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Comparison of biomass partitioning and transpiration for water-stressed shortleaf, loblolly, and shortleaf x loblolly pine hybrid seedlings

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Abstract: Hybridization between shortleaf (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.) has dramatically increased and may threaten the genetic integrity of shortleaf pine. Shortleaf pine is presumed to be more drought tolerant than loblolly pine but the drought hardiness of the hybrid pine is not known. We determined biomass partitioning in response to water stress and measured whole-plant transpiration of shortleaf, hybrid, and loblolly pine seedlings. Water stress decreased total seedling biomass, increased biomass partitioning to foliage, and decreased biomass partitioning to coarse roots. Shortleaf pine seedlings partitioned more biomass to coarse roots than loblolly pine and hybrid pine was intermediate between the parent species. We found no differences in the level of soil moisture at which seedlings of different species began to limit transpiration. Our results suggest that the transpiration response of shortleaf pine and hybrid pine is similar to loblolly pine when exposed to water stress. However, greater partitioning to coarse root may allow shortleaf and hybrid pines to better withstand drought due to greater potential belowground carbohydrate supply.

Key words: *Pinus echinata*, *Pinus taeda*, biomass partitioning, drought, transpiration

Introduction

Compared to loblolly pine (*Pinus taeda* L.), shortleaf pine (*Pinus echinata* Mill.) is more cold tolerant (Lawson 1990), ice tolerant (Boggess and McMillan 1954), fire tolerant (Stewart et al. 2015), and presumably, drought tolerant (Zak 1961). Recent increases in hybridization between shortleaf pine and loblolly pine might lead to lower resilience of pine forests in the southeastern USA (Tauer et al. 2012) where hybrids are displacing shortleaf pine. How shortleaf x loblolly pine hybrids respond to drought and water stress is not known. Given the potential for climate change to make droughts worse, understanding how shortleaf x loblolly pine hybrids withstand drought compared to their parent species is important to predict future forest dynamics and to make management decisions accordingly.

Shortleaf pine and loblolly pine are closely related (Gernandt et al. 2005). Historically, the species were usually separated by site preference with shortleaf pine on drier sites and loblolly pine on more mesic sites (Mattoon 1915). Given shortleaf pine's close association with fire (Mattoon 1915), previous habitat preferences may have been reinforced by more frequent fire on drier sites. Loblolly and shortleaf pine are both ecologically and economically important in the southern forests of the United States (Schowalter et al. 1981). Loblolly pine is the most commercially important pine in the southern United States, with 1.2 billion seedlings planted annually (McKeand and Allen 2005). Shortleaf pine has the widest range among pine species in the southeastern United States, is a major timber species, and is an important component of fire-adapted ecosystems which provide important habitat for fauna.

Of the two species, shortleaf pine is presumed to be more drought tolerant than loblolly pine as it more frequently occurs and exhibits greater competitiveness on drier sites (e.g., Mattoon 1915; Lawson 1990) in part likely due to its relatively larger root system (Mattoon

1915; Zak 1961). Shortleaf pine is slower growing than loblolly pine (e.g., Dipesh et al. 2015) which is perhaps a tradeoff related to greater drought tolerance (Parsons 1968). In Scots pine (*Pinus sylvestris* L.), genotypes with slow growth rates also had higher drought tolerance characteristics, i.e., more biomass allocated to roots and higher water use efficiency (Cregg and Zhang 2001). The slower growth of the shortleaf pine seedlings (e.g., Kramer 1943) may put it at a competitive disadvantage on sites where loblolly pine (or the shortleaf x loblolly pine hybrids) can successfully establish. However, this slower growth rate may be an adaptation that allows shortleaf pine to persist on sites with low water or nutrient availability (Lawson and Kitchens 1983).

Given their genetic similarity, shortleaf and loblolly pine naturally hybridize. Hybrids typically are intermediate in morphological traits and may have a resistance to pathogens which are deleterious to the parent species (Cotton et al. 1975). While hybridization between shortleaf and loblolly pine previously occurred, the rate of hybridization has recently increased (Tauer et al. 2012). By comparing the genetic makeup of trees from the 1950's to current seedling cohorts from the same locations, Stewart et al. (2012) found that hybrid seedling numbers have dramatically increased in the last 60 years. The 1950's shortleaf pine and loblolly pine were only 3% and 4% hybrid, respectively, while the present seedling cohort contained 46% and 23% hybrids. This increase in hybridization indicates an ecosystem in flux and may lead to loss of species identity through introgression (Tauer et al. 2012).

Replacement of shortleaf and loblolly pines by hybrids may result in less resilient pine forests as introgression between the two species might dilute species-specific phenotypic traits that are important to withstand stress or recover from disturbance. Displacement of shortleaf pines by hybrid pines reduces resilience to fire (Stewart et al. 2015; Bradley et al. 2016) because

hybrid pines don't possess a basal crook that is an important adaptation for shortleaf pine to resprout following topkill (Lilly et al. 2012a). However, little is known regarding the drought tolerance of shortleaf x loblolly pine hybrids. This is especially important given future climate predictions. In the southeastern United States, temperature is predicted to increase by 2.5 to 4.0 °C by the end of this century (Collins et al. 2013). In addition to direct effects, higher temperatures increase vapor pressure deficit and potential water stress (Breshears et al. 2013) which can increase seedling mortality during drought (Will et al. 2013b). Also, more intense droughts are predicted due to longer dry periods between precipitation events (Collins et al. 2013; Walsh et al. 2014).

How will an increasingly hybrid southern pine forest component respond to drought and future climate change? If hybrid pines are as drought tolerant as shortleaf pine, the faster growth rate of the hybrid pines (e.g., Will et al. 2013a) might lead to the displacement of shortleaf pine on more xeric sites. If hybrid pine seedlings are not as drought tolerant as their shortleaf pine parents, they may be susceptible to mortality during future intense droughts. This could either shift seedling populations on drier sites back towards pure shortleaf pine or lead to loss of pine regeneration if the presence of shortleaf pine seedlings is insufficient. The increased presence of hybrid pine on what were historically shortleaf pine sites (Stewart et al. 2012) suggests that hybrid pine may have similar tolerance to water stress as shortleaf pine. There is some evidence that shortleaf x loblolly pine seedlings have a leaf-level water use efficiency (carbon gain per water loss) that is more similar to shortleaf pine than loblolly pine (Lilly et al. 2012b). However, the seedlings in that study were not subjected to water stress.

We sought to determine the drought tolerance of the shortleaf x loblolly hybrid pine seedlings. We compared the response of transpiration under declining water availability and

biomass partitioning to water stress of hybrid pine seedlings to both loblolly and shortleaf pine seedlings. In regards to biomass partitioning, we hypothesized that regardless of water availability, shortleaf pine seedlings would partition more to roots while loblolly pine would partition more to shoots, with the hybrid pines intermediate. We predicted that water-stressed shortleaf pine would have a larger shift in biomass partitioning towards root in response to drought than loblolly pine, with hybrid pine intermediate. To address seedling water use, we measured the normalized transpiration rate (NTR) (Jyostna Devi et al. 2009) which compares whole-seedling transpiration under water-stressed conditions to its previous transpiration under well-watered conditions. We hypothesized that shortleaf pine would exhibit a more isohydric response, i.e., greater stomatal closure during water stress to maintain internal water status, than loblolly pine and reduce its NTR at higher soil moisture content during soil drying, with hybrid pine's response intermediate.

Material and Methods

Shortleaf pine, loblolly pine, and shortleaf x loblolly pine hybrid seeds were produced at the Oklahoma Forestry Services seed orchard in Idabel, OK, USA (33.8942, -94.7519). Both shortleaf pine and loblolly pine seeds were collected from open pollinated cones for six shortleaf and six loblolly pine clones. Hybrid pine seeds were created through controlled pollination of six of the loblolly pine and five of the shortleaf pine clones. Eight hybrid families were produced from the crosses. Shortleaf pine pollen was used to fertilize loblolly pine. Seeds were collected from cones and stored in a -20 °C freezer.

On 24 January 2013, seeds were removed from the freezer and allowed to soak in water for 12 hours at approximately 4 °C. After 12 hours, seeds that sank were placed in stratification.

109 To stratify, seeds were wrapped in a moist paper towel, placed in a plastic bag, and then put in a
110 refrigerator at 4 °C for 75 days.

111 Planting medium was prepared by mixing 2 parts of soil from Idabel, OK USA with 2
112 parts coarse sand (Quikrete, OH, USA), and 1 part peat moss (Majestic Earth, Sun Gro, MA,
113 USA). The native soil was a mixture of Adaton loam (Fine-silty, mixed, active, thermic Typic
114 Endoaqualfs) and Kullit fine sandy loam (Fine-loamy, siliceous, semiactive, thermic Aquic
115 Paleudults). Before mixing, soil was sieved through a screen with 1 x 1 cm openings to remove
116 large clumps, roots, or other debris. Components were mixed in a ~100 liter concrete mixer and
117 poured into 6.59 liter pots (22 cm height x 22 cm top diameter). Before planting, pots were
118 watered until water drained from the bottoms.

119 In total, there were eight hybrid pine full-siblings families, six loblolly pine half-sibling
120 families, and six shortleaf pine half-sibling families. Each family was planted in four pots with
121 (4-5 seeds per pot) on 10 April 2013 in a greenhouse in Stillwater, OK, USA (36.119379,
122 -97.104179). In total, 80 pots were planted. Each pot had a matching partner of the same family,
123 yielding 40 pairs. Partner pots were placed adjacent to each other on greenhouse benches along a
124 north-south axis. Throughout the study, no supplemental light was provided.

125 Pots were watered daily with a fine mist until seedlings established, at which point they
126 were watered every-other day until the beginning of the experiment. Seedlings were treated
127 every 14 to 21 days with a (24-8-16) NPK fertilizer with micronutrients (B, Fe, Cu, Mn, Mo, Zn)
128 (Scotts Miracle Grow Products Inc, OH, USA). Fertilizer was applied by watering until water
129 drained from the pots using a fertilizer concentration of 0.66 ml l⁻¹.

130 All germinants were allowed to grow until July 2013, at which point the hardiest seedling
131 was selected and the others were either gently removed from the soil or clipped. Seedlings were

selected based on their height, the number of needles, and the coloration of their foliage. After thinning, steel welding rods were installed in the pots to measure time-domain reflectometry (TDR). Rods were 15 cm in length, had a diameter of 1.59 mm, and were inserted 3 cm apart from one another near the base of the remaining seedling. For each pair of pots, one pot was randomly selected for water-stress treatment. After the seedling thinning, but before the treatments began, two seedlings died. This left 78 total seedlings in the study and 38 pairs. All of the remaining seedlings survived until the end of the study. Just before the beginning of the first water-stress period, we measured total height (to the nearest mm) and ground line diameter (GLD; to the nearest 0.1 mm).

The seedlings were exposed to three episodes of water stress: the first from 4 September 2013 to 15 October 2013, the second from 18 October 2013 to 15 November 2013, and the third from 17 November 2013 to 8 January 2014. Between each water stress period, all pots were watered until water drained from the pots and then fertilized. No fertilizer was added during the dry down events. After the third dry down period, seedlings were harvested. In total, water-stress seedlings were exposed to 122 days of treatment.

During the first dry down period, drought treatment pots were allowed to reach 2% soil volumetric water content (VWC). Volumetric water content was measured every two or three days using TDR (Tektronix TDR Metallic Cable Tester, Model 1502B). In addition to measurements of TDR, pots were weighed to the nearest gram. When TDR measured less than 2% soil VWC, water-stress treatment seedlings were given 300 ml of water which returned them to approximately 14% VWC. After every addition of 300 ml of water, pots were not watered again until they once again reached 2% VWC. During this water-stress period, control pots were

watered every two or three days to bring them back to their initial weight. The VWC of the control pots was measured by TDR and ranged from 25-35%.

All pots were fully watered on 16 October 2013 to end the first dry down period. Two days later, on 18 October 2013, all pots were weighed. During the second water-stress period, TDR was not measured. Rather, the pot weight corresponding to 2% VWC for each seedling from the first dry down period was used as a trigger to add water (250 ml) to water-stress treatment pots. On 15 November 2013, all pots were watered, fertilized, and allowed to drain.

The third dry down period began on 17 November 2013. The weight of each pot was recorded as the starting weight, and a plastic bag was put around each pot to prevent evaporation of water from the soil surface such that the change in pot weight was considered to be solely transpiration. All pots were weighed every two days after sunset (around 19:00 CST). Average temperature during the third dry down period in the greenhouse was 10.0 °C with a standard deviation of 3.4 °C. Maximum and minimum temperatures were 23.5 and 0.5 °C, respectively.

The daily change in weight of water-stress treatment pots was compared to well-watered companion pots (same family located adjacent to one another) to calculate a ratio for the pair called the Normalized Transpiration Ratio (NTR), which was expressed as a fraction (Equation 1) (Jyostna Devi et al. 2009). Initial water use of partner trees was not the same as they varied in size, leaf area, and transpiration rate. In order to account for initial differences among partner pots, the first three days' water use for each pot was averaged and used in the denominator for calculation of NTR.

Equation 1: *Normalized Transpiration Rate (NTR)* =
$$\frac{\text{daily water stress use/daily well watered use}}{\text{first three day average of (water stress/well watered)}}$$

After the initial three days of the dry down period, well-watered pots were returned to their initial weights minus 100 g every-other-day. Water-stress treatment pots were given no water until their NTR reached 0.10. When a water-stress treatment pot reached 0.10 NTR, the soil was assumed to have no transpirable soil moisture left (Sinclair and Ludlow 1986). At this NTR, pots were periodically provided minimal water as described above based on pot weight corresponding to VWC of 2%.

The fraction of transpirable soil water (FTSW) was calculated using Equation 2 where the FTSW at NTR of 0.10 was assumed to be 0 (Jyostna Devi et al. 2009) (Equation 2).

Equation 2: *Fraction of Transpirable Soil Water (FTSW)* =
$$\frac{(\text{daily weight} - \text{final weight})}{(\text{initial weight} - \text{final weight})}$$

The NTR was plotted against FTSW for each water-stressed seedling and the points were fit into a segmented regression using SAS 9.3. During the early part of the dry down, the first line segment was fairly horizontal, as the NTR was fairly constant at high soil water content. During the later stages of the dry down, the second line segment sloped downward because the water-stressed treatment seedlings reduced their transpiration compared to the well-watered treatment trees. The point where the horizontal and diagonal line crossed was the ‘join’ and indicated the FTSW at which transpiration began to decline.

The FTSW corresponding to the ‘join’ was used as the experimental variable in a one-way ANOVA with species as the main effects (n=16 for hybrid pine, n=12 for loblolly pine, n=10 for shortleaf pine). The last pot reached an NTR of 0.10 on 8 January 2014.

Beginning on 21 January 2014, each seedling was harvested and separated into component parts: needle, branch, stem, coarse root, and fine root. Fine roots were classified as roots with a diameter less than or equal to 2.0 mm. Seedling pairs were harvested within the same hour. The components were placed into a paper bag and put in a drying oven at 60 °C. The last seedlings were harvested on 31 January 2014. After the bags reached a constant weight, their contents then weighed to the nearest 0.01 g.

Partitioning to different biomass components changes with seedling size (Bongarten and Teskey 1987; Ledig et al. 1970). For example, a larger tree may devote a larger proportion of biomass development to stem growth than a smaller tree. To correct for size differences in biomass partitioning, ANCOVA (Proc Mixed, SAS 9.3) with the main effects of species and watering treatment was used to compare the component values at the same total biomass thus allowing direct comparison among species at the same size (Ledig et al. 1970). The data were transformed using natural log to normalize the residuals. After the transformation, the components' LS means were directly compared if there was no difference in the slope of the relationship between the component biomass and total biomass.

Results

Both species ($p < 0.04$) and water treatment ($p < 0.001$) significantly affected ground line diameter (GLD) growth during the course of the water-stress treatments. The hybrid pine seedlings (5.26 ± 0.29 s.e. mm) and shortleaf pine seedlings (5.38 ± 0.39 s.e. mm) grew less in GLD than did loblolly pine (6.13 ± 0.39 s.e. mm). Water-stress treatment seedlings grew significantly less in GLD (4.28 ± 0.13 s.e. mm) than the well-watered seedlings (6.79 ± 0.24 s.e. mm). There was an interaction between treatment and species effects on height growth during

the course of the water-stress treatments ($p = 0.01$). Well-watered loblolly pine seedling height growth (38.8 ± 14.2 s.e. mm) was less than well-watered hybrid pine (78.2 ± 9.8 s.e. mm) and shortleaf pine seedlings (95.0 ± 16.9 s.e. mm). In contrast, water-stress treated loblolly pine seedlings (33.4 ± 10.3 s.e. mm) experienced similar height gains as water-stress treated hybrid pine (21.1 ± 4.4 mm) and shortleaf pine seedlings (28.1 ± 5.6 s.e. mm).

Because of these differences in growth, size at harvest also differed due to species ($p = 0.02$) and water treatment ($p < 0.0001$) for final GLD and a treatment x species interaction occurred ($p = 0.02$) for final height (Fig. 1). Loblolly pine seedlings (12.5 mm) had significantly greater final GLD than shortleaf pine (10.6 mm) while hybrid pines (11.5 mm) were statistically similar to both parent species. The water-stress treatment had a smaller final GLD (10.1 mm) than the well-watered treatment (12.9 mm). The interaction for height between species and treatment occurred because shortleaf pine and hybrid pine seedling heights were negatively affected by the water-stress treatment, but loblolly pine seedling heights were not (Fig. 1). Water-stress treatment shortleaf pine seedlings were 36% shorter than their well-watered siblings ($p < 0.0001$) and hybrid pine seedling height was 13% lower ($p = 0.05$). In contrast, loblolly pine seedling height was not significantly diminished by water stress (5% reduction) ($p = 0.46$).

Loblolly pine and hybrid pine seedlings had more biomass at the end of the experiment than shortleaf pine seedlings for total biomass ($p = 0.02$), fine root biomass ($p = 0.03$), stem biomass ($p = 0.008$), and foliage biomass ($p = 0.03$). Total biomass for loblolly (51.7 g) and hybrid pines (51.6 g) were both larger than for shortleaf pine (40.3 g) (Table 1). Fine root biomass was greater in loblolly (16.0 g) and hybrid (14.9 g) pines compared to shortleaf (12.1 g) pine. Stem biomass was greater in loblolly (8.5 g) and hybrid (6.9 g) pines than in shortleaf (4.5 g) pine. Foliage biomass was greater in loblolly (17.8 g) and hybrid (18.8 g) pines than in

shortleaf (12.6 g) pine. In contrast, shortleaf pine (9.4 g) had greater coarse root biomass than loblolly pine (7.3 g) ($p = 0.05$) while hybrid pine (8.5 g) had intermediate values that were statistically similar to both parent species. Branch biomass did not differ among species ($p = 0.08$), however species and treatment exhibited a significant interaction ($p = 0.009$) because water stress seedlings had less branch biomass for hybrid pine ($p < 0.0001$) and shortleaf pine ($p < 0.0001$), but not loblolly pine ($p = 0.19$). Water-stress seedlings had less total biomass (35% less), fine root biomass (34% less), coarse root biomass (48% less), stem biomass (45% less), and foliage biomass (24% less) compared to the well-watered treatment seedlings ($p < 0.0001$) (Table 1).

When measured at final harvest, the slope of relationship between $\ln(\text{total biomass})$ and $\ln(\text{fine root})$ did not statistically differ among species or water treatments (Table 2; Fig. 2a). When the amount of fine root per total biomass was compared to the mean total biomass (LSMean), differences for fine root biomass were not statistically different between species or water treatments (Table 3; Table 4) indicating that partitioning to fine root was similar among seedlings in the study.

For coarse root biomass, the slope of the relationship between $\ln(\text{total biomass})$ and $\ln(\text{coarse root})$ did not statistically differ among species or treatments (Table 2; Fig. 2b, c). Both treatment and species influenced coarse root biomass partitioning as indicated by differences in LSMeans (Table 3; Table 4). Shortleaf pine had significantly more coarse root biomass partitioning than hybrid pine (13% greater for shortleaf) and loblolly pine (21% greater for shortleaf) and hybrid pine had significantly more coarse root biomass partitioning than loblolly pine (8% greater for hybrid). Partitioning to coarse root was greater for the well-watered treatment seedlings than for the water-stressed treatment seedlings (12% greater).

For stem biomass, the slope of the relationship between $\ln(\text{total biomass})$ and $\ln(\text{stem})$ differed significantly among species (Table 2). At low total biomass, loblolly pine seedlings had greater stem biomass. However, this partitioning difference decreased when comparing the larger seedlings across the three species (Table 5; Fig. 2d). The slope of the relationship for shortleaf pine seedlings (1.36) was statistically similar to hybrid pine (1.45). The slope for shortleaf and hybrid pines were both greater than for loblolly pine seedlings (1.11) (Table 5). The slopes for the different watering treatments did not statistically differ when comparing the relationship between $\ln(\text{total})$ biomass and $\ln(\text{stem})$.

The slope of the relationship between $\ln(\text{total biomass})$ and $\ln(\text{branch})$ did not statistically differ among species or treatments (Table 2; Fig. 2e). There was a significant interaction between species and treatment when comparing LSMeans (Table 3). The interaction occurred because loblolly pine had larger branch biomass for the water-stress seedlings (18% increase) compared to the well-watered seedlings ($p = 0.01$) while shortleaf ($p = 0.05$) and hybrid pine ($p = 0.05$) seedlings had 5% and 10% less, respectively, in branch biomass for the water-stress seedlings (Table 4).

The slope of $\ln(\text{total biomass})$ and $\ln(\text{foliage})$ did not statistically differ between species or treatments (Table 2; Fig. 2f). Water-stress treatment significantly increased partitioning to foliage by 5% (Table 3; Table 4). Partitioning among species did not statistically differ.

During the third water-stress period, the normalized transpiration rate (NTR) was initially stable as the fraction of transpirable soil water (FTSW) declined. When the seedlings began to limit transpiration in response to soil drying, NTR declined linearly. Figure 3 portrays a representative relationship between NTR and FTSW. Loblolly pine seedlings began to limit transpiration at a FTSW of 0.73 (s.e.=0.02), hybrid pine at FTSW of 0.70 (s.e.=0.02), and

shortleaf pine at FTSW of 0.77 (s.e.=0.01); these differences were not significantly different ($p = 0.15$) from one another, indicating seedling for the three species began to reduce transpiration at approximately the same soil moisture content.

Discussion

There were not differences among loblolly pine, shortleaf pine, and the hybrid pine in the response to water stress for traits associated with water relations. Transpiration of all three species decreased at approximately the same soil moisture and there were not differences among species in partitioning to organs associated with water uptake (fine roots) or water use (leaves). There may exist differences among the species related to drought survival. However, we did not impose water stress severe enough to kill the seedlings. Shortleaf pine had more coarse root than loblolly pine with the hybrids intermediate. Assuming coarse root biomass is proportional to carbohydrate storage, additional coarse root development could be associated with greater ability to withstand or recover from drought for shortleaf and the hybrid pines than for loblolly pine. If so, this may help facilitate the spread of hybrid pines to areas that might otherwise be too xeric for pure loblolly pine. Alternatively, the perception of differences in drought tolerance between loblolly and shortleaf pine might be overstated and confounded with previous fire history as drier, more xeric sites were more likely to burn which favors shortleaf pine (Stewart et al. 2015).

The reduction in growth due to water stress we measured was consistent with previous research on loblolly pine seedlings (e.g. Teskey et al. 1987; Torreano and Morris 1998). The water-stress treatments did not start until early September, after much of the seedling height growth and foliage development had been completed. The water-stressed and well-watered seedlings gained 8.5% and 16.5% of their final height during the experimental period. Even so,

water stress reduced height growth in shortleaf and hybrid pines. In contrast to height growth, GLD gained 47% of its final size across all treatments during the experimental period and water stress reduced GLD growth for loblolly, shortleaf, and hybrid pine seedlings. Greater size of loblolly pine than shortleaf pine (Williston 1972; Dipesh et al. 2015), with the hybrids more similar to loblolly pine (Lilly et al. 2012b; Will et al. 2013a), was expected. In the current study, the hybrid pine seedling growth response was intermediate between the two parent species for height and diameter and similar to loblolly pine for fine root, stem, foliage, and total biomass accumulation. The greater size of shortleaf x loblolly pine hybrids than shortleaf pine regardless of water treatment may lead to greater competitiveness of hybrid pines over shortleaf pine on a broad range of sites.

The similarity in fine root partitioning among species and treatments was unexpected. Because of the role of fine roots in water uptake, we hypothesized that shortleaf pine, which is presumably more drought tolerant than loblolly pine (Lawson 1990), would have proportionately more fine root biomass than loblolly pine or the shortleaf x loblolly pine hybrids. A previous study comparing loblolly and shortleaf pine seedling growth found no significant differences in aboveground biomass or size, but that shortleaf pine had greater belowground biomass (Zak 1961). However, this study did not differentiate fine roots from coarse roots. If we had combined coarse and fine roots, partitioning to root for shortleaf pine would have been greater than for loblolly pine, with hybrids intermediate.

We had expected the water-stressed seedlings to have proportionally more fine root biomass (Rodrigues et al. 1995), but did not find any differences in partitioning. Previous studies finding water-stressed loblolly pine increased partitioning to root biomass did not separate fine and coarse roots (Bongarten and Teskey 1987; Barnes 2002). Specifically related to

fine roots, there is evidence that acute drought does not increase biomass allocation to roots in southern pine seedlings (Hallgren et al. 1991; Torreano and Morris 1998). A possible explanation for the similarity in fine root biomass partitioning between the well-watered and water-stressed seedlings could be related to differences in fine root turnover or pot confinement. However, Hallgren et al. (1991) found no evidence that moisture stress altered fine root mortality in loblolly pine seedlings and our pot sizes (6.59 liter) were relatively large compared to our seedlings and should not have interfered with root biomass growth.

Partitioning to coarse root exhibited differences between species as we hypothesized (shortleaf > hybrid > loblolly). Shortleaf pine is a species that tolerates fire and is presumably more drought tolerant than loblolly pine (Lawson 1990). Greater partitioning to coarse root biomass of shortleaf pine (and the hybrids) could result in greater non-structural carbohydrate reserves because coarse roots store the largest pool of carbohydrate in southern pine seedlings (Ludovici et al. 2002). Carbohydrate reserves are linked to drought survival (Galiano et al. 2011; McDowell et al. 2011). The greater potential reserve of carbohydrate in shortleaf pine could provide it resources for resprouting following topkill from fire or a greater ability to withstand reduced photosynthesis during drought. Loblolly pine's lower partitioning to coarse root suggests that loblolly pine devotes more of its carbon resources to other biomass components and less to storage. Hybrid pine's intermediate coarse root partitioning likely makes it better suited to tolerate drought than loblolly pine.

Given the general expectation of greater partitioning belowground with water stress (e.g., Bongarten and Teskey 1987; Barnes 2002), lower coarse root biomass partitioning in the water-stressed treatment compared to the well-watered treatment was surprising. The lower partitioning to coarse root in the water-stress treatment was probably a response to the lower photosynthetic

activity. Hartmann et al. (2013) reported a significant reduction in nonstructural carbohydrates (NSC) in belowground tissue and suggested that root biomass may have decreased during drought. Using ^{14}C as a tracer, Kuhns and Gjerstad (1988) found that loblolly pine seedlings exposed to water stress allocated little newly fixed carbon to their root system.

Loblolly pine partitioned more biomass to stem than shortleaf or hybrid pine when compared at similar seedlings size, which indicates that loblolly pine may invest more resources to outgrow competition aboveground during the first year. While this difference in stem biomass partitioning declined in larger seedlings, it could be an important feature of a young seedling's development. The stem stores less NSC than fine and coarse roots in southern pine (Gholz and Cropper Jr 1991). Therefore, the shift in partitioning to stem from coarse root in loblolly pine might decrease the ability of loblolly pine seedlings to withstand drought as compared to shortleaf and the hybrid pines. Biomass partitioning to stem has been shown to decrease under water-stressed conditions (Ledig et al. 1970). However, we found no effects of drought on partitioning to stem in our study.

Partitioning to foliage increased in water-stress treatments which was likely an effect of decreased coarse root biomass in water-stressed seedlings. As partitioning is zero-sum, if coarse root biomass decreases, biomass in another component must increase on a relative basis and the foliage component was probably least affected by water stress. Late season drought does not affect needle biomass with the same intensity as early season drought (Dougherty et al. 1994). If the experiment were conducted during the early part of the growing season, effects of drought on foliage development may have been greater and partitioning to plant components may have differed.

Loblolly, shortleaf, and hybrid pines stopped transpiring at the same soil moisture level, which does not support a hypothesis that shortleaf pine or hybrid pines are either: A) more isohydric, i.e., stops transpiring earlier to conserve water, or B) more anisohydric, i.e., continues to transpire (and gain carbon) to a lower soil moisture than loblolly pine. Schopmeyer (1939) came to a similar conclusion when comparing loblolly and shortleaf pine transpiration rates across well-watered and water-stressed treatments. While we found no differences, other species do show genetic differences in FTSW values at which transpiration declines. For instance, Jyostna Devi et al. (2009) described wide variation in critical soil moisture when testing peanut varieties (*Arachis hypogaea* L.) and Sinclair and Ludlow (1986) were able to compare the water balance of soybean (*Glycine max*), cowpea (*Vigna uniuiculata*), black gram (*Vigna mungo*), and pigeon pea (*Cajanus cajan*) using the FTSW method.

Like most other characteristics of the shortleaf x loblolly hybrid pine, biomass partitioning was generally intermediate between the two parent species. This is similar to many other physical traits, such as the intermediate basal crook, needle length, male strobili length, needle sheath length, needles per fascicle, cone size, bud size, etc. (Schultz 1997; Lilly et al. 2012b). The greater size of the hybrid pines than shortleaf pine likely provides a competitive advantage under optimal conditions. We found coarse root partitioning for the hybrids intermediate between shortleaf and loblolly pine that might make the hybrids more competitive than loblolly pine on xeric sites and facilitate its persistence on sites traditionally dominated by shortleaf pine. If so, the use of prescribed fire to eliminate the hybrids (Stewart et al. 2015; Bradley et al. 2016) is critical to conserve shortleaf pine.

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Table 1. Arithmetic mean biomass (g) of each seedling component for shortleaf, shortleaf x loblolly hybrid, and loblolly pine seedlings grown under well-watered and water-stressed conditions (s.e. in parentheses).

	Fine Root	Coarse Root	Stem	Branch	Foliage	Total
Shortleaf Stressed	9.15 (1.10)	5.83 (0.69)	2.64 (0.38)	0.91 (0.16)	8.82 (1.07)	27.35 (2.81)
Shortleaf Watered	14.96 (1.64)	13.01 (0.82)	6.32 (0.67)	2.54 (0.20)	16.39 (1.12)	53.22 (2.55)
Hybrid Stressed	12.33 (0.70)	6.28 (0.42)	5.08 (0.48)	1.80 (0.19)	17.22 (1.49)	42.71 (2.52)
Hybrid Watered	17.50 (1.30)	10.64 (0.73)	8.77 (0.79)	3.11 (0.19)	20.40 (1.08)	60.42 (3.50)
Loblolly Stressed	12.70 (0.72)	5.04 (0.29)	6.31 (0.36)	1.90 (0.25)	16.64 (1.18)	42.59 (2.10)
Loblolly Watered	19.33 (2.00)	9.48 (0.83)	10.63 (1.35)	2.27 (0.33)	19.06 (1.67)	60.77 (5.07)

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Table 2. The p values of the Type 3 test of Fixed Effects and slope differences for the relationship between $\ln(\text{biomass component})$ and $\ln(\text{total biomass})$. If p values of the interaction terms (slopes) were > 0.05 , a simplified model (Table 3) was used to evaluate significance of species and treatments using LSMeans.

Effect	Fine Root	Coarse Root	Stem	Branch	Foliage
Species	0.96	0.37	0.02	0.10	0.74
$\ln(\text{total})$	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001
$\ln(\text{total}) * \text{Species}$	0.96	0.63	0.02	0.11	0.88
Treatment	0.38	0.98	0.08	0.82	0.27
$\ln(\text{total}) * \text{Treatment}$	0.37	0.72	0.07	0.85	0.15
$\text{Species} * \text{Treatment}$	0.23	0.16	0.22	0.32	0.21
$\ln(\text{tot}) * \text{Species} * \text{Treat}$	0.25	0.16	0.21	0.27	0.21

Table 3. The *p* values of the model without slopes from the test of ln(biomass component) and ln(total biomass). Stem was not tested because of the slope differences among species for the relationship between ln(stem) and ln(total) was significant.

Effect	Fine Root	Coarse Root	Stem	Branch	Foliage
Species	0.55	≤0.0001	NA	0.13	0.06
ln(total)	≤0.0001	≤0.0001	NA	≤0.0001	≤0.0001
Treatment	0.62	≤0.0001	NA	0.99	≤0.0001
Species*Treat	0.28	0.48	NA	<0.01	0.09

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Table 4. LSMeans estimates of the natural log transformed data ($\ln(\text{grams})$) for each species and treatment combination.

	Fine Root	Coarse Root	Stem	Branch*	Foliage
Shortleaf Stressed	2.63	2.20	1.65	1.07	2.71
Shortleaf Watered	2.57	2.43	1.55	1.13	2.62
Hybrid Stressed	2.57	1.88	1.69	1.07	2.89
Hybrid Watered	2.61	2.11	1.77	1.19	2.72
Loblolly Stressed	2.60	1.68	1.86	1.10	2.88
Loblolly Watered	2.71	1.99	2.00	0.92	2.66

*branch weights were sometimes less than 1.0 g, means of Branch were calculated as $\ln(1+\text{branch})$

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Table 5. Regression equations predicting ln(stem biomass) from ln(total biomass). Loblolly pine's slope was less steep than shortleaf and hybrid pines. Stem biomass at 1.5 and 1.8 ln(total biomass) were calculated for comparison.

	Regression Equation	r^2	Stem biomass at 1.5 g total	Stem biomass at 1.8 g total
Shortleaf	$\hat{y} = -1.56 + 1.36x$	0.93	0.48	0.89
Hybrid	$\hat{y} = -1.67 + 1.46x$	0.81	0.51	0.95
Loblolly	$\hat{y} = -0.98 + 1.11x$	0.66	0.68	1.01

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Figure Captions

Fig. 1. Heights and ground line diameters of one-year-old, loblolly, shortleaf x loblolly hybrid, and shortleaf pine seedlings growing under well-watered or water-stressed conditions. Vertical bars are standard error.

Fig. 2. Relationship between $\ln(\text{total seedling biomass})$ and a) $\ln(\text{fine roots})$, b) and c) $\ln(\text{coarse roots})$, d) $\ln(\text{stem})$, e) $\ln(\text{branches})$, and f) $\ln(\text{foliage})$ for well-watered and water-stressed seedlings of shortleaf, hybrid, and loblolly pine seedlings. Multiple lines are displayed when there is a significant difference related to either watering treatment or species.

Fig. 3. Relative transpiration rates plotted against soil moisture (Normalized transpiration rate vs. Fraction of transpirable water). This is a representative graph from a loblolly pine seedling.

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