Southern Forest
Nursery Management Cooperative

RESEARCH REPORT 21-04

IMPACTS OF PLANTING DROUGHT HARDENED LOBLOLLY PINE SEEDLINGS UNDER VARIOUS DROUGHT CONDITIONS

by
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INTRODUCTION

Increases in temperature combined with greater frequency of drought events has led to more unfavorable planting conditions for forest seedlings (Souden et al. 2019, Rehschuh et al. 2020) in the southern United States. At outplanting, seedlings may experience abiotic and biotic stresses that lead to limited growth or seedling mortality (Kozolwski and Davies 1975). Several factors can play a role in a seedling's ability to overcome such stresses, such as root system size and architecture, root-soil contact, and root hydraulic conductivity (Grossnickle 2005). This is required, as a recently planted seedling's root system must adequately supply water throughout the stem and out the transpiring foliage (Grossnickle 2005).

Forest nurseries sometimes use the practice of drought hardening seedlings to reduce the adverse effects of moisture stress when planted in dry conditions (Zhang et al. 2018). Drought hardening is achieved by exposing seedlings to drought conditions in the later stages of seedling culture by reducing irrigation thereby, theoretically, increasing the seedling's ability to adapt to subsequent droughts (Thomas 2009). The practice of drought hardening seedlings began in the first half of the 20th century with the establishment of a nursery in a relatively arid region in the Pacific Northwest with the hopes of producing seedlings that would have greater resistance to drought (Engstrom 1949, South and Nadel 2020). This practice was further validated when Lavender and Cleary (1974) published a graph indicating greater survival of drought stressed seedlings compared to non-stressed seedlings although no data was presented to support their findings (South and Nadel 2020).

While some studies have shown morphological and physiological adjustments in seedlings from drought hardening that could be beneficial (Sloan et al. 2020, Seiler and Johnson, 1985), very few studies have shown increased growth and survival of seedlings planted under dry conditions after drought hardening has occurred (Salvador et al. 2004, Morgan et al. 1984, Pita and Pardos 2001). In fact, studies have shown that increased survival does not occur with reductions in summer or fall irrigation in many bareroot *Pinus* species (South and Nadel 2020, Minko 1976, Williams et al. 1988, Dierauf and Chandler 1991).

Even with the possibility of beneficial physiological adjustments to seedlings, there are still a number of questions on the short- and long-term effects of drought hardening on forest tree seedlings. For example, drought stressing seedlings can lead to significant damage to their hydraulic system through xylem embolisms that may result in drought induced mortality (Choat et al. 2018, Choat et al. 2014). Drought induced mortality does not always occur at the time of stress, but injury to the hydraulic system caused by drought hardening could predispose plants to subsequent damage from other stresses (Rehschug et al. 2020, Anderegg et al. 2015, Choat et al. 2018). Because of the conflicting studies, the ability of a seedling to recover their hydraulic capacity (fix xylem embolisms) has become a research focus in recent years. While rapid refilling of embolized xylem conduits has been documented, most cases involve temperate deciduous species and usually occurs with winter freeze-thaw cycles and positive root pressures (Choat et al 2019, Sperry et al. 1987, Utsumi et al 1998, Cobb et al. 2007). However, many recent studies, have shown that embolisms are not repaired despite recovery in plant water status to pre-drought levels, especially in conifer species (Choat et al. 2019, Choat et al. 2014, Rehshuh et al. 2020). Choat et al. (2018) suggested that if a plant cannot fix the xylem as part of its recovery strategy, subsequent seedling growth and survival is therefore dependent on production and growth of new xylem that would incur greater carbon costs (photosynthate) to the plant.

In the southeastern U.S., loblolly pine is the most widely produced and planted timber species (Ramirez et al. 2021, Haase et al. 2020, Schultz 1997). Given that several studies have shown that conifers lack a mechanism to rapidly refill embolized xylem after drought, our objective was to determine the physiological effects of reduced water availability on drought hardened one-year-old bareroot loblolly pine seedlings and subsequent xylem recovery after rewatering.

MATERIALS AND METHODS

For this study, one-year-old bareroot loblolly pine seedlings were obtained from the ArborGen nursery in Shellman, Ga in February 2019. Seedlings were immediately planted in an outdoor covered area on Auburn University consisting of 12 sand filled boxes measuring 70” long, 36” wide and 36” deep, referred to as “stress facility”. The roof of the stress facility was greenhouse plastic and light measurements conducted showed that average photosynthetically active radiation (PAR) under the roof (measured at 1100 and 1430 hrs) was 970 μmol m^{-2} s^{-1} compared to ambient of 1365 μmol m^{-2} s^{-1} (27.5 % reduction) and there was no difference in PAR over each box (data not shown). Seedlings were watered to saturation for two weeks between planting and treatment initiation.

On February 19, 2019, treatments were randomly assigned to each of the 12 boxes. Treatments included saturation, historical and drought. Each treatment was replicated 4 times. The saturation treatment was watered to saturation daily. This historical treatment was based of the 58-year average (1958-2016) precipitation from Marshallville, GA for December, January, and February which resulted in each seedling receiving 16.38 ml of water daily. Since 2016 was an extreme drought year for much of the southeastern U.S., we based the drought treatment of weekly precipitation from December 2016-Febraury 2017 in Marshallville, Ga. Drought treatment seedling received from 0 to 19.66 ml of water a day. Treatment continued until April 23, 2019, then all treatments were watered to saturation to explore drought recovery.

A random sample of 12 seedlings were collected before outplanting and average root collar diameter (RCD) was 4.86 mm. Once outplanted, one seedling from each treatment replication combination (12 total) was randomly selected for measurements of RCD, height, biomass, and midday shoot water potential (Ψ_x) measurements 3, 5, 7, 9, 11 and 13 weeks after treatment initiation. For each measurement period, the seedling was cut just above RCD and placed inside a PMS pressure chamber (PMS Instrument Company, Albany, OR) for Ψ_x measurements for an indication of whole seedling water stress. RCD and height measurements were taken, and biomass separated into roots and shoots and dried to constant mass at 70˚C.

Following treatment termination, seedlings were watered to saturation for 6 months. In November 2019, the remaining seedlings were collected and measured for RCD, height, biomass and percent embolized xylem. To examine the percent of embolized xylem conduits 6 months after drought treatment, and rewatering, stems were cut at the base then recut underwater at 2 cm above the initial cut. Samples were placed in a dye solution of 0.5% Safranin O. under ambient conditions and allowed to transpire. The transpiring seedling would uptake the dye solution and non-embolized xylem would be red. Digital images were analyzed with imageJ software to determine the proportion of conducting and non-conducting xylem.

The study was a randomized complete block design with 4 replicates. The plot was considered the experimental unit. To test for main and interactive effects of treatment on time during the treated phase of the study, repeated measures of analysis of variance (Proc Mixed, SAS Inc., Cary, NC) was used. A visual assessment of the residuals and minimizing the Akaike Information Criterion was used for selection of the appropriate covariance structure. Main and interactive effects were considered significant at $\alpha = 0.05$. Tukey's paired comparison procedure was used for mean separation and the SLICE option of Proc Mixed was used for means separation when an interaction was significant. To test for main effects of treatment 6 months after treatment termination, analysis of variance (Proc GLM, SAS Inc, Cary NC) was used. Linear regression analysis was used to examine relationships between percent embolism, growth, and midday leaf water potential.

RESULTS

Growth

The application of water to seedlings to a point of saturation resulted in an 11% increase in RCD over the historical moisture treatment. (Figure 1). Six months after treatment termination, RCD was decreased on average 20% in the historical and drought treatment (Table 1). However, there were no significant differences in RCD growth between the end of treatment and six months after treatment termination (Table 1). There was a significant treatment by measurement date interaction in shoot mass 4 out of 6 measurement dates (Figure 2). As treatment progressed, the saturation treatment began to increase shoot mass over historical and/or saturation treatments. During the final measurement of the treated phase of the study, drought decreased shoot mass by 49.6% compared to the saturation treatment. Both the drought and historical treatments decreased root mass on average 37% compared to the saturation treatment (Figure 3). There was no significant difference in shoot mass, shoot growth, root mass or root growth 6 months after treatment termination (Table 1).

Midday Shoot Water Potential

Midday Ψ_x (a measure of drought stress) ranged from -0.25 to -1.37, -0.37 to -2.00, and -0.34 to -3.88 MPa in the saturation, historical and drought treatments, respectively (Figure 4). There was an interaction of measurement date and treatment on midday Ψ_x with a decrease in midday Ψ_x in the drought and sometimes historical treatments compared to the saturation treatment on the majority of measurement dates. On the final measurement date during the treatment phase of the study, midday Ψ_x was reduced by 355% in the drought treatment compared to the saturation treatment (Figure 4).

Loss of Hydraulic Conductance

To determine long-term effects of the three moisture treatments, we examined loss of hydraulicity six months after treatment termination by measuring the percent of embolized conducting tissue in the stem. Six months after treatment termination, the percent of embolized conducting tissue in the drought treatment was higher (33%) compared to the historical and saturation treatments (15 and 10%, respectively) (Figure 5). The percent of embolized conductive tissue six months after treatment termination was negatively related ($p=0.009$, $R^2=0.53$) to the final midday Ψ_x during the treatment phase (Figure 6). Across all treatments, the increase in RCD and shoot mass (but not root mass) was negatively related to percent embolized xylem six months after treatment termination (Figure 7).

DISCUSSION

The objective of this study was to determine if the practice of drought hardening loblolly pine seedlings in the summer or fall in nurseries was detrimental to the physiological state and growth potential of seedlings when planted at different levels of water availabilities. In practice, drought hardened seedlings should be better equipped to handle drought conditions during plantation establishment. Care was taken to make sure that planting of seedling occurred properly, and the soil was saturated and remained saturated for the first two weeks of establishment.

After about 10 weeks of limited water availability, severe reductions in midday Ψ_x began to appear. Midday Ψ_x was -2.1 and -3.9 MPa after 11 and 13 weeks of treatment, respectively. Midday leaf water potential at the turgor loss point (Ψ_{tlp}) has been reported as high as -1.16 MPa for loblolly pine (Hart et al. 2020), however, under an extensive review, Bartlett et al. (2016) reported and average Ψ_{tlp} of -2.22 MPa for loblolly pine. Osmotic adjustments of Ψ_{tlp} can occur under drought conditions but even accounting for the average adjustment of 0.4 MPa shown for temperate conifers (resulting in an average Ψ_{tlp} of -2.62 MPa) (Bartlett et al. 2014), by 13 weeks of treatment, Ψ_x was below Ψ_{tlp} in the drought treatment. This can be damaging both physiologically and on a cellular level (Clearly 1971, South and Nadel 2020).

Shoot and root biomass began showing significant reductions in the drought treatment by week eleven corresponding the extreme low Ψ_x. Martin-StPaul et al. (2017) proposed a mechanism which plants limited decreases in Ψ_x to resist extreme droughts by closing their stomata (Ψ_{close}). Simply, Ψ_{close} is the Ψ_x where stomatal conductance would reach zero. Samuelson et al. (2019)

reported a Ψ_{close} at approximately -3.0 MPa for longleaf pine. Longleaf pine is considered more resilient to drought than loblolly pine, so it may be safe to assume that Ψ_{close} for loblolly pine may be a bit higher as evident by a study with 2-year-old loblolly pine that showed Ψ_x at 90% stomatal closure to be -2.3 MPa (Hammond et al. 2019). This could explain the reductions in biomass as stomata closed to reduce further water loss resulting in less carbon for production.

As discussed, hydraulic integrity is important for plants to function and survive. Embolized xylem conduits decrease hydraulic integrity that is necessary for the roots to absorb water from the soil, translocate through the xylem eventually transpiring through the leaf stomata. Cavitation in conifers, with their tracheid-based xylem, can spread rapidly and have as much as 40% loss in conductive tracheids in a single cavitation event (Choat et al. 2014). In a study with 2-year-old loblolly pine, Hammond et al. (2019) found that 80% loss of hydraulic conductance would result in >50% mortality. In that report, they did speculate that this could be lower given that drought alleviation by rewatering in their study was immediate and continuous, as in our study, and that (continuous watering) in nature rarely happens. Using these studies, a vulnerability curves can be established to predict levels of loss of hydraulic conductance (50% loss-P_{50}, 88% loss-P_{88}) based on Ψ_x. For mature loblolly pine, average P_{50} and P_{88} has been reported as -3.3 and -4.4 MPa for stems and a P_{50} of −1.7 MPa for roots (Bartlett et al. 2016). Early developmental stages in juvenile trees may lead to different levels of tension in the xylem to invoke loss of hydraulic conductance (Hammond et al. 2019), thus it may be reasonable to assume that P_{50} and P_{88} in this study could be higher (less negative). This may explain the abrupt decline in Ψ_x between 11 and 13 weeks as seedlings may have been experiencing catastrophic hydraulic failure (Augustine and Reinhardt 2019).

Although root mass and seedling growth had recovered after 6 months of rewatering, the drought treatment still had significantly higher embolized xylem compared to the historical and saturation treatments indicating that xylem refilling did not occur. While RCD and shoot growth was not different for the 6 months of rewatering after treatment, both were negatively related to percent of embolized xylem. Several studies have also shown, with various species, that even though plant water status and gas exchange had recovered after drought alleviation, a mechanism for rapid xylem refilling was not evident (Villar-Salvador et al. 2004, Choat et al. 2014, Choat et al 2016, Hart et al. 2020). This can be detrimental by possibly predisposing plant to increased damaged caused by future droughts or other disturbances (Rehschug et al. 2020, Anderegg et al. 2015, Choat et al. 2018).

MANAGEMENT IMPLICATIONS

- The practice of drought hardening loblolly pine in nurseries may be more harmful than beneficial to outplanting success if these seedlings are planted in drought conditions.
- Under drought conditions, drought hardened seedlings had reduced size, growth, and Ψ_x reached critical levels of hydraulic failure.

- In this study, loblolly pine demonstrated the lack of a mechanism to refill embolized xylem conduits, which can delay growth of the seedling, making them more vulnerable to future droughts.
- Additional work is required to determine whether one can identify a specific level of drought hardening, or if no drought hardening, would be optimum for the survival of loblolly pine after outplanting.

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Table 1. Mean (±SE) root collar diameter (RCD), shoot mass and root mass along with growth of RCD, shoot mass and root mass 6 months after treatment termination and rewatering for loblolly pine exposed to different levels of irrigation. Different letters indicate a significant difference between treatments at α=0.05.

Treatment	RCD (mm)	Shoot Mass (g)	Root Mass (g)	RCD Growth (mm)	Shoot Mass Growth (g)	Root Mass Growth (g)
Saturation	12.06 ± 0.42 a	51.09 ± 3.69	7.20 ± 0.59	5.16 ± 0.67	38.08 ± 3.63	4.10 ± 0.63
Historical	10.07 ± 0.59 b	42.76 ± 5.57	5.61 ± 0.64	4.22 ± 0.47	34.79 ± 6.38	4.22 ± 0.69
Drought	9.18 ± 0.42 b	33.92 ± 2.27	5.02 ± 0.74	3.88 ± 0.28	27.36 ± 1.58	3.86 ± 0.85
p-value	**0.023**	0.088	0.178	0.133	0.249	0.902

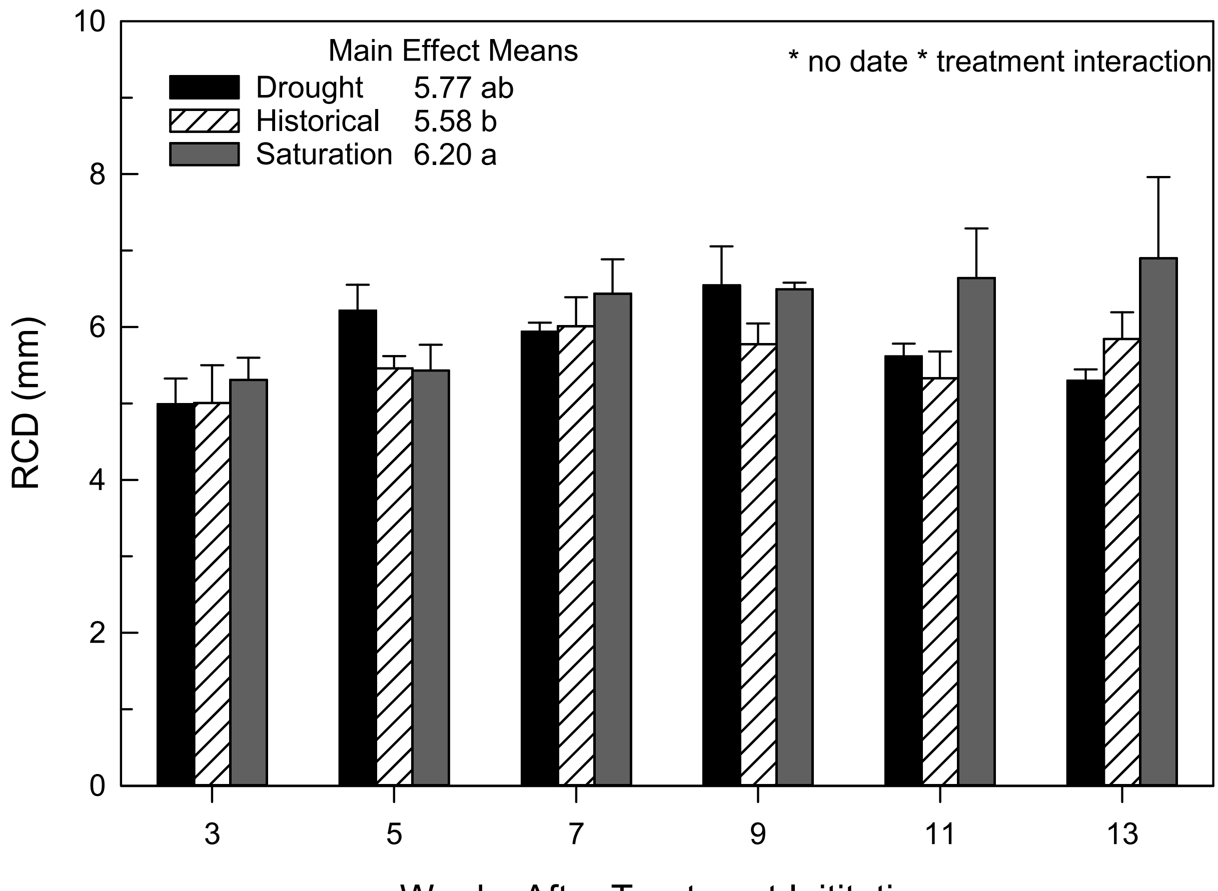

Figure 1. Mean (±SE) root collar diameters (RCD) by weeks of treatment for loblolly pine seedlings under different levels of irrigation. Main effect means are given in the legend. Different letters indicate significant difference between treatments at α=0.05.

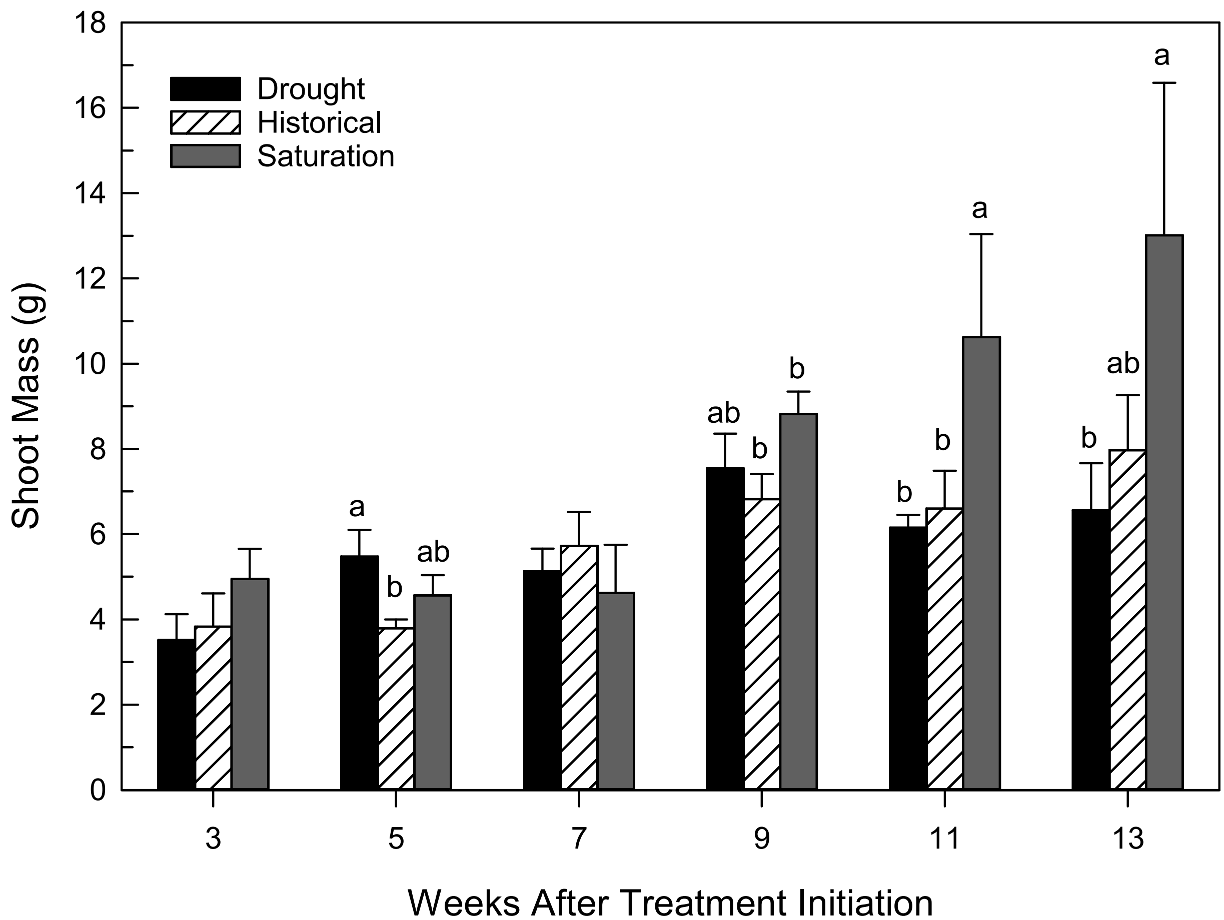

Figure 2. Mean (±SE) shoot mass by weeks of treatment for loblolly pine seedlings under different levels of irrigation. Different letters indicate a significant difference between treatments at α=0.05.

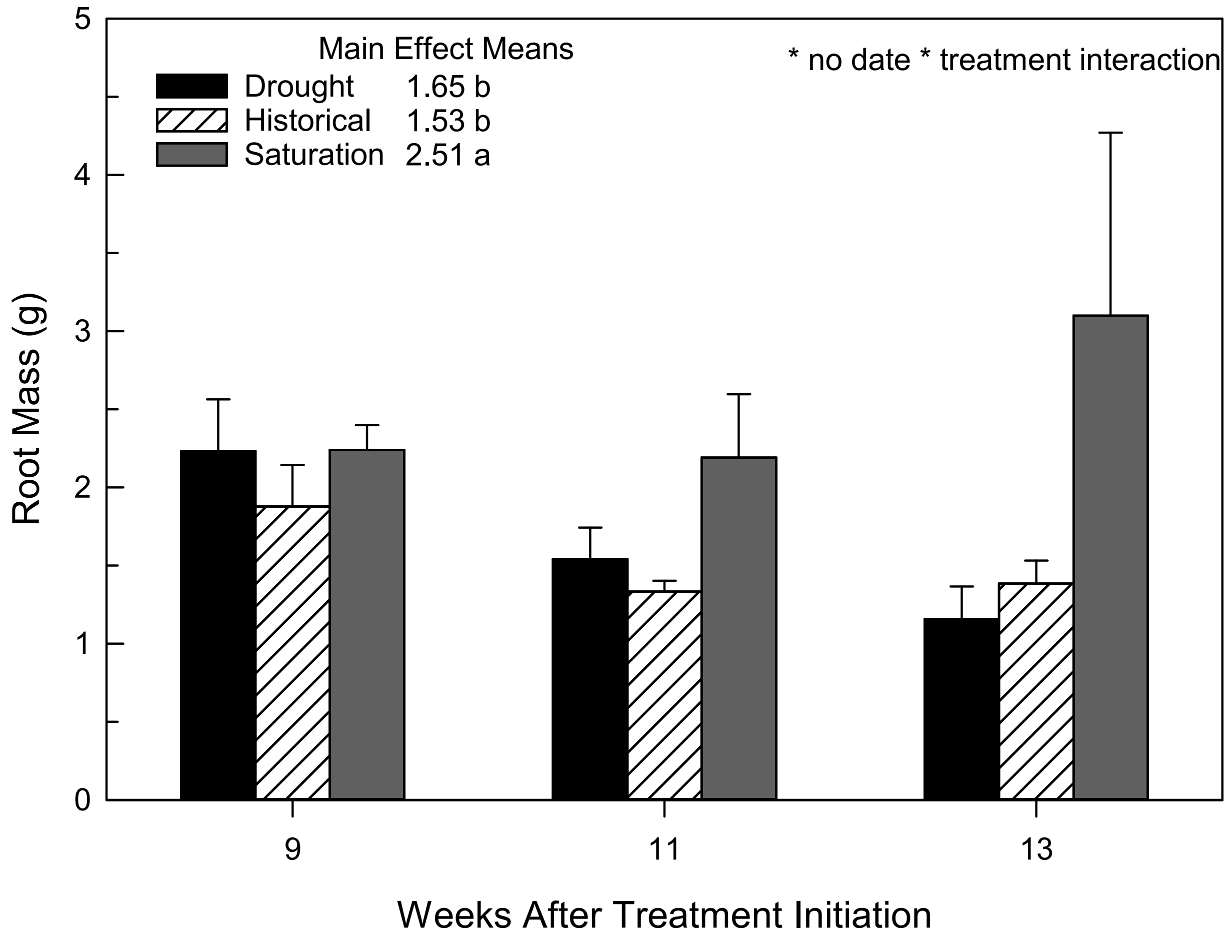

Figure 3. Mean (±SE) root mass by weeks of treatment for loblolly pine seedlings under different levels of irrigation. Main effect means are given in the legend. Different letters indicate significant difference between treatments at α=0.05.

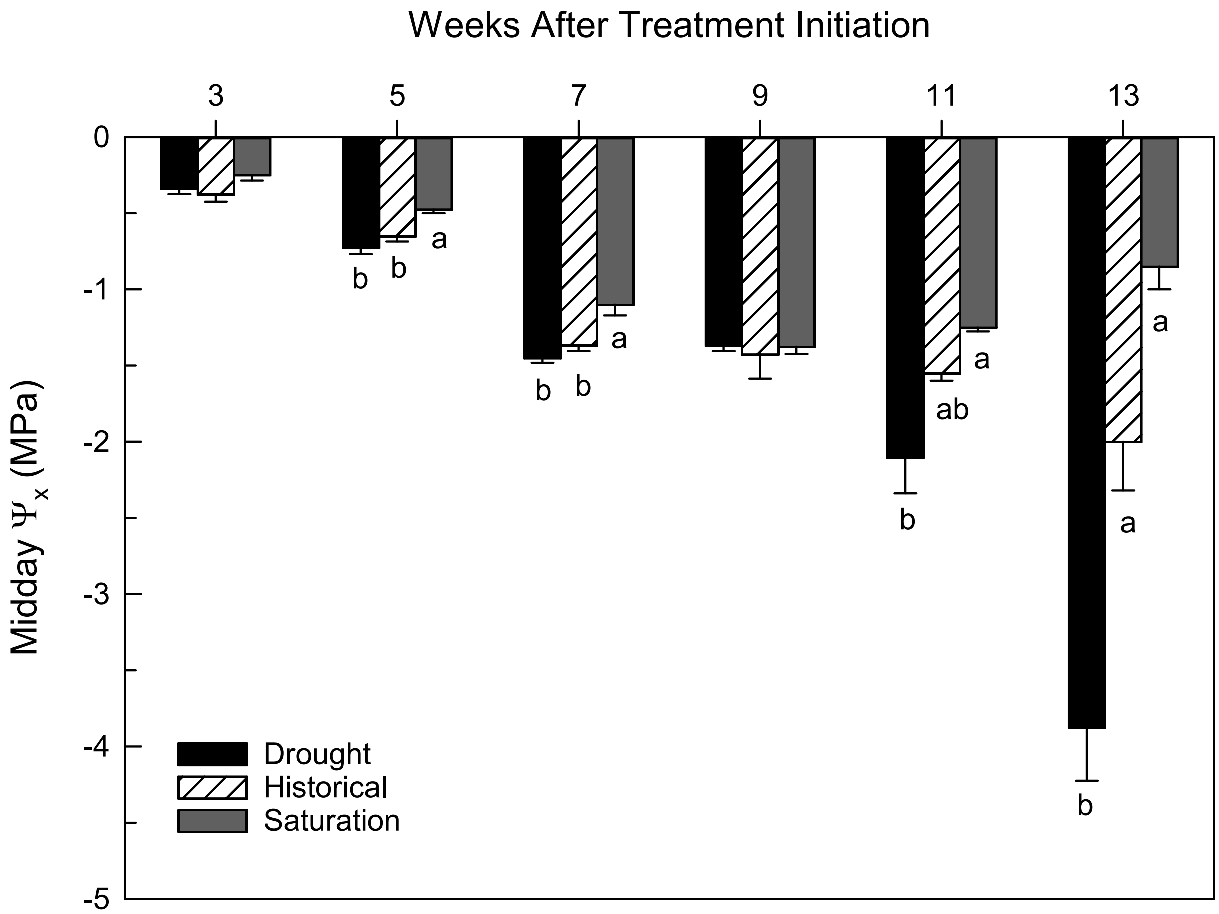

Figure 4. Mean (±SE) midday shoot water potential (Ψ_x) by weeks of treatment for loblolly pine seedlings under different levels of irrigation. Different letters indicate significant difference between treatments at α=0.05.

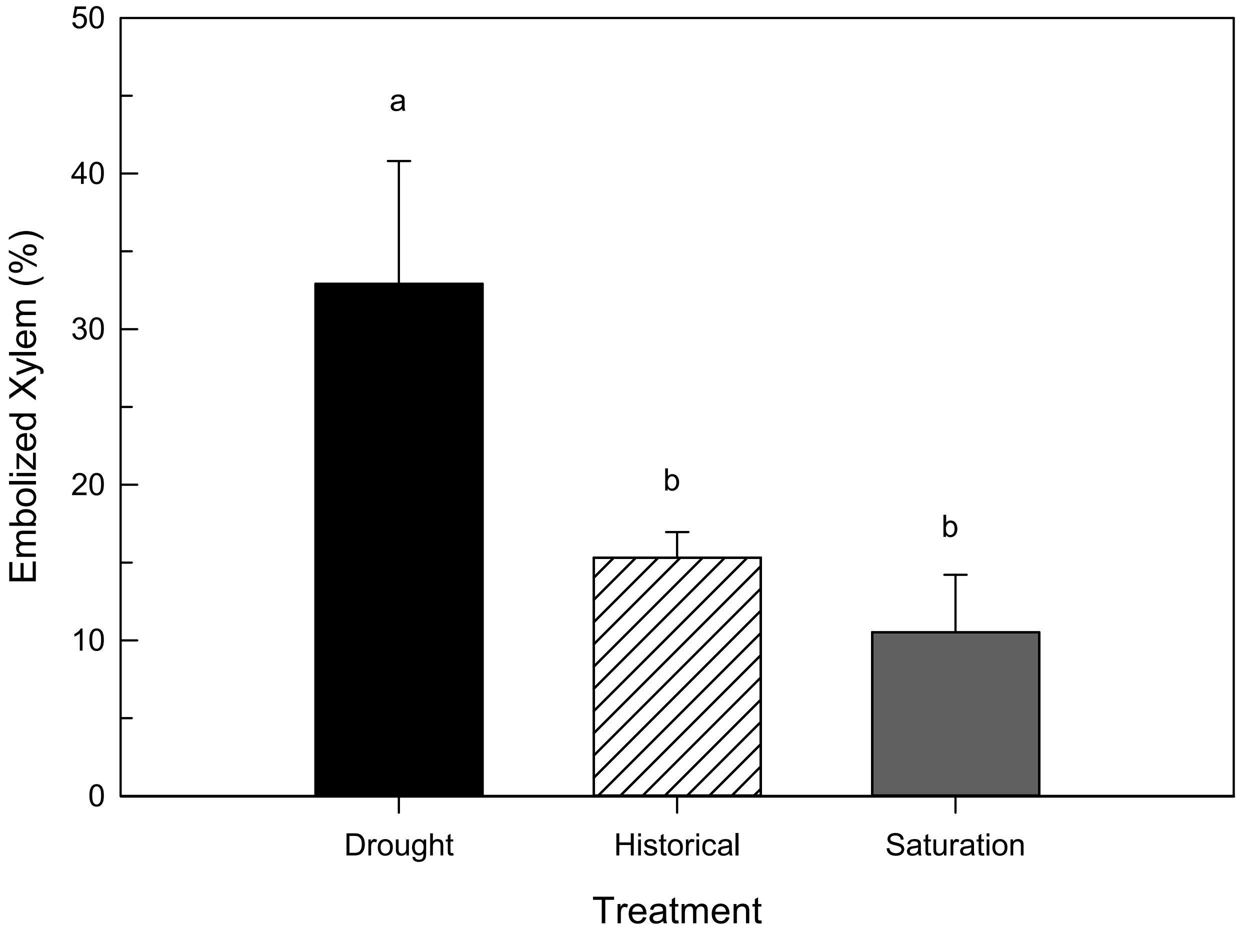

Figure 5. Mean (±SE) percent of embolized xylem by treatment 6 months after treatment termination and rewatering for loblolly pine. Different letters indicate a significant difference at α=0.05.

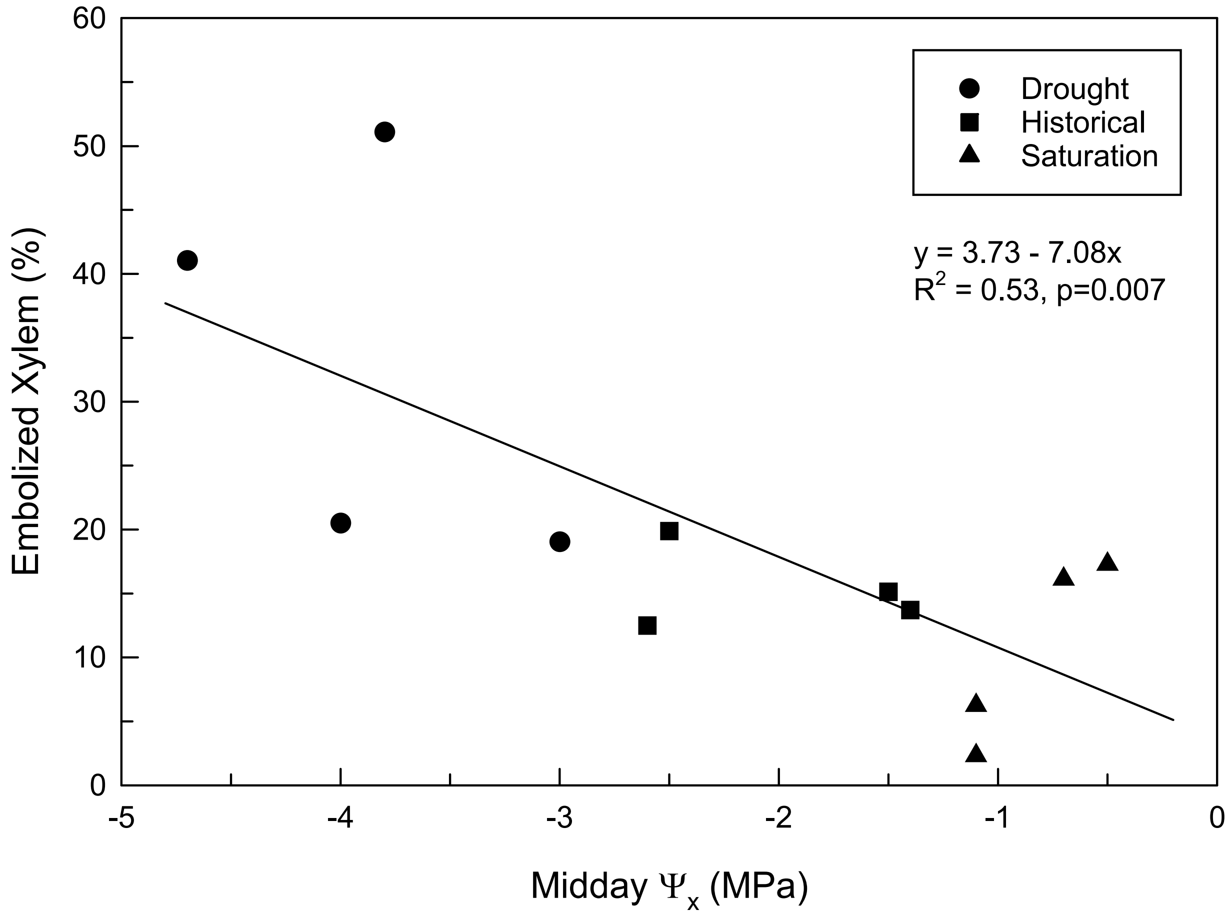

Figure 6. Relationship between midday shoot water potential (Ψ_x) on last day of treatment and percent of embolized xylem 6 months after treatment termination and rewatering for loblolly pine under different levels of irrigation.

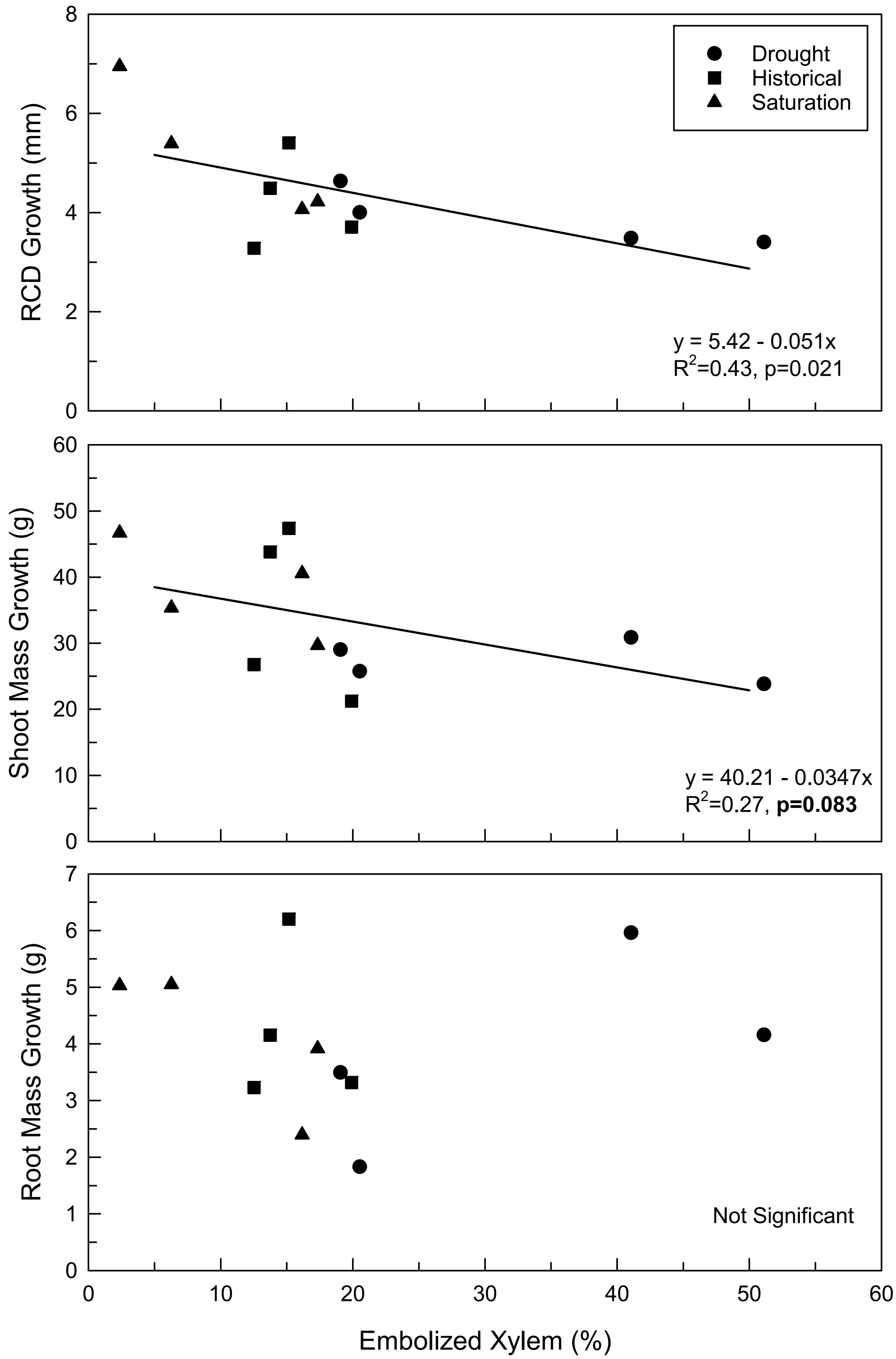

Figure 7. Relationship between percent embolized xylem and either root collar diameter (RCD), shoot mass growth or root mass growth 6 months after treatment termination and rewatering for loblolly pine under different levels of irrigation.