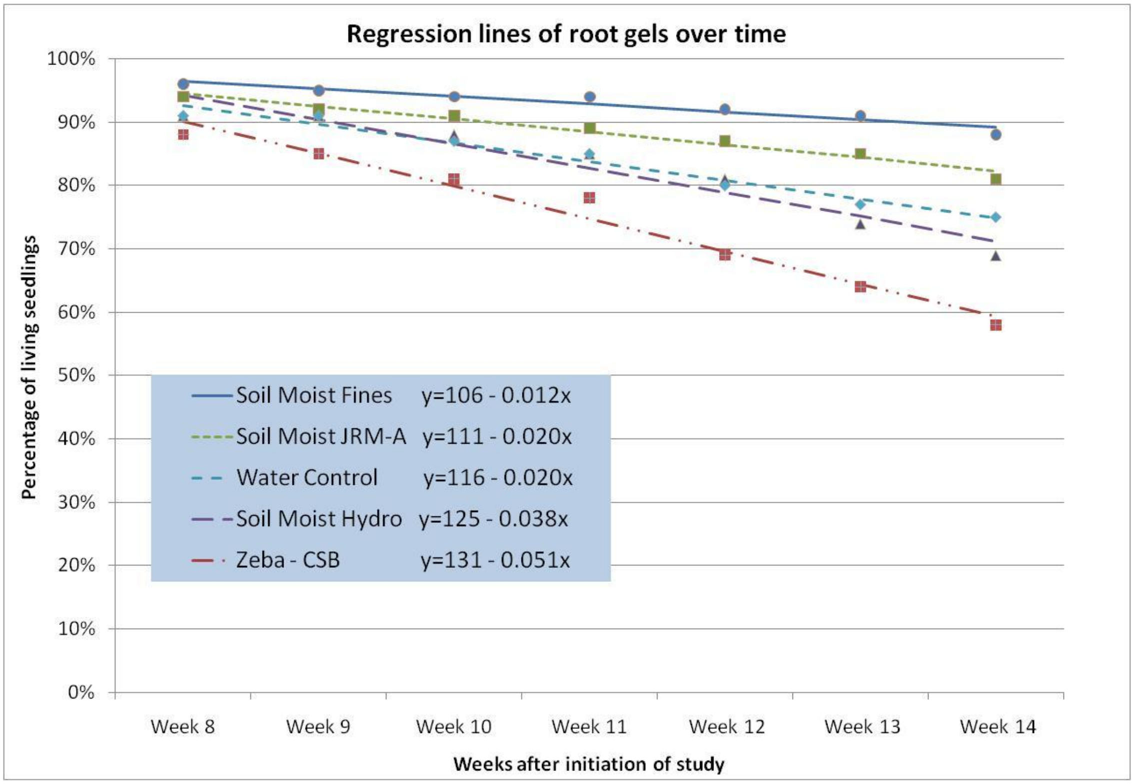

Figure 5. Regression analyses of treatment root gels and control over time.

Table 1. Results of Gel Bag study #2.

Treatment	Proportion Alive	Root Dry Wt (g)
Gel on roots @ lifting plus whole gel bag in planting hole (TRT #3)	0.61	1.8
Gel on roots @ lifting - No gel bag(TRT #1)	0.68	1.0
Gel on roots @ lifting plus gel bag emptied into planting hole (no bag in hole)(TRT #4)	0.70	1.5
No Gel on roots @ lifting plus whole gel bag in planting hole (TRT #5)	0.76	1.9
No Gel on roots @ lifting plus gel bag emptied into planting hole (no bag in hole) (TRT #6)	0.82	1.8
No Gel on roots @ lifting – No gel bag (*Designated as Control*) (TRT # 2)	0.88	1.2
lsd (α=0.05)	0.17	0.47

Table 2. Individual contrast tested comparing Control (TRT # 2) with other treatments or treatment combinations

Source	df	Contrast Means	Probability of a greater F-value
Replication	10		0.9504
Treatment	5		0.0381
Control vs gel *(#2 vs #1)	(1)	0.88 vs 0.68	0.0278
Control vs trees w root gel (#2 vs #1, #3, #4)	(1)	0.88 vs 0.66	0.0036
Whole bag vs broken bag (#3 #5 vs #4, #6)	(1)	0.69 vs 0.76	0.2260
Gel on Roots vs whole bag or broken bag (#1 vs #3, #4)	(1)	0.68 vs 0.66	0.6924
Control vs no root gel plus whole bag or broken bag (#2 vs #5, #6)	(1)	0.88 vs 0.79	0.2289
Error	50		

Table 3. Percentage of living seedlings at week 14, root dry weights and root morphology at the end of the study.

			Root Morphology				
Treatments	**Proportion of Living Seedlings @ Wk. 14**	**Root Dry Weight (g)**	**Length (cm)**	**Area (cm^2)**	**Diameter (mm)**	**Volume (cm^3)**	**Tips**
Soil Moist® Fines	0.88 a	3.16 a	578.2 a	143.7 a	7.76 a	2.88 a	1241 ab
Soil Moist® JRMA	0.82 a	2.48 a	555.7 a	122.9 a	7.06 bc	2.19 a	1232 ab
Water Control	0.75 ab	2.24 a	614.5 a	129.3 a	6.69 c	2.18 a	1286 a
Soil Moist® Hydro	0.69 ab	2.63 a	525.8 a	118.7 a	7.19 b	2.16 a	1216 ab
Zeba® CSB	0.58 b	*2.74 a*	487.7 a	117.4 a	7.51 ab	2.31 a	978 b
lsd (α=0.05)	*0.21*	*0.77*	*120.5*	*30.6*	*0.45*	*0.66*	*248*