Auburn University
Southern Forest Nursery
Management Cooperative

RESEARCH REPORT 16-06

SEEDLING QUALITY AND ROOT ARCHITECTURE OF LOBLOLLY AND LONGLEAF PINE CONTAINER SEEDLINGS

by
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INTRODUCTION

Container seedling production was one million in 1974, climbed to 3.5 million seedlings in 1980 (South 2015) and now exceeds over 200 million seedlings annually (Enebak 2015). While, accurately quantifying container seedling production is difficult, as a number of small longleaf pine (*Pinus palustris* P. Mill.) nurseries do not participate in any type of survey, container production in the South now accounts for more than 20% percent of the total forest tree seedlings produced in the Southern United States.

Another interesting facet of seedling production is stock type used for reforestation differs dramatically for the three major pine species grown in the Southern United States. For example, 1 percent of slash pine (*P. elliottii* Engelm.) and 9 percent of loblolly pine (*P. taeda* L.) seedlings are produced in containers whereas 97 percent of longleaf pine seedlings are produced in containers (Enebak 2015). Longleaf, loblolly, and slash pine account for 99 percent of all conifers grown as container stock in the South, with longleaf pine accounting for 50 percent of the container total (Enebak 2015).

There are several unique features of container grown seedlings.

1. Each seedling is in grown in an individual media plug as part of a container set that may contain anywhere from 40 to 130 plugs.
2. The length of the container plug ranges from 3.4” to 6”.
3. The growing media is soil-less, composed primarily of peat moss.
4. The seedling taproot is air pruned when it reaches the bottom of the container and stops growing until outplanting.
5. Hard plastic and polystyrene container sets are used in the south. The hard plastic containers account for more than 85% of all southern seedling production. Some growers use copper-treated polystyrene trays that results in lateral roots to be chemically pruned when the growing tip touches the copper treated cell wall.
6. All commercially used containers had small vertical “ribs” to prevent root spiraling of the lateral roots.
7. Some hard plastic containers have air pruning holes in the side of the container wall to encourage fibrous root growth.

Root architecture of the container seedling refers to the spatial orientation of the root system with respect to the extent and configuration of the various components. Each root system is composed of a taproot, lateral roots and a fibrous root system. The types of container as well as cell wall treatments or openings alter the root architecture within the cell plug. Overall container seedling root architecture can effect seedling survival on outplanting as both Hatchell (1987) and Carlson (1986) reported that the root fibrosity and number of lateral roots can effect outplanting survival of longleaf and loblolly pine. The re-establishment of conifer stands using container stock has been successful across the southern United State. Planting container-grown pine seedlings yields well-stocked stands and increases both the planting window and seedling survival. In some circumstances, however, young conifers planted as container-grown seedlings become unstable in high winds. This instability, referred to as toppling, has been associated with poor root morphology in container stock, improper site preparation, and soil type. The amount of toppling varies within any particular container-planted stand. Although toppling of young trees has been correlated with container seedlings that do not form a tap root following outplanting, there has not been a link proven between specific root architecture following planting and the incidence of toppling. The spatial root growth formation from containers used in the southern US following outplanting is not known and has been at times a point of heated discussion. The purpose of this study is to evaluate the root architecture of loblolly and longleaf pine grown in container sets used in the Southern United States. An outplanting study of the different container sets was included for loblolly pine as part of this container root architecture evaluation.

METHODOLOGY

2013-2014 Loblolly pine seedling quality, outplanting, and root morphology

On April 1, 2013, loblolly pine was sown into 20 container types represented in Table 1 that contained the same media mix for all containers. The seedlings were maintained in the same production area at the nursery using standard operating procedures for growing container seedlings. Beginning in September, 2013, and every month for four months, 30 seedlings of each container type were selected at random for seedling quality measurements including root collar diameter (RCD), height, dry weight. In addition, for each month, 5 seedlings, replicated 4 times, for each container type, were placed in root growth chambers and evaluated for root growth potential (RGP) by counting the number of white root tips after 30 days in aerated water.

In early November, 2013 four seedlings were selected from each container type in the nursery and placed in the center of a one cubic foot plexiglass box strung with fishing line to support root growth (Figure 1). Each box was then filled with a combination of washed river sand and crushed clay particles to provide both soil porosity and water saturation. The boxes were randomly placed on a greenhouse bench and watered and fertilized as needed. Each side of the plexiglass box was covered with one inch of insulation to maintain constant soil temperatures within the box and inhibit algae growth within the soil. In March, 2014 5 months after planting, the sand / clay mixture was dropped out of the bottom of each box leaving the seedling and roots suspended on the fishing line. Seedling roots growing out of each plug that were greater than 0.5” were counted, weighed and root architecture studied for each one inch horizontal segment of the root plug. Although not part of the original study plan, 4 bareroot seedlings were also placed in the boxes at the same times as the container seedlings. The bareroot seedlings were the same genotype as the container seedlings but were grown at the different nursery under different growing regimes and thus were not included in the statistical analysis of the container types. The spatial distribution of the root system of the bareroot seedlings was approximated based upon point of origin on the tap root when the seedlings were placed in the plexiglass boxes in November.

In early December, 2014, loblolly pine seedlings from the different container types were outplanted in Moultrie, GA and Evans, LA. Six seedlings from each container type were planted in a randomized complete block design replicated five times. Seedling survival and growth will be measured over a two year period.

2014-2015 Longleaf pine root morphology

On April 1, 2014, longleaf pine was sown into container types represented in Table 1 using the same media mix for all containers. The seedlings were maintained in the same production area and using standard operating procedures for growing container seedlings. In early November, 2014 five seedlings were selected and each placed in the center of a one cubic foot plexiglass box strung with fishing line to support root growth (Figure 1). Each box was then filled with a combination of washed river sand and crushed clay particles to provide both soil porosity and water saturation. The boxes were randomly placed on a greenhouse bench and watered and fertilized as needed. Each side of the plexiglass box was covered with one inch of insulation to maintain constant soil temperatures within the box and inhibit algae growth within the soil. In March, 2015, 5 months after planting, the sand / clay mixture was dropped out of the bottom of each box leaving the seedling and roots suspended on the fishing line. Roots growing out of the plug that were greater than 0.5” in length were counted, weighed and root architecture studied for each one inch horizontal segment of the root plug.

RESULTS AND DISCUSSION

2013-2014 Loblolly pine seedling quality

In September 2013, container type C1 had greatest height and RCD (Table 2). The average RCD for the container types examined increased about 1mm from September to December. In December 2013 container type C1 had the largest RCD. There was no difference in root growth potential (Table 3) between the container types for September and October. In December 2013container type C1, C2, C4, and C5 had the largest number of white root tips (Table 3). Container type C2 had the largest seedling biomass in three of the four months examined (Table 4). The four container types with the greatest number of white root tips also had the greatest amount of seedling biomass (Table 3, 4).

2013-2014 Loblolly pine seedling root morphology

The root weight for each one inch horizontal segment of plug length for the four container types is represented in Figure 2. The segment denoted as “bottom” reflects all vertical root growth out the bottom of the container plug. Container types C1, C2, and C6 had 5 horizontal 1” segments whereas C7, the longest plug, had 6-1” horizontal segments (Table 1).

Container type C1 had more than 62% of the root weight in the top 1” and the bottom segment. All other container types produced 50% of the root weight in the bottom two segments (Figures 2, 4, 5, 6, 7, Table 5). Container types C1 and C2 had the greatest root weight in the center portion (horizontal segments) of the plug. Container types C6 and C7 had the lowest root weight in the center portion (horizontal segments) of the plug. This growth habit (low root weight in the center portion) confirms field observations by the senior author that longer plugs appear to have an area in the center of the plug lacking in root growth (Figure 7). This may be caused by a lack of oxygen or a segment of the plug that stays too wet thereby discouraging active root growth.

The use of the median as a measure of central tendency would not be influenced as the average by large root weight values found in the first or bottom segment. Thus, an unbiased method to compare the root growth for each container type would be to compare the median root growth for each container. When this is done, the median root weight for the container types C1, C2, C6, and C7 are 0.35, 0.35, 0.27 and 0.18 g, respectively. Although the root weight varied by level for C1 and C2 (Figure 2) especially in the top of the root plug the median root weight for these two containers was the same. It is interesting to note that container type, C7, was an inch longer than any of the other container types but still had the lowest median root weight of the four containers.

The average number of loblolly roots and average root weight for each of the container types are presented in Table 6. There were no significant differences for the average number of seedling roots produced by the different container types, however, the bareroot seedlings had more than 60% fewer roots than any of the container types examined. The difference in the average number of roots among bareroot vs container types was also evident in the average root biomass with the bareroot seedling the lowest and the four container types with similar root biomass. The magnitude of difference between the average root weight for the bareroot and the container seedlings is similar to root weight biomass calculated by the Southern Forest Nursery Management Cooperative in other unpublished research.

The root and top dry weights and root weight ratios were calculated for each of the container types at the end of the study (Figure 3). Container type C2 had the greatest dry seedling weight. The root weight ratio for all four containers was similar but greater than the bareroot.

2014-2015 Longleaf pine root morphology

Two of the six container sets used in this study were 6” in length (Table 1) and as a result had an extra horizontal 1” data segment as represented in Figure 8. Container type C1 had the most uniform average root weight across all of the plug levels (Figure 8). No one segment of this container set had more than 25% of the total root biomass. In all the container types (Figure 12, 13, 14, 15, 16, 17) except for C1, the top 2 or 3 segments had significantly less root weight than the bottom segment of the plug (Figure 8,9). In this study with longleaf, more so than with loblolly pine, the root growth in the hard plastic containers had a sweeping appearance from low root weight at the top of the plug to high root weight in the bottom 1” segments. The greatest differential was in container type C5 where the average root weight closest to the root collar had 1% of the total root mass with the bottom two 1” segments accounting for 85% of the total root biomass.

The median root weight for container type C1, C2, C4, C5, C6, and C7 were 0.38, 0.27, 0.31, 0.09, 0.20 and 0.25 g respectively. Container type C1 had a similar median root weight of 0.35 g for the loblolly. Container type, C1, had the most uniform root weight distribution at all levels of the plug (Figure 8) and the greatest total median root weight. The three hard plastic containers that had side air pruning holes (C2, C4 and C7) all had about the same median root weight. Container type C5 had significantly less total median root mass than any of the container types.

The longleaf pine seedling root analysis was partitioned into roots greater than 2.0 mm (designated as primary roots) and those less than 2.0 mm (designated as secondary roots). Container type C1 had nearly equal amounts of primary and secondary at each 1” segment level. The larger primary roots in container type C5 were nearly lacking except for the bottom two 1” segments. The three container types with side air pruning (C2, C4, C7) holes had an increase in the number of primary roots at the segment levels with the air pruning holes. Container type C2 had air pruning holes at two levels which correlated with the increase in the number of primary roots at those segment levels.

The number of longleaf roots and root weight for each of the container types used in this trial is presented in Table 7. There was no significant difference in the number of roots for the two 6 inch plugs (C5 and C7) nor was there a significant difference between the other 4 plugs that were less than 6 inches. Container types C1 and C6 had numerically less number of roots than the other container types. Container types C5 had significantly more root mass than the other 6 inch container type, C7. There was no significant difference in root mass of the container types less than 6 inches.

The root and shoot dry weights and root weight ratios were calculated for each of the container types at the end of the study (Figure 11). Container type C5 had the greatest dry seedling weight, with the root weight ratios similar for all six container types.

An interesting growth habit was observed after all the roots were removed and the soil-less media was washed out of the plug. The remaining root mass from the hard plastic container types (C2, C4, C5, C6, C7) contained a mass of fine roots, large primary roots (and tap root) extending down the length of the plug (Figure 18). Container type C1 which was the copper-treated polystyrene container lacked the mass of fine roots that was present in the other container types (Figure 19). In this container, all the primary roots extended horizontally approximately 0.5 inches. While the seedling was in the container plug the developing lateral roots were chemically air pruned by the copper which halted their growth until outplanted and the copper removed. Once the effect of the copper was removed, lateral root growth developed. The reduced fine root mass in the initial plug may be caused by root tips killed by the copper resulting in the cessation of cytokinin flow needed for lateral root growth (Aloni and others, 2006)

Management Implications

The choice of container set composition is limited to either polystyrene or hard plastic. The trend over the past 15 years has been toward hard plastic due to the longevity, ease of extraction, and reduced price differential between polystyrene and hard plastic. In 2016, over 85% of all seedlings were produced in hard plastic container sets. When root morphology for loblolly and longleaf pines are examined from the various container types, the copper treated polystyrene container produces a uniform root system. As a result of these studies, some container set manufacturers are modifying new container sets to include more side root pruning holes that will increase the number of fibrous roots around the area of their placement. Although some growers do not like the side air pruning holes, since it requires more attention to irrigation, the best hard plastic containers with respect to root architecture and root growth were those with side air pruning holes. Container sets without the side air pruning holes had regions of the seedling plug with little to no lateral root growth.

Toppling of young stands is defined as an instability in young stands that occurs when the trees are not completely wind-thrown, but lean at various angles and continue to grow. Of the documented cases of wind-thrown trees dating back to the late 1980's, nearly every case has been associated with container seedlings. When toppled seedlings have been examined, many do not have a normal tap root extending into the soil profile. There is a lack of documentation of wind-thrown trees correlating the seedlings to the type of container in which they were grown. This study has shown that polystyrene containers have a relatively uniform distribution of roots throughout the horizontal profile of the plug, whereas, hard plastic containers have the greatest proportion of root numbers and weight toward the bottom of the plug. Does an even distribution of roots throughout the plug provide better stability than when the majority of roots are located toward the bottom of the container? Further research is needed to determine which root morphology form provides the best stability in times of high winds. The outplanting trials associated with these same seedlings may shed some light on the subject when they are taken down in the next few years.

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Table 1. Comparison of container type used in study.

	C1	C2	C3	C4	C5	C6	C7
2013 - 2014 Loblolly Seedling Quality and Outplanting	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**No**
2013 - 2014 Loblolly Root Morphology	**Yes**	**Yes**	**No**	**No**	**No**	**Yes**	**Yes**
2014- 2015 Longleaf Root Morphology	**Yes**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**Yes**
Container Specification							
Seedling/sq foot	49.4	51.7	49.0	52.0	52.0	54.0	50.0
Cavity Diameter (in)	1.40	1.50	1.60	1.55	1.55	1.50	1.40
Cavity Length (in)	5.9	4.7	3.4	5.3	6.0	5.0	6.0
Cavity Volume (ml)	108	110	93	110	131	113	100
Cavities/tray	112	128	120	128	128	135	112
Chemical root pruning?	Yes	No	No	No	No	No	no
Side root pruning holes?	No	Yes	No	Yes	No	No	yes
# root pruning holes	0	8	0	4	0	0	3

Table 2. Container types RCD (mm) and height (in) by month, n=30.

	Sept		**Oct**		**Nov**		**Dec**	
Container	**Ht**	**RCD**	**Ht**	**RCD**	**Ht**	**RCD**	**Ht**	**RCD**
C1	12.1 B	3.8 A	11.9 AB	3.9 AB	11.9 C	4.9 A	12.9 A	4.9 A
C2	11.2C	3.2 D	11.9 AB	3.7 BC	12.8 A	4.3 B	13.4 A	4.3 B
C3	10.5 D	3.3 CD	11.3 C	3.5 C	12.7 A	4.0 C	13.4 A	4.2 B
C4	11.7 BC	3.5 B	12.3 A	3.6 BC	12.5 AB	4.4 B	13.0 A	4.3 B
C5	11.2 C	3.4 BC	11.3 C	3.9 A	11.8 C	4.2 BC	11.6 C	4.3 B
C6	12.9 A	3.7 A	11.8 B	3.8 AB	12.2 BC	4.2 BC	12.4 B	4.2 B
lsd	*0.57*	*0.18*	*0.39*	*0.21*	*0.44*	*0.25*	*0.46*	*0.31*

Table 3. Number of white root tips (RGP) by container type.

Container	Sept	Oct	Nov	Dec
C1	32.2 A	41.6 A	50.7 AB	57.3 A
C2	37.5 A	44.1 A	51.1 AB	43.2 AB
C3	44.7 A	33.7 A	42.3 B	36.0 B
C4	46.1 A	41.9 A	64.5 A	42.8 AB
C5	33.3 A	39.4 A	57.0 AB	41.1 AB
C6	27.9 A	36.1 A	54.1 AB	30.1 B
lsd	*19.8*	*12.3*	*16.5*	*15.6*

Table 4. Seedling dry weight (g) by container type.

	Sept			Oct			Nov			Dec		
Container	Top	Root	Total	Top	Root	Total	Top	Root	Total	Top	Root	Total
C1	1.5	0.85	2.35	2.17	1.21	3.38	2.57	1.42	3.99	2.74	0.91	3.65
C2	1.9	0.71	2.61	2.36	0.82	3.18	3.38	1.81	5.19	3.36	0.99	4.35
C3	1.46	0.58	2.04	1.8	0.85	2.65	2	0.88	2.88	2.5	0.86	3.36
C4	1.72	0.87	2.59	2.04	1.03	3.07	2.87	1.2	4.07	2.59	0.86	3.45
C5	1.6	0.61	2.21	2.48	1.03	3.51	2.59	1.88	4.47	2.75	0.86	3.61
C6	1.86	0.69	2.55	2.23	0.85	3.08	2.4	0.97	3.37	2.59	0.84	3.43

Table 5 Location of the first 50% of the loblolly pine roots.

Level	C1	C2	C6	Level	C7
0-1"				0-1"	
1"-2"				1"-2"	
2"-3"				2"-3"	
3"-4"				3"-4"	
4"-5"				4"-5"	
Bottom				5"-6"	
				Bottom	

Table 6. Average number of roots and root weight (plus taproot) after 5 months – Loblolly pine.

Container	Average total number of roots	Average weight of roots (g) (Total)
Bareroot	20	3.5
C1	64 A	6.1
C2	82 A	7.7
C6	71 A	6.3
C7*	82 A	5.3

* Container C7 was 6 inches in length. All other containers were shorter.

Table 7. Number of roots and root weight (plus taproot) after 5 months for longleaf pine seedlings by container type.

Container	Average total number of roots	Average weight of roots (g) (total)
C1	36 B	5.6 BC
C2	54 AB	5.7 BC
C4	50 AB	7.3 AB
C5*	64 A	8.3 A
C6	30 B	5.4 C
C7*	54 AB	6.0 BC

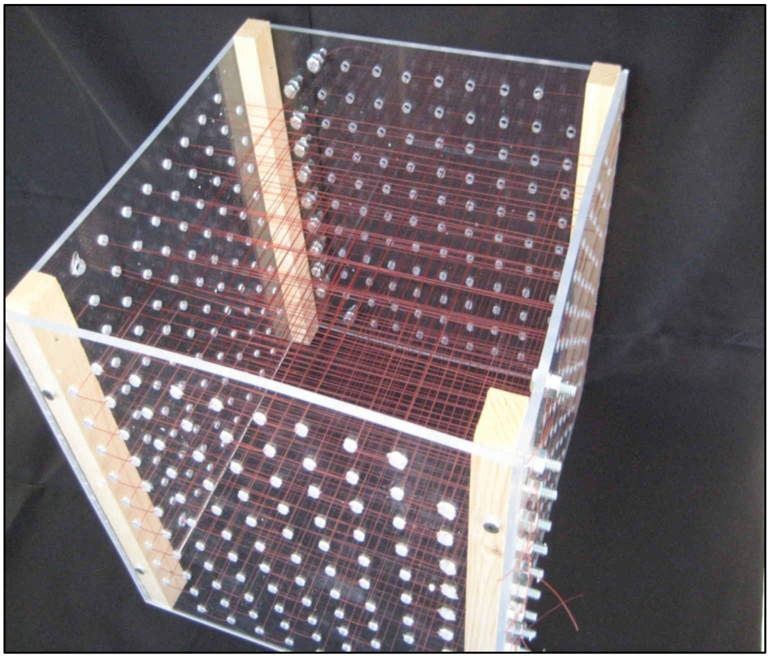

Figure 1. Plexiglass box strung with fishing line used to study root architecture.

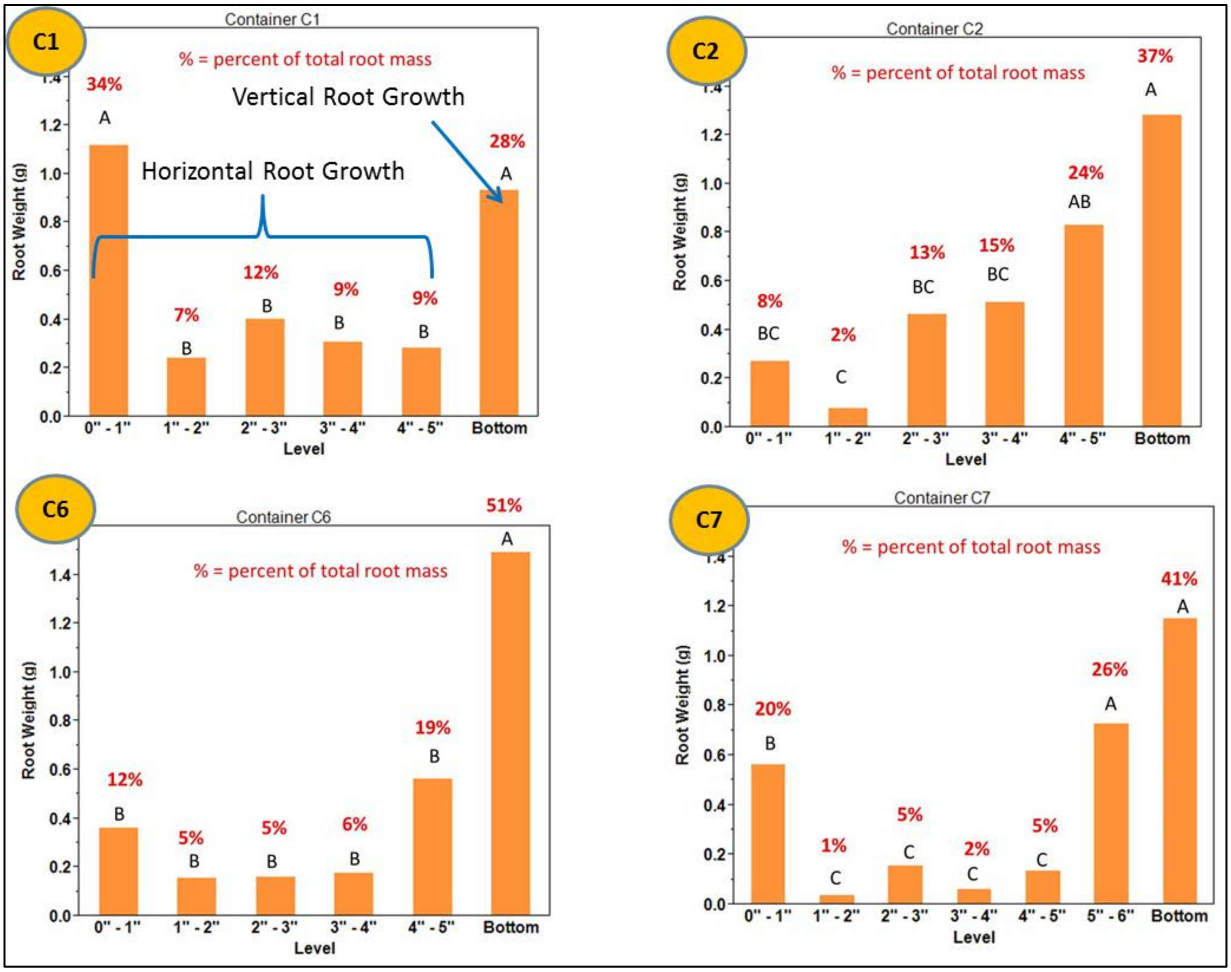

Figure 2. Root weight by plug level for Loblolly Pine for container types examined.

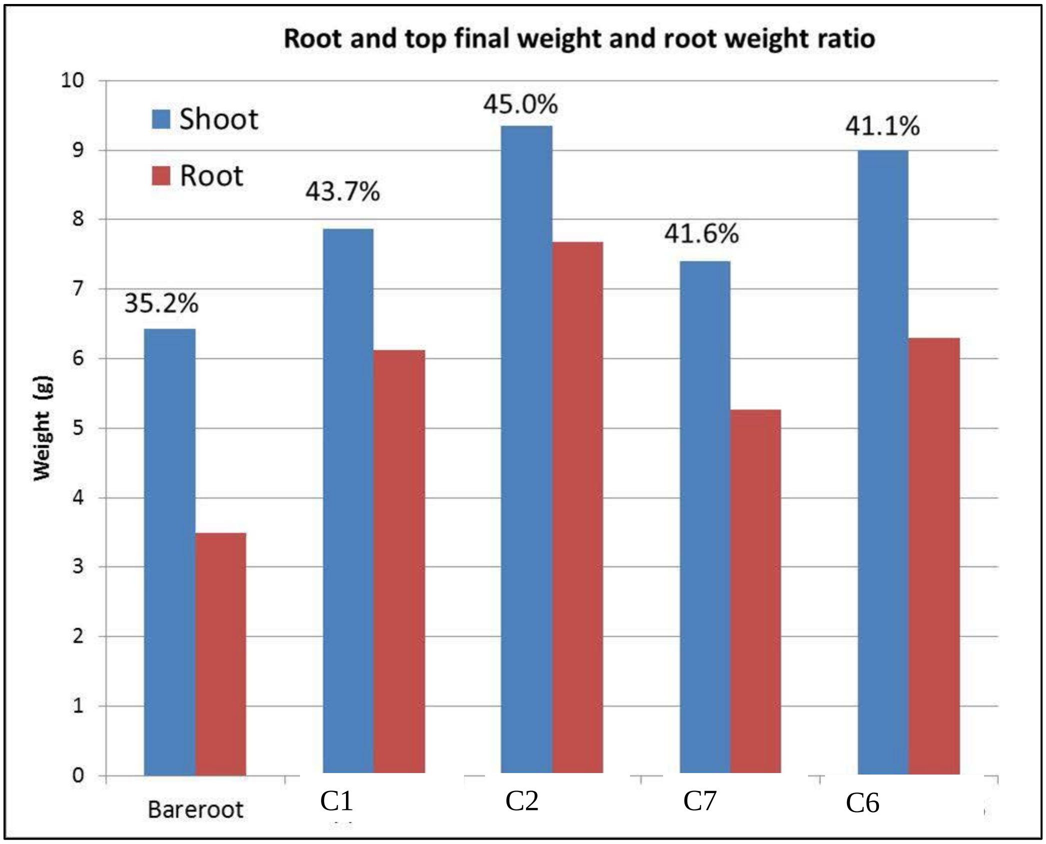

Figure 3. Final root and shoot weight and root weight ratio for loblolly pine seedlings.

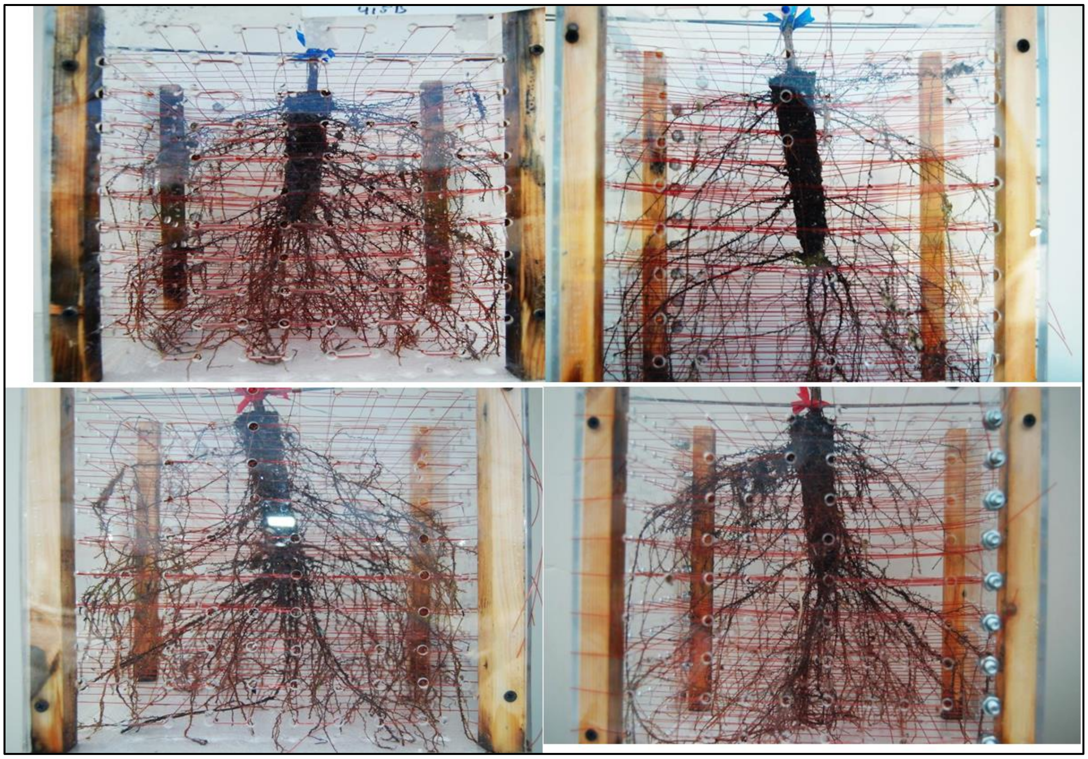

Figure 4. Loblolly root growth representing 4 replications for container type C1.

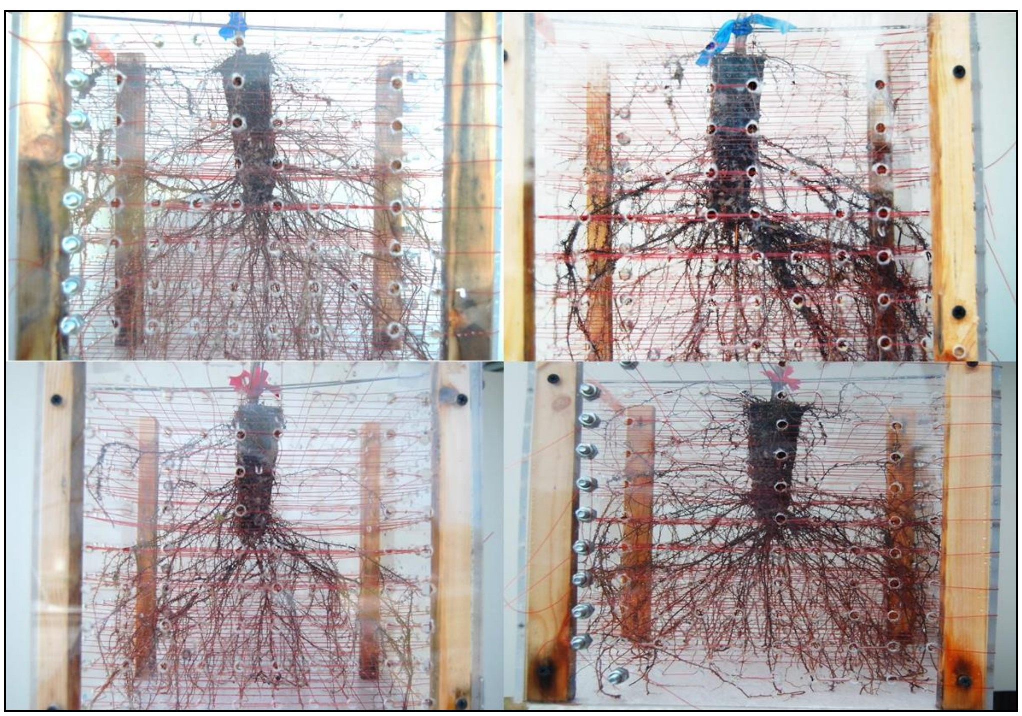

Figure 5. Loblolly root growth representing 4 replications for container type C2.

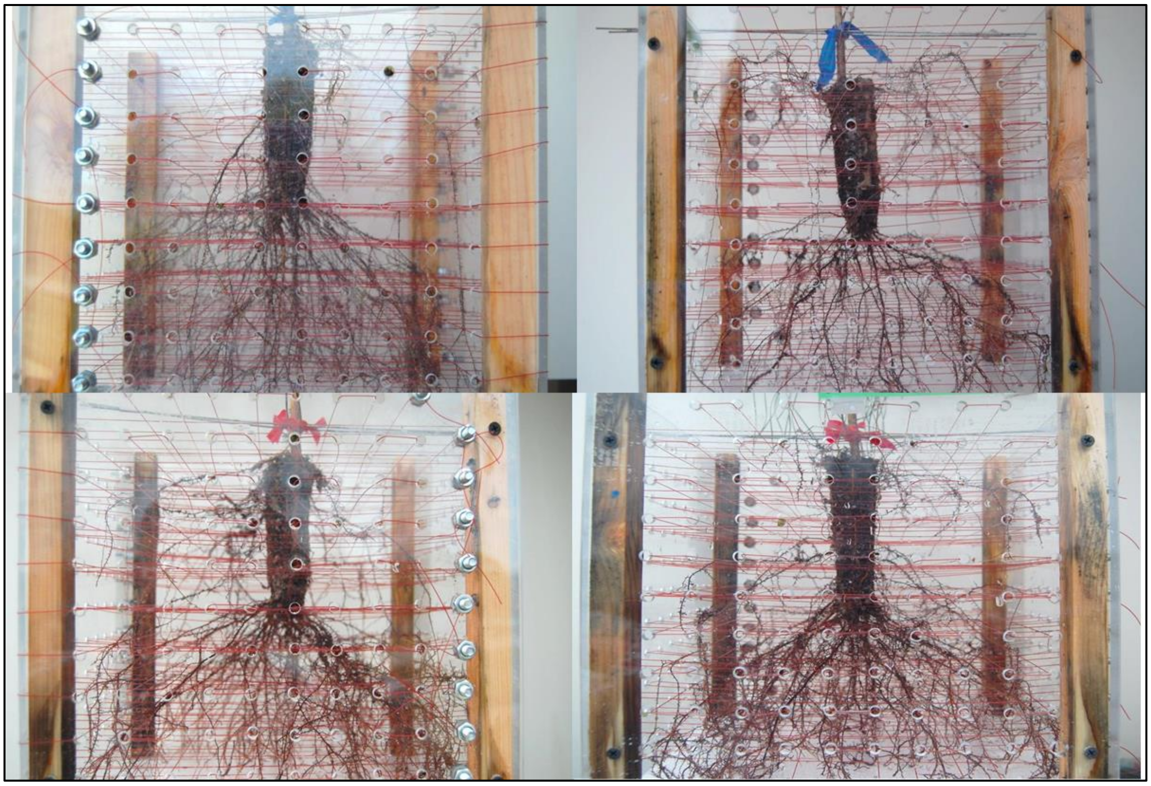

Figure 6. Loblolly root growth representing 4 replications for container type C6.

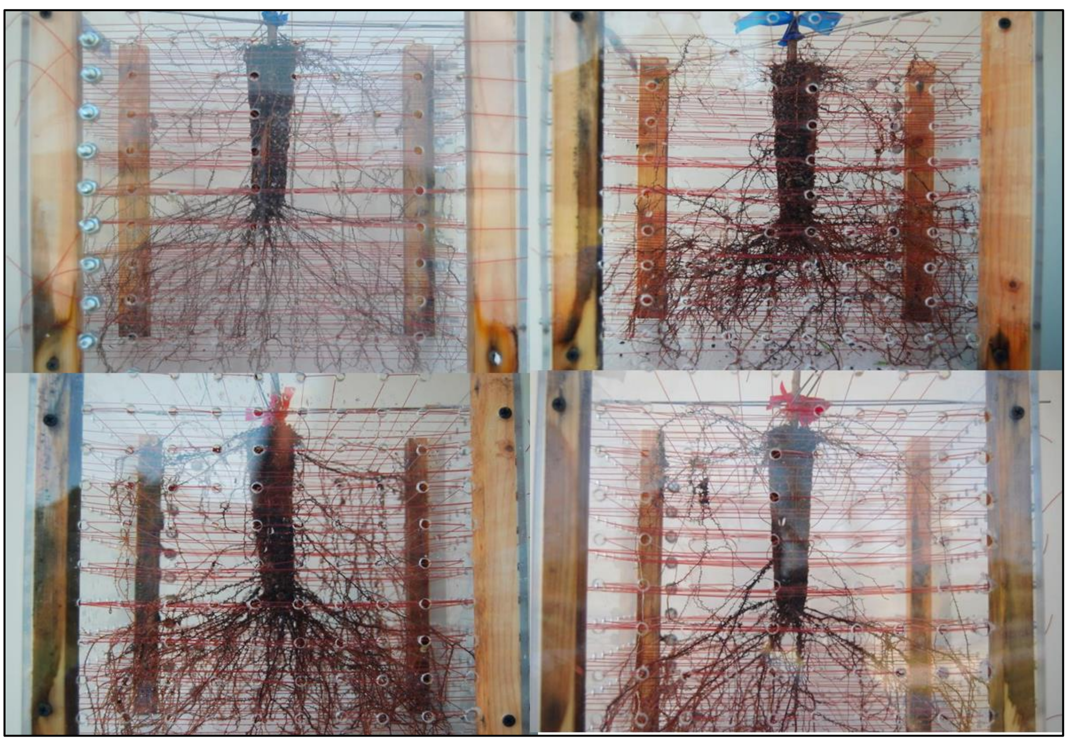

Figure 7. Loblolly root growth representing 4 replications for container type C7.

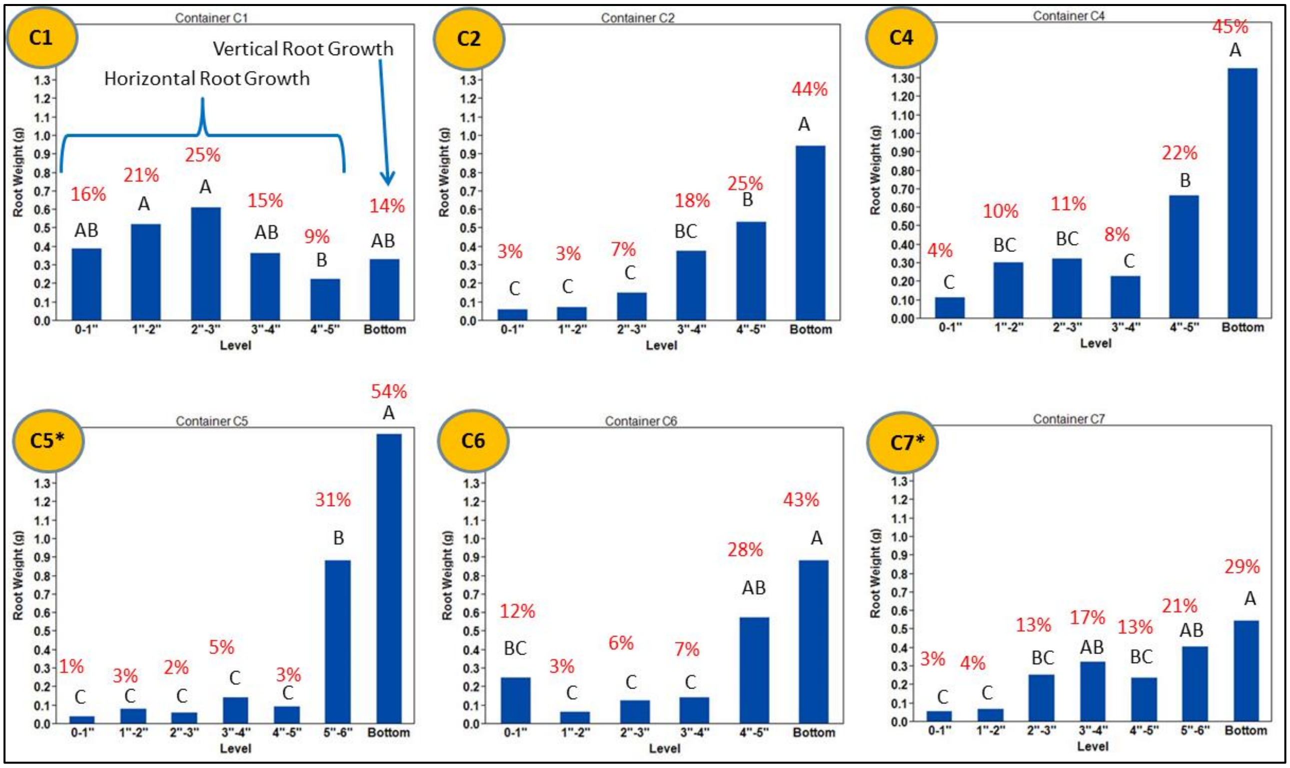

Figure 8. Root weight by plug level for longleaf pine for container types examined .

Level	C1	C2	C4	C6	Level	C5	C7
0-1"					0-1"		
1"-2"					1"-2"		
2"-3"					2"-3"		
3"-4"					3"-4"		
4"-5"					4"-5"		
Bottom					5"-6"		
					Bottom		

Figure 9. Location of the first 50% of the longleaf pine roots.

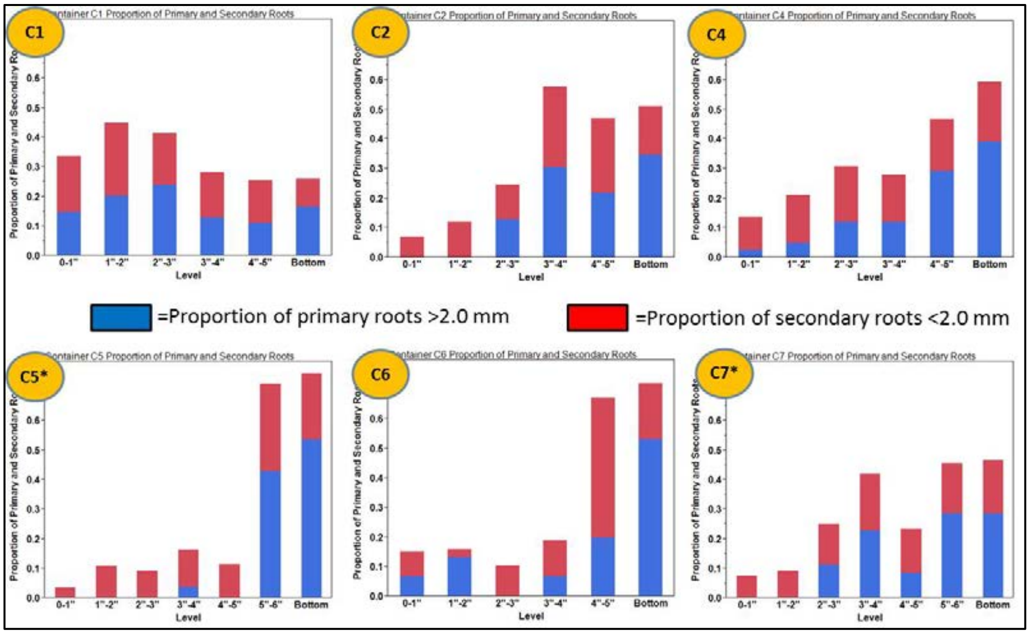

Figure 10. Proportion of primary and secondary longleaf roots by level for container types examined.

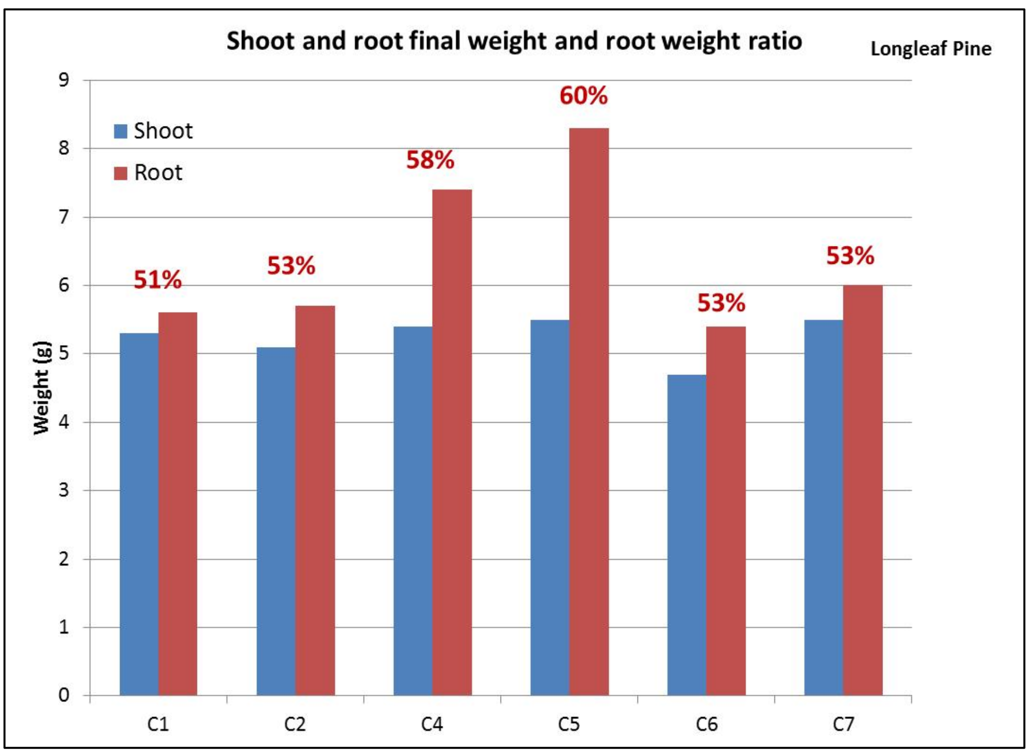

Figure 11. Final root and shoot weight and root weight ratio for longleaf pine seedlings.

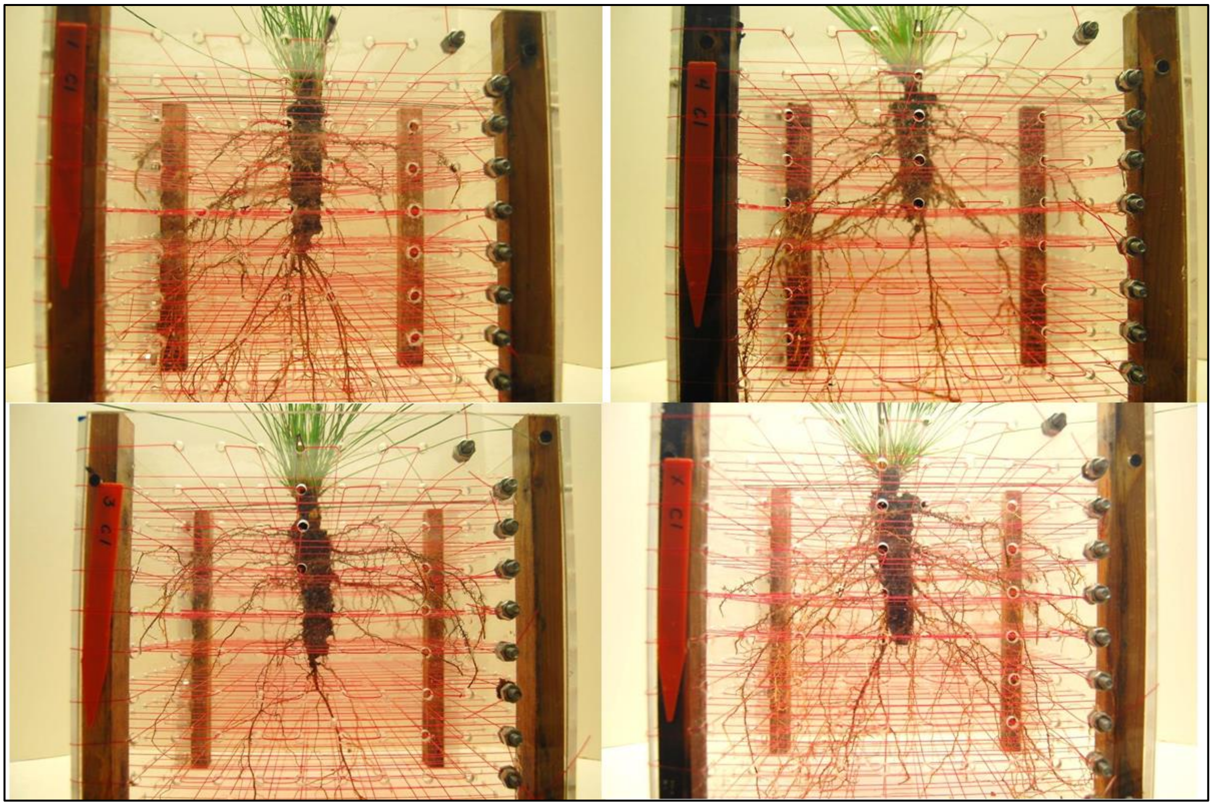

Figure 12. Longleaf root growth representing 4 replications for container type C1.

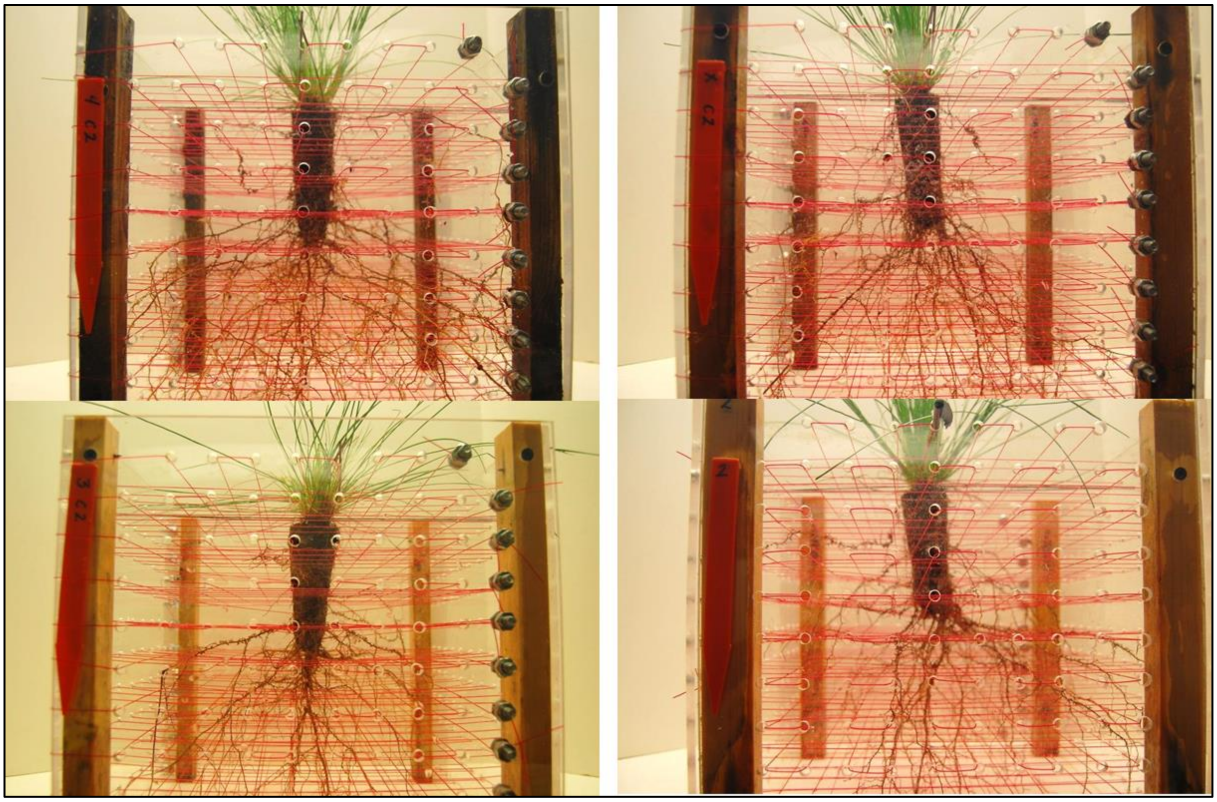

Figure 13. Longleaf root growth representing 4 replications for container type C2.

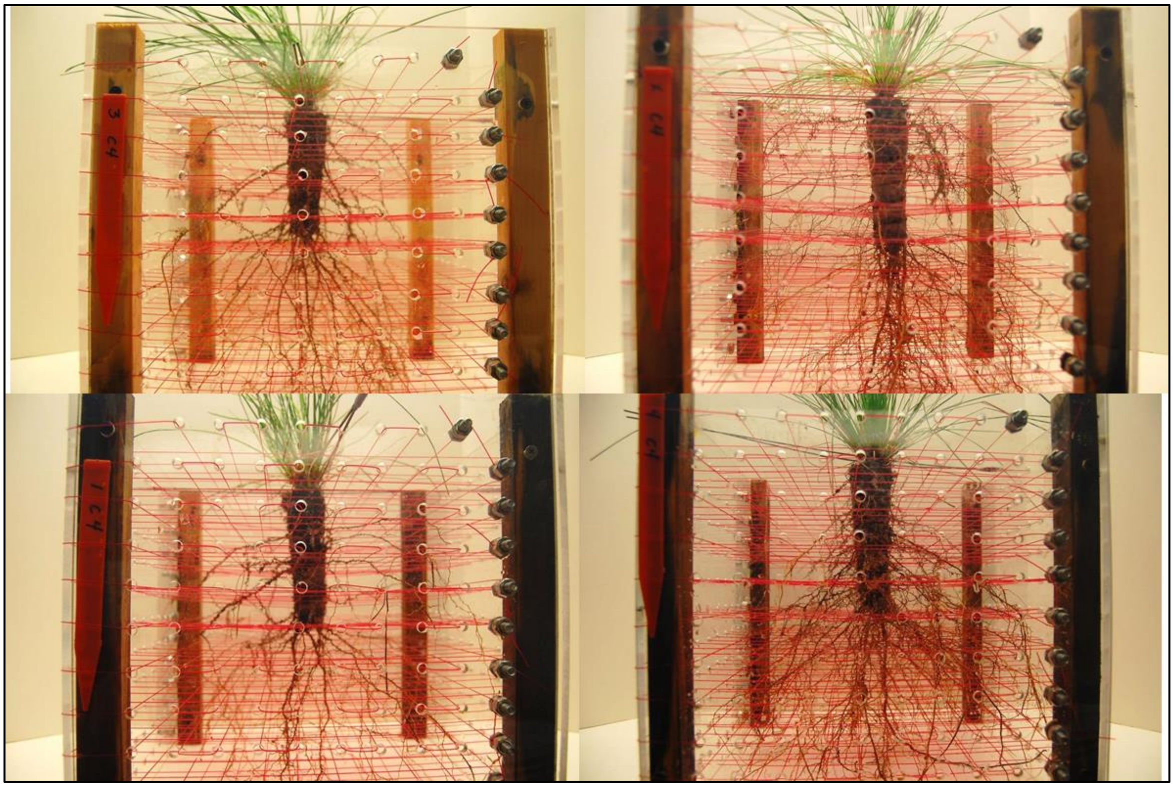

Figure 14. Longleaf root growth representing 4 replications for container type C4.

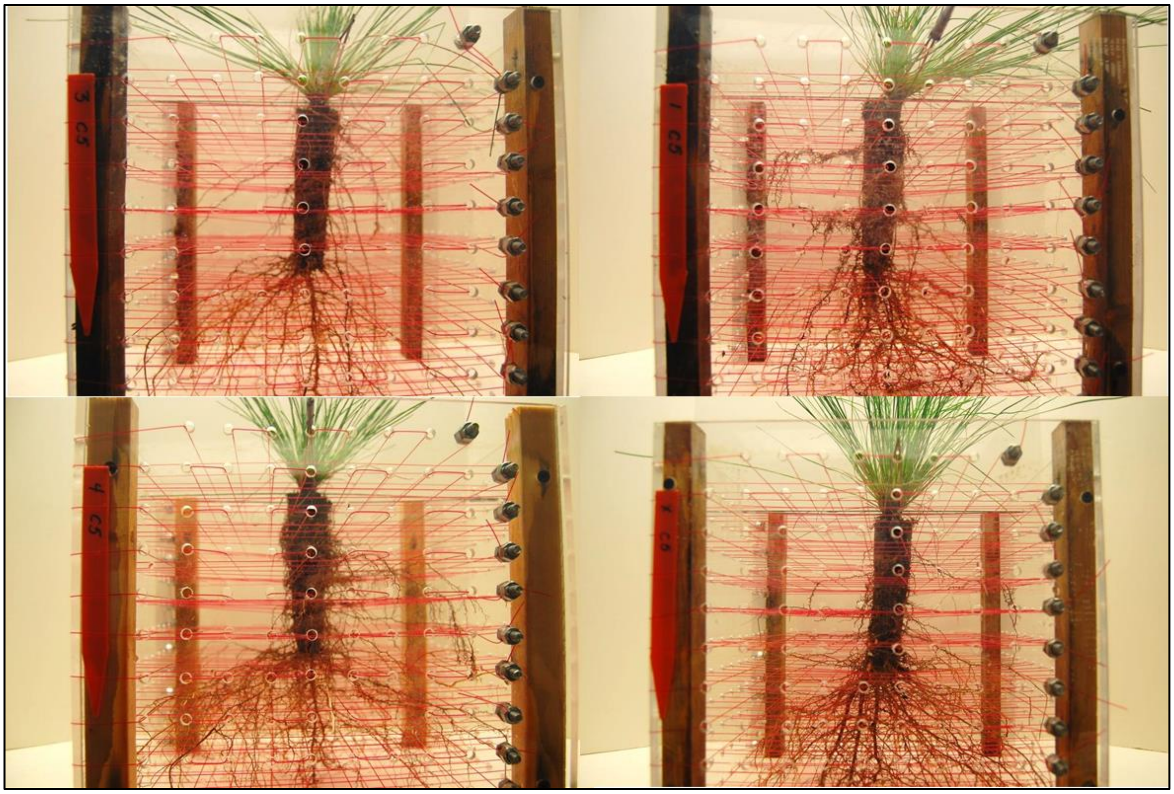

Figure 15. Longleaf root growth representing 4 replications for container type C5.

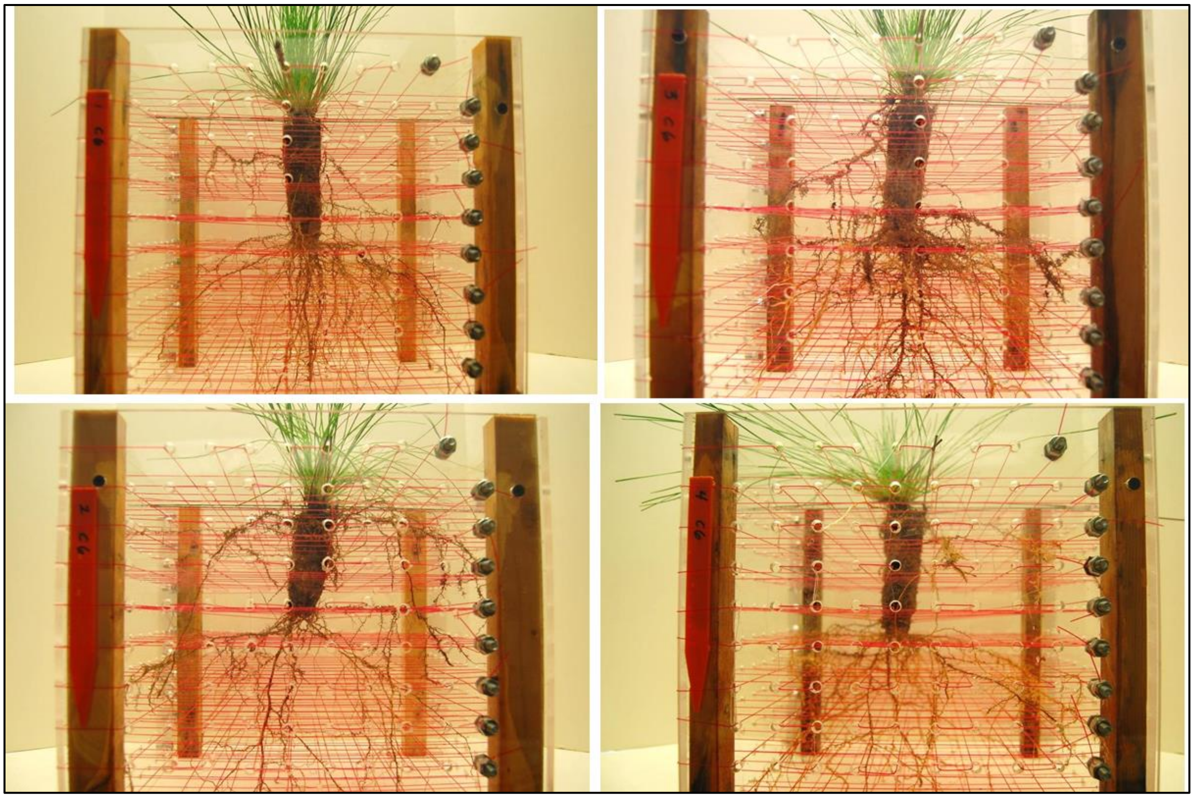

Figure 16. Longleaf root growth representing 4 replications for container type C6.

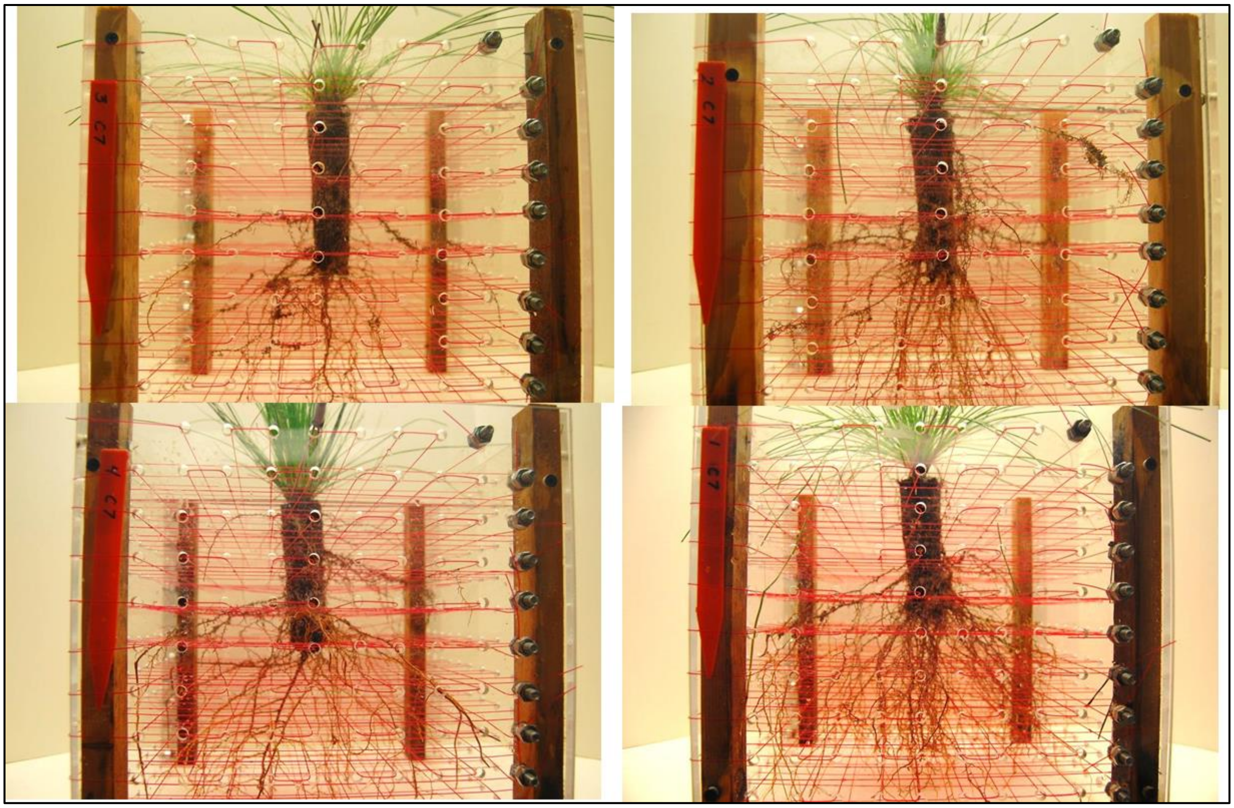

Figure 17. Longleaf root growth representing 4 replications for container type C7.

Figure 18. Longleaf container plugs after soil-less growing media was removed. .

Figure 19. Longleaf container plug from polystyrene and hard plastic container after growing media was removed.