PHYSIOLOGICAL ATTRIBUTES ASSOCIATED WITH DROUGHT RESISTANCE AND PRODUCTIVITY IN LOBLOLLY PINE

by

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(Under the Direction of Robert O. Teskey)

ABSTRACT

Clonal forestry allows for selection of trees and traits that may increase productivity in different environmental conditions. The first part of this research project utilized ramets from three half-sibling loblolly pine (*Pinus taeda* L.) clones to evaluate intraspecific responses to moisture stress. The second part used ramets from a single clone to better understand how four needle fascicles differed physiologically and morphologically from three needle fascicles. In the first set of experiments, we subjected the clones to three soil moisture treatments: low water (-1.5 MPa), high water (-0.3 MPa), and an alternating water treatment of cyclical low and high water conditions. Traits associated with moisture stress and productivity such as needle level gas exchange, carbon allocation and carbon isotope discrimination were measured. A rank order change in biomass accumulation was observed among the clones between the low and high soil moisture treatments. One clone, relative to the other two clones, had more biomass accumulation in the low soil moisture treatment, but less biomass accumulation in the high soil moisture treatments. The two clones that had the most biomass accumulation under high soil moisture conditions had greater below ground carbon allocation and higher rates of morning gas exchange under the high soil moisture conditions. Carbon isotope discrimination, an index for water use efficiency, was higher in the clone that had the most biomass accumulation under low water conditions. The clone that had the most biomass accumulation under the low water conditions also had an abnormally high abundance of four-needle fascicles, which was the subject of the second set of experiments. Four-needle fascicles, compared to three needle fascicles, had equal diameter, volume and specific leaf area as well as similar anatomy, yet needle tissue density and fascicle surface area were greater. The changes in fascicle anatomy did not affect most of the physiological gas exchange parameters measured with A/Ci and light response curves. The maximum rate of RuBisCo-mediated carboxylation was, however, significantly higher in the four-needle fascicles. This higher rate of carboxylation may have accounted for the greater amount of biomass accumulation observed in this clone in the low water treatment.

INDEX WORDS: Loblolly pine, water use efficiency, photosynthesis, transpiration, biomass accumulation, needle number, tissue density, ramets

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DEDICATION

I dedicate my dissertation to my wife, Laura Ingwers, whose love, support, and untold sacrifices have made it possible for me to finish my PhD.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Literature Review

Loblolly pine background and importance

Loblolly pine (*Pinus taeda* L.) is a species of major economic and ecological importance in the Southeastern USA and abroad. An estimated 13.4 million hectares of loblolly pine are planted in the southeastern USA alone (Schultz, 1997). Presently, 50% of all global wood production is provided by plantation forests (Miller et al., 2009), half of which are located in the Southeastern USA (Fox et al., 2007). Among the approximately 1.2 billion seedlings annually planted in the Southeastern USA, 80% are loblolly pine (Li et al., 1999). A combination of selective tree breeding and silvicultural techniques has dramatically increased loblolly pine plantation productivity (Wear and Greis, 2002). In the past half century, mean annual increment (an index of tree growth) has more than doubled, while rotation time (the amount of time between planting and harvesting) has been nearly halved (Fox et al., 2004). Selective tree improvement has resulted in straighter, faster growing and more disease resistant loblolly pine (Li et al., 1999). Improved trees from second generation seed orchards alone have a 12 to 20% gain in volume (Li et al., 1999). The genetic gain from loblolly pine tree improvement has an estimated value of $2.5 billion USD (McKeand, 2015). Ninety-five percent of all loblolly pine seedlings are genetically improved, and full-sib families, half-sib families and clonal varieties are widely planted across the Southeastern USA (McKeand et al., 2003).

Climate change, drought and future tree improvement

Future tree improvement efforts will need to focus on the effects of higher temperatures, greater atmospheric CO_2 concentration and an increased frequency and severity of drought due to global climate change. Multiple climate change models predict that there will be drier conditions in the future for much of North America, including the southeastern USA (Cook et al., 2015). Loblolly pine growth is reduced in moisture-limiting conditions (Wertin et al., 2012; Bauweraerts et al., 2014), therefore, the predicted drier climates will likely have a deleterious effect on loblolly pine productivity. To help tree improvement efforts, a better understanding of intraspecific variation in drought resistance and the physiological traits associated with drought resistance is needed.

Genetic gain in loblolly pine plantations has already increased from the use of clonal material (McKeand et al., 2003; Bettinger et al., 2009). There is no genetic variation among ramets within a clone and, therefore, there is greater uniformity in stands planted with a single clone. That uniformity may be a key reason for the increased planation productivity when using clones. The potential genetic gain of practicing clonal forestry was demonstrated in a field study conducted on 450 clones of different families at two sites, which found that the elite clones, compared to unimproved seedlings, had a 52% greater volume yield (Isik et al., 2005). Likewise, a study comparing clones to seedlings from the same full sibling families found that there is a greater amount of uniformity of traits within clones (Isik et al., 2003). The uniformity of traits within a specific clone can allow for the selection and creation of clonal varieties with specific desired traits, for example, fusiform rust resistance s (McKeand et al., 2003; Isik et al., 2005). The main limitation to the implementation of clonal forestry in loblolly pine is the cost of young bareroot ramets, which is about \$0.30-\$0.40 (USA dollars) each, which is substantially more expensive than a bareroot seedling, which only costs about \$0.05 (Bettinger et al., 2009). However, decreasing costs of ramet production coupled with increased genetic gain will make clonal forestry more viable and lucrative in the future (Bettinger et al., 2009).

Productivity of loblolly pine may be increased in the future by selective deployment of particular clones or genotypes to specific environments. A genetic by environment interaction (G x E) occurs when a specific clone or genotype performs better in one environment compared to another. Currently it is unclear if G x E is an important factor in improving loblolly pine productivity. A field study of 1200 different clones across six different sites in Georgia and Florida (USA) found very few G x E interactions among clones (Baltunis et al., 2006). In a review, McKeand et al. (2006) concluded that while G x E interactions existed among loblolly genotypes at different locations, soil types, and silvicultural treatments, they are not of practical concern as highly productive families and genotypes maintained their productivity across different environments and treatments. Still, G x E interactions have been found in multiple studies. Specifically, G x E interactions have been found in response to silvicultural treatments at different sites within the southeastern USA (Roth et al., 2007). Genetic by environment interactions have also been shown to be more pronounced among clones than among families, likely due to an increase in uniformity of traits within clones. Clonal studies with different fertilization regimes demonstrated that G x E interactions affected leaf area (Tyree et al., 2009), biomass accumulation (King et al., 2008; Stovall et al., 2013), gas exchange (King et al., 2008), and carbon allocation (Stovall et al., 2013). While G x E interactions have been studied among loblolly pine clones at different locations within the loblolly pine range, the effects of drought have not been specifically investigated in loblolly pine clones.

Growth as a function of physiology

Biomass growth, which is reduced under drought conditions, is a function of photosynthesis per unit leaf area, respiration per unit photosynthetic area, leaf area and the rate of respiration per unit of non-photosynthetic area (Teskey et al., 1987a). Some factors that affect these attributes are strongly correlated with growth; for example, a meta-analysis of numerous loblolly pine stands found that productivity (growth) is strongly correlated with needle biomass (Teskey et al., 1987a). When seedlings from six different geographic origins were compared, differences in transpiration and leaf conductance were found between coastal and interior provenances (Bongarten and Teskey, 1986). Differences in growth and gas exchange have also been observed among the same provenances (Boltz et al., 1986), demonstrating considerable genetic and physiological variation within the species. Comparisons of loblolly pine half-sib families have further demonstrated that transpiration can vary even among closely related individuals (Seiler and Johnson, 1988). Multiple physiological and morphological traits in loblolly pine are linked to productivity and biomass accumulation. A set of related studies using five loblolly pine families reported that needle biomass, and its distribution and longevity, as well as leaf area index and photosynthetically active radiation interception were all correlated with genetic differences in aboveground growth

(McCrady and Jokela, 1996; 1998). In the private sector, trials on 120 different clones by MeadWestvaco Corporation demonstrated correlations between stem wood growth and several traits including needle nitrogen content, root vs. shoot carbon allocation, crown area, branch size and frequency, internode length, and the ratio of biomass to crown area, needle area, and nitrogen content (Martin et al., 2005). However, in that study, stem growth did not correlate well with net assimilation. While net assimilation is clearly a fundamental component of biomass accumulation, measurements are taken at a single point in time and, therefore, lack time-integration. As net assimilation varies throughout the day and in response to numerous environmental conditions, thus it is not surprising that these measurements did not correlate as well with growth rate as more time-integrative measures such as leaf area and crown attributes. More information and data are needed to understand the complex relationships between physiology, morphology, and growth.

Variation in growth

Genetic variation in biomass accumulation has been observed in loblolly pine. In an early study, 15 loblolly pine sources, representing populations across the native loblolly pine range, were grown at multiple localities throughout the southeastern USA and were analyzed after 10 years of growth (Wells and Wakeley, 1966) and 25 years of growth (Wells and Lambeth, 1983; Wells, 1983). The first measurements, at age 10, demonstrated that, trees from coastal populations grew faster than the majority of populations from elsewhere in the region, except at the coldest trial sites, while trees from inland sources had higher rates of survival (Wells and Wakeley, 1966). These results were corroborated by the study at age 25, which reported that the individuals from the coastal populations continued to grow faster than their counterparts from more interior provenances, but were more susceptible to drought and less disease resistant (Wells, 1983). In southern Arkansas, an inland region, after 25 years of growth, trees from coastal provenances were, on average, five to eight feet taller than trees from provenances native to the area (Wells and Lambeth, 1983). Comparisons of clonal material from multiple provenances in several common garden experiments indicated that clones from some regions were more adaptable to a broad range of climates than individuals from other regions (McKeand et al., 1989). A common garden experiment that compared growth rates of bulk seed lots, open-pollinated families, full-sib families, and clones at different locations and with different silvicultural inputs demonstrated that some clones and families tended to be consistently more productive than others (McKeand et al., 2006). Also in a common garden study, differences were found in growth traits among full-sibling clones within the same family (Paul et al., 1997). Intraspecific differences in growth rates and productivity were again demonstrated in a study with 1200 clones and 1400 seedlings from 61 half-sibling families (Baltunis et al., 2006). Different families of loblolly pine exhibit different growth responses to fertilization (King et al., 2008; Stovall et al., 2013) with differences detected among clones within the same family (King et al., 2008).

Moisture stress and genetic variation in drought resistance

In response to moisture stress, loblolly pine is known to reduce photosynthesis, transpiration, stomatal conductance, and tissue water potential (Seiler and Johnson, 1985; Bongarten and Teskey, 1986; Seiler and Johnson, 1988; Wertin et al., 2012; Bauweraerts et al., 2014). Lower rates of transpiration allow loblolly pine to avoid moisture stress by minimizing water loss. Loblolly pine can further tolerate moisture stress via osmotic adjustment (Seiler and Johnson, 1985; Bongarten and Teskey, 1986; Meier et al., 1992). Loblolly pine may also respond to moisture stress by altering its root/shoot carbon allocation ratio; however, the relationship between moisture availability and root/shoot ratios is not consistent in the literature. For example, Seiler and Johnson (1988) found that moisture stress reduced root/shoot ratio of loblolly pine seedlings. In contrast, in an irrigation field study, the ratio of woody root tissue to woody shoot tissue decreased in loblolly pine trees under higher soil moisture (Samuelson et al., 2004). However, another loblolly pine field study found that belowground carbon allocation did not change in response to irrigation (Coyle et al., 2008).

Moisture availability varies across the native range of loblolly pine, which extends from Delaware in the north to Florida in the south and East Texas in the west (Schultz, 1997), resulting in different levels of drought resistance among loblolly pine trees from different regions (Eckert et al., 2010). The “Lost Pines” is a genetically unique loblolly pine population (Al-Rabab’ah and Williams, 2004) in a region of east Texas that is much drier than other areas in the native loblolly pine range (Bilan et al., 1977). When compared to their counterparts from wetter regions in common garden experiments, the “Lost Pines” had less drought induced mortality (Zobel and Goddard, 1955; Goddard and Brown, 1959). A separate study comparing the “Lost Pines” to their counterparts from wetter regions found that the “Lost Pines” had a lower needle moisture content, greater stomatal closure and lower transpiration (Bilan et al., 1977). Compared to loblolly pine populations in different regions, the “Lost Pine” population has deeper roots, possibly to allow better access to water (Van Buijtenen et al., 1976), thicker needle cuticles and lower stomatal densities in cotyledons (Knauf and Bilan, 1977) and seedlings (Knauf and Bilan, 1974), as well as more hypodermal cells and a greater cross-sectional needle area (Thames, 1963), all of which may be adaptations to help prevent water loss. However, growth rates of individuals from the “Lost Pines” population were lower than individuals from outside the “Lost Pines” population in a common garden experiment (McKeand et al., 2000), possibly indicating a fundamental tradeoff between growth and drought resistance.

A genetic analysis across loblolly pine populations further determined that there is variation in five loci that are correlated with soil aridity and drought (Eckert et al., 2010). These results indicate that there are genetic influences on the physiological processes that could affect drought resistance in loblolly pine. Variation in loblolly pine genes associated with drought response has been further characterized via association mapping (González-Martínez et al., 2006). Water relations among loblolly pine populations are also known to vary. For example, when loblolly seedlings from diverse geographic origins were compared, differences were found in leaf conductance and osmotic potential (Bongarten and Teskey, 1986; Teskey et al., 1987b). In addition, in that same study, average net photosynthesis varied across the duration of the experiment (Boltz et al., 1986). Likewise, when seedlings from seven diverse geographic origins were compared under high and low soil moisture conditions, the above and below ground carbon allocation differed (Bongarten and Teskey, 1987). Intraspecific variation in drought resistance and water relations has also been observed in other tree species. For example, a growth chamber study comparing three Ponderosa pine (*Pinus ponderosa*) populations from environments of low, moderate and high moisture found that individuals from the low moisture environment were better able to avoid drought by more rapidly closing their stomata relative to individuals from other populations (Zhang et al., 1997). A similar study on Scots pine (*Pinus sylvestris* L.) from 12 different diverse geographic regions under a cyclical drought treatment also found differences in water use efficiency, below ground allocation and biomass accumulation among populations (Cregg and Zhang, 2001). Furthermore, differences in water use efficiency have been found among populations of Douglas-fir (Aitken et al., 1995) and rose gum (*Eucalyptus grandis*) clones (Osorio and Pereira, 1994). A better understanding of how genetic variation affects physiological traits associated with drought resistance and water relations is needed to further improve loblolly pine breeding efforts.

Chapter Overview

Chapter 2 explores intraspecific variation in biomass accumulation and whole tree physiology among three loblolly pine half-sibling clones under different soil moisture regimes. Ramets from each clone were exposed to either a high soil moisture treatment (-0.3 MPa), a low soil moisture treatment (-1.5 MPa), or a third treatment that alternated between the low and high soil moisture levels. Growth and physiological characteristics, including leaf gas exchange, shoot water potential and $\delta^{13}C$, were compared among the clones. The objective of Chapter 2 was to determine how some of the physiological and morphological traits affecting drought resistance and productivity varied among closely related clones exposed to different water availability treatments. We hypothesized that: 1) compared to the high soil moisture treatment, the response to the low soil moisture treatment would result in a genetic by environment (G x E) interaction in which some clones, relative to others, could have higher productivity in one moisture regime and lower productivity in another; 2) the rank order of biomass accumulation would differ in the wetting-drying treatment relative to the constant moisture treatments, and 3) clones would differ in water use efficiency, gas exchange, and carbon allocation reflecting different strategies to maximize drought resistance and productivity. Chapter 3 focuses on the physiology of four-needle fascicles, compared to three-needle fascicles, in order to determine if this difference in morphology affects needle level physiology and/or productivity/biomass accumulation. We hypothesized that four-needle fascicles, compared with three-needle fascicles, would differ in mass, surface area and tissue density and that these differences would affect net assimilation, stomatal conductance, and transpiration. Chapter 4 is a synthesis of Chapters 2 and 3.

References

Aitken, S.N., Kavanagh, K.L., and Yoder, B.J. (1995). Genetic variation in seedling water-use efficiency as estimated by carbon isotope ratios and its relationship to sapling growth in Douglas-fir. *Forest Genetics* 2**,** 199-206.

Al-Rabab'ah, M.A., and Williams, C.G. (2004). An ancient bottleneck in the Lost Pines of central Texas. *Molecular Ecology* 13**,** 1075-1084.

Baltunis, B.S., Huber, D.A., White, T.L., Goldfarb, B., and Stelzer, H.E. (2006). Genetic analysis of early field growth of loblolly pine clones and seedlings from the same full-sib families. *Canadian Journal of Forest Research* 37**,** 195-205.

Bauweraerts, I., Ameye, M., Wertin, T.M., McGuire, M.A., Teskey, R.O., and Steppe, K. (2014). Water availability is the decisive factor for the growth of two tree species in the occurrence of consecutive heat waves. *Agricultural and Forest Meteorology* 189**,** 19-29.

Bettinger, P., Clutter, M., Siry, J., Kane, M., and Pait, J. (2009). Broad implications of southern United States pine clonal forestry on planning and management of forests. *International Forestry Review* 11**,** 331-345.

Bilan, M.V., Hogan, C.T., and Carter, H.B. (1977). Stomatal opening, transpiration, and needle moisture in loblolly pine seedlings from two Texas seed sources. *Forest Science* 23**,** 457-462.

Boltz, B.A., Bongarten, B.C., and Teskey, R.O. (1986). Seasonal patterns of net photosynthesis of loblolly pine from diverse origins. *Canadian Journal of Forest Research* 16**,** 1063-1068.

Bongarten, B.C., and Teskey, R.O. (1986). Water relations of loblolly pine seedlings from diverse geographic origins. *Tree Physiology* 1**,** 265-276.

Bongarten, B.C., and Teskey, R.O. (1987). Dry weight partitioning and its relationship to productivity in loblolly pine seedlings from seven sources. *Forest Science* 33**,** 255-267.

Cook, B.I., Ault, T.R., and Smerdon, J.E. (2015). Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Science Advances* 1**,** e1400082.

Coyle, D.R., Coleman, M.D., and Aubrey, D.P. (2008). Above-and below-ground biomass accumulation, production, and distribution of sweetgum and loblolly pine grown with irrigation and fertilization. *Canadian Journal of Forest Research* 38**,** 1335-1348.

Cregg, B., and Zhang, J. (2001). Physiology and morphology of *Pinus sylvestris* seedlings from diverse sources under cyclic drought stress. *Forest Ecology and Management* 154**,** 131-139.

Eckert, A.J., van Heerwaarden, J., Wegrzyn, J.L., Nelson, C.D., Ross-Ibarra, J., González-Martínez, S.C., and Neale, D.B. (2010). Patterns of population structure and environmental associations to aridity across the range of loblolly pine (*Pinus taeda* L., Pinaceae). *Genetics* 185**,** 969-982.

Fox, T.R., Allen, H.L., Albaugh, T.J., Rubilar, R., and Carlson, C.A. (2007). Tree nutrition and forest fertilization of pine plantations in the southern United States. *Southern Journal of Applied Forestry* 31**,** 5-11.

Fox, T.R., Jokela, E.J., and Allen, H.L. (2004). The evolution of pine plantation silviculture in the southern United States. *In* Southern forest science: Past, present, future. USDA Forest Service General Technical Report. SRS-75.

Goddard, R.E., and Brown, C.L. (1959). Growth of Drought Resistant Loblolly Pines. Texas Forest Service Research Note 23. Texas Forest Service, College Station, Texas.

González-Martínez, S.C., Ersoz, E., Brown, G.R., Wheeler, N.C., and Neale, D.B. (2006). DNA sequence variation and selection of tag single-nucleotide polymorphisms at candidate genes for drought-stress response in *Pinus taed*a L. *Genetics* 172**,** 1915-1926.

Isik, F., Goldfarb, B., LeBude, A., Li, B., and McKeand, S. (2005). Predicted genetic gains and testing efficiency from two loblolly pine clonal trials. *Canadian Journal of Forest Research* 35**,** 1754-1766.

Isik, F., Li, B., and Frampton, J. (2003). Estimates of additive, dominance and epistatic genetic variances from a clonally replicated test of loblolly pine. *Forest Science* 49**,** 77-88.

King, N., Seiler, J., Fox, T., and Johnsen, K. (2008). Post-fertilization physiology and growth performance of loblolly pine clones. *Tree Physiology* 28**,** 703-711.

Knauf, T., and Bilan, M.V. (1974). Needle variation in loblolly pine from mesic and xeric seed sources. *Forest Science* 20**,** 88-90.

Knauf, T., and Bilan, M.V. (1977). Cotyledon and primary needle variation in loblolly pine from mesic and xeric seed sources. *Forest Science* 23**,** 33-36.

Li, B., McKeand, S., and Weir, R. (1999). Tree improvement and sustainable forestry–impact of two cycles of loblolly pine breeding in the USA. *Forest Genetics* 6**,** 229-234.

Martin, T.A., Dougherty, P.M., Topa, M., and McKeand, S.E. (2005). Strategies and case studies for incorporating ecophysiology into southern pine tree improvement programs. *Southern Journal of Applied Forestry* 29, 39-58.

McCrady, R., and Jokela, E. (1996). Growth phenology and crown structure of selected loblolly pine families planted at two spacings. *Forest Science* 42**,** 46-57.

McCrady, R., and Jokela, E. (1998). Canopy dynamics, light interception, and radiation use efficiency of selected loblolly pine families. *Forest Science* 44**,** 64-72.

McKeand, S. (2015). The success of tree breeding in the southern US. *BioResources* 10**,** 1-2.

McKeand, S., Grissom, J., Handest, J., O'malley, D., and Allen, H. (2000). Responsiveness of diverse provenances of loblolly pine to fertilization-age 4 results. *Journal of Sustainable Forestry* 10**,** 87-94.

McKeand, S., Mullin, T., Byram, T., and White, T. (2003). Deployment of genetically improved loblolly and slash pines in the south. *Journal of Forestry* 101**,** 32-37.

McKeand, S., Weir, R., and Hatcher, A. (1989). Performance of diverse provenances of loblolly pine throughout the southeastern United States. *Southern Journal of Applied Forestry* 13**,** 46-51.

McKeand, S.E., Jokela, E.J., Huber, D.A., Byram, T.D., Allen, H.L., Li, B., and Mullin, T.J. (2006). Performance of improved genotypes of loblolly pine across different soils, climates, and silvicultural inputs. *Forest Ecology and Management* 227**,** 178-184.

Meier, C., Newton, R., Puryear, J., and Sen, S. (1992). Physiological responses of loblolly pine (*Pinus taeda* L.) seedlings to drought stress: osmotic adjustment and tissue elasticity. *Journal of Plant Physiology* 140**,** 754-760.

Miller, D.A., Wigley, T.B., and Miller, K.V. (2009). Managed forests and conservation of terrestrial biodiversity in the southern United States. *Journal of Forestry* 107**,** 197-203.

Osorio, J., and Pereira, J. (1994). Genotypic differences in water use efficiency and ^{13}C discrimination in *Eucalyptus globulus*. *Tree Physiology* 14**,** 871-882.

Paul, A., Foster, G.S., Caldwell, T., and McRae, J. (1997). Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine. *Forest Science* 43**,** 87-98.

Roth, B.E., Jokela, E.J., Martin, T.A., Huber, D.A., and White, T.L. (2007). Genotype× environment interactions in selected loblolly and slash pine plantations in the Southeastern United States. *Forest Ecology and Management* 238**,** 175-188.

Samuelson, L.J., Johnsen, K., and Stokes, T. (2004). Production, allocation, and stemwood growth efficiency of *Pinus taeda* L. stands in response to 6 years of intensive management. *Forest Ecology and Management* 192**,** 59-70.

Schultz, R.P. (1997). Loblolly pine: the ecology and culture of loblolly pine (*Pinus taeda* L.). *Agriculture Handbook (Washington).*

Seiler, J.R., and Johnson, J.D. (1985). Photosynthesis and transpiration of loblolly pine seedlings as influenced by moisture-stress conditioning. *Forest Science* 31**,** 742-749.

Seiler, J.R., and Johnson, J.D. (1988). Physiological and morphological responses of three half-sib families of loblolly pine to water-stress conditioning. *Forest Science* 34**,** 487-495.

Stovall, J.P., Fox, T.R., and Seiler, J.R. (2013). Allometry varies among 6-year-old *Pinus taeda* (L.) clones in the Virginia Piedmont. *Forest Science* 59**,** 50-62.

Teskey, R., Bongarten, B., Cregg, B., Dougherty, P., and Hennessey, T. (1987a). Physiology and genetics of tree growth response to moisture and temperature stress: an examination of the characteristics of loblolly pine (*Pinus taeda* L.). *Tree Physiology* 3**,** 41-61.

Teskey, R.O., Bongarten, B.C., Cregg, B.M., Dougherty, P.M., and Hennessey, T.C. (1987b). Physiology and genetics of tree growth response to moisture and temperature stress: an examination of the characteristics of loblolly pine (*Pinus taeda* L.). *Tree Physiology* 3**,** 41-61.

Thames, J.L. (1963). Needle variation in loblolly pine from four geographic seed sources. *Ecology* 44**,** 168-169.

Tyree, M.C., Seiler, J.R., Maier, C.A., and Johnsen, K.H. (2009). *Pinus taeda* clones and soil nutrient availability: effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology. *Tree Physiology* 29**,** 1117-1131.

Van Buijtenen, J., Bilan, M.V., and Zimmerman, R.H. (1976). Morpho physiological characteristics, related to drought resistance in *Pinus taeda*. *In* Tree Physiology and Yield Improvement. M.G.R. Canell and F.T. Last. Acedemic Press, London. pp. 349-359.

Wear, D.N., and Greis, J.G. (2002). Southern forest resource assessment. General Technical Report. SRS-53. United States Department of Agriculture, Forest Service, Southern Research Station, Asheville, North Carolina

Wells, O., and Lambeth, C. (1983). Loblolly pine provenance test in southern Arkansas 25th Year results. *Southern Journal of Applied Forestry* 7**,** 71-75.

Wells, O.O. (1983). Southwide pine seed source study -loblolly pine at 25 years. *Southern Journal of Applied Forestry* 7**,** 63-71.

Wells, O.O., and Wakeley, P.C. (1966). Geographic variation in survival, growth, and fusiform-rust infection of planted loblolly pine. *Forest Science Mongraph* 11**.**

Wertin, T.M., McGuire, M.A., and Teskey, R.O. (2012). Effects of predicted future and current atmospheric temperature and [CO2] and high and low soil moisture on gas exchange and growth of *Pinus taeda* seedlings at cool and warm sites in the species range. *Tree Physiology* 32**,** 847-858.

Zhang, J., Feng, Z., Cregg, B., and Schumann, C. (1997). Carbon isotopic composition, gas exchange, and growth of three populations of Ponderosa pine differing in drought tolerance. *Tree Physiology* 17**,** 461-466.

Zobel, B.J., and Goddard, R.E. (1955). Preliminary Results of Tests of Drought Hardy Strains of Loblolly Pine *(Pinus taeda* L.). Texas Forest Service, Texas A. & M. College System.

CHAPTER 2[1]

A PHYSIOLOGICAL ANALYSIS OF INTRASPECIFIC VARIATION, G X E INTERACTIONS, AND ^{13}C DISCRIMINATION OF THREE LOBLOLLY PINE (*PINUS TAEDA* L.) HALF-SIBLING CLONES IN RESPONSE TO DROUGHT

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[1] Ingwers MW, McGuire MA, Aubrey DP, Bhuiyan RA and Teskey RO. To be submitted to *Tree Physiology*.

Abstract

To better understand the intraspecific response of loblolly pine (*Pinus taeda* L.) to drought, we conducted a greenhouse study of 171 two-year-old ramets from each of three loblolly pine half-sibling clones (hereafter referred to as Clone 1, 2, and 3). Three soil moisture treatments were imposed. Two were constant soil moisture treatments at either high (0.3 MPa) or low (-1.5 MPa) soil water potential and the third was a wetting-drying treatment that alternated between three weeks of low soil water potential (-1.5 MPa) and one week at field capacity (-0.3 MPa). Biomass accumulation was measured by harvesting a subset of plants every four weeks. Root and shoot carbon allocation and carbon isotope discrimination ($\delta^{13}C$), an index of water use efficiency, were measured at the end of the experiment. In addition, pre-dawn needle water potential, afternoon needle water potential, dark respiration, morning and afternoon photosynthesis, stomatal conductance, intercellular CO_2, and transpiration were measured approximately bi-weekly throughout the experiment.

All clones accumulated more biomass under the well-watered treatment compared with the drought treatment, but the rank order of biomass accumulation among the clones depended on the soil moisture treatment. There was a genetic by environment (G x E) interaction: Clone 1 accumulated more biomass than the other clones in the low soil moisture treatment and less in the high soil moisture treatment. Differences in biomass accumulation among clones maybe related to the observed positive correlation between $\delta^{13}C$ and biomass accumulation under high water conditions and negative correlation under low water conditions. Carbon isotope discrimination was significantly higher in Clone 2, compared to Clone 1, across all treatments, which correlated to more biomass accumulation in the high soil moisture treatment and less biomass accumulation in the low soil moisture treatment. Gas exchange and needle water potential were higher in the high soil moisture treatment compared to the other treatments across all clones. Morning gas exchange was higher in Clones 2 and 3 than in Clone 1 across all treatments. The results from this study demonstrate that there is variation in drought response, even among closely related clones, that could be used to breed for increased drought resistance or to identify drought resistant genotypes for selective deployment.

Introduction

A recent analysis of 17 climate change models (Cook et al., 2015) indicates drier conditions in the future in much of North America, including the southeastern USA. A drier climate in the southeastern USA will likely have a deleterious effect on the productivity of loblolly pine (*Pinus taeda* L.), a species of major economic importance in the region (Schultz, 1997). Selection for drought resistant trees, and water relations traits that may affect drought resistance, are needed to maintain or improve loblolly pine production under future drought scenarios. Loblolly pine exhibits significant genetic (Eckert et al., 2010) and physiological (Teskey et al., 1987a) diversity associated with moisture regimes across its native range. Genetically improved loblolly pine half-sibling families, full-sib families and clonal varieties are widely distributed across the southeastern USA (McKeand et al., 2003); however, there has been little genetic selection for drought resistance.

Intraspecific variation in drought resistance and water relations has been frequently observed in loblolly pine (Van Buijtenen, 1966; Wells and Wakeley, 1966;

Kissee et al., 1985; Bongarten and Teskey, 1986a; Bongarten and Teskey, 1987; Teskey et al., 1987b; Aspinwall et al., 2011a). For example, the “Lost Pines” of East Texas is a genetically unique population (Al-Rabab’ah and Williams, 2004) that has persisted in a climate that is moisture-limited compared to typical climates in other parts of the loblolly pine native range (Bilan et al., 1977). The “Lost Pines” have undergone selective pressures resulting in adaptations that confer drought resistance. Seedlings from the “Lost Pines” population exhibited less drought-induced mortality than their counterparts from wetter regions when grown in a common garden (Zobel and Goddard, 1955; Goddard and Brown, 1959). When loblolly pine seedling from populations in drier regions and wetter regions were compared in a common garden experiment, those from drier regions had increased root biomass allocation and differences in root architecture (Van Buijtenen et al., 1976; Bilan et al., 1977; Cannell et al., 1978; Bongarten and Teskey, 1987; Seiler and Johnson, 1988), and decreased stomatal density and a greater reduction in transpiration in response to moisture deficit (Van Buijtenen et al., 1976; Bilan et al., 1977; Seiler and Johnson, 1988).

Loblolly pine displays many drought resistance characteristics. Traits associated with drought resistance include differences in root and leaf biomass and morphology (Pallardy, 1981), a reduction in leaf surface area (Wells and Wakeley, 1966), decreased stomatal conductance and transpiration (Newton et al., 1991), increased leaf cuticular wax and lower cuticular transpiration (Oppenheimer and Shomer-Ilan, 1963) and osmotic adjustment in needles (Seiler and Johnson, 1985), shoots (Bongarten and Teskey, 1986b), and roots (Meier et al., 1992). Loblolly pine can also acclimate to moisture stress. Loblolly pine seedlings pre-exposed to moisture stress conditioning have been demonstrated to photosynthesize at lower needle water potential and had higher water use efficiency than their counterparts that were not pre-exposed to moisture stress (Seiler and Johnson, 1988).

Carbon isotope discrimination is an index of drought stress (Farquhar et al., 1982) and water use efficiency and stomatal aperture (Farquhar et al., 1982; Farquhar and Richards, 1984; Martin and Thorstenson, 1988; Farquhar et al., 1989). Relationships between carbon isotope discrimination (Farquhar et al., 1989) and height or biomass have been observed in many species including *Picea mariana* (Flanagan and Johnsen, 1995; Johnsen et al., 1999), *P. radiata* (Li et al., 2015) and *P. elliotti* Engleman var. *elliotti* x. *P. caribaea* hybrids (Xu et al., 2000). A recent meta-analysis reported a consistent correlation between carbon isotope discrimination and height in 36 different datasets on forest trees (McDowell et al., 2011). If a strong relationship between carbon isotope discrimination and biomass accumulation exists in loblolly pine, then this relationship could be exploited for selection of clones and genotypes with superior drought resistance, as has already been done in some agricultural plant species, such as wheat (Farquhar and Richards, 1984; Condon et al., 1987).

Genetic variation among loblolly pine populations may result in physiological differences in their response to drought, or genetic by environment (G x E) interactions, that could be used to select clones, genotypes, and traits better suited to drought conditions. When G x E interactions were studied in loblolly pine genotypes across climatic conditions, soil types, and silvicultural inputs, it was concluded that while G x E interactions occurred, they were not of substantial concern, as productive families tended to maintain high productivity across all sites and treatments (McKeand et al., 2006).

Likewise, a field study comparing growth of 1200 clones from 61 half-sibling families found few G x E interactions among clonal populations (Baltunis et al., 2006). However, others have concluded that there are significant G x E interactions among loblolly pine genotypes and silvicultural treatments (Roth et al., 2007). Furthermore, G x E interactions for loblolly pine growth traits were found to be more significant among clones than among full-sibling families (Isik et al., 2003). Increased use of clonal varieties and selective breeding for specific traits, coupled with dramatic environmental changes such as severe or persistent drought events, may necessitate the evaluation of G x E interactions for future tree improvement.

This study examined the relationship between biomass accumulation and drought resistance traits among three half-sibling clones. Although loblolly pine clones have been the subject of numerous studies, to date no work has been done comparing productivity and carbon isotope discrimination and other physiological drought responses of loblolly pine clones. Differences in traits can be more easily identified among clones than among populations, genotypes, and families because there is less biological variation among individuals in the same clone. Experiments with clones have allowed for more precise determination of genetic and phenotypic variability in *Populus deltoides* (Funk et al., 2007). In addition, the use of clones has resulted in greater stand uniformity, productivity, and genetic gain in loblolly pine (Isik et al., 2003). Traits we examined include water use efficiency, gas exchange, and carbon allocation. The objective of this study was to determine how productivity, and some of the physiological factors that contribute to productivity, vary under well-watered and water-deficit conditions among a select group of highly productive and closely related clones. We had three hypotheses: 1) compared to the high soil moisture treatment, the response to the low soil moisture treatment would result in a genetic by environment (G x E) interaction in which some clones, relative to others, could have higher productivity in one moisture regime and lower productivity in another; 2) the rank order of biomass accumulation would differ in the wetting-drying treatment relative to the constant moisture treatments, and 3) clones would differ in water use efficiency, gas exchange, and carbon allocation reflecting different strategies to maximize drought resistance and productivity.

Methods

Plant material

This experiment was conducted on two-year-old ramets of three half-sibling clones of loblolly pine (ArborGen Inc., Summerville, SC, USA) bred from populations in the Atlantic Coastal Plain. Ramets, obtained as containerized stock, were transplanted into 4.3 L tree pots (CP612R, Steuwe and Son, Inc., Tangent, OR, USA) in a mix of crushed fritted clay (Turface Field and Fairway, Profile Products LLC, Buffalo, IL, USA) and fine sand (3:1, v:v). Drainage in the pots was increased by drilling five holes (diameter 0.9 cm) in the base of each pot in addition to the two existing holes (diameter 1.5 cm). Ramets were grown in a greenhouse at the Whitehall Experimental Forest in Athens, GA, USA, at approximately ambient humidity and air temperature. Air temperature was measured every five minutes with a shielded thermocouple and recorded with a data logger (CR23X, Campbell Scientific, Logan, UT, USA). Average ambient air temperature over the course of the experiment was 25.6°C. Ramets were planted and fertilized in late March 2013 and grown under well-watered conditions for approximately

11 weeks. During this time, the ramets were watered by hand daily to field capacity and fertilized once with 40 g of 15-9-12 NPK extended release fertilizer (Osmocote Plus #903286, Scotts-Sierra Horticultural Products, Marysville, OH, USA) and 0.2 g of chelated iron (Sprint 138, Becker Underwood, Ames, IA, USA).

Experimental design

The experiment was arranged as a split-plot design with clone as the whole-plot factor and moisture regime as the sub-plot factor with three replications. Each block (replicate) was on a separate greenhouse bench. Fifty-seven ramets were randomly chosen from each clone for assignment to each of the three blocks. Within each block x clone combination, each of the three soil moisture treatments was randomly assigned to a subplot of 19 ramets, resulting in a total of 27 clone x treatment x replicate combinations (hereafter called "plots"). To minimize any potential greenhouse location effects, the blocks were rotated among the benches at four-week intervals. Due to a solenoid malfunction, one of the replicates of the low water treatment in Clone 2 was removed from the analysis.

Experimental treatments

Three soil moisture treatments were imposed by controlling soil water potential at three levels: a constant high water treatment (-0.3 MPa), a constant low water treatment (-1.5 MPa), and an alternating water treatment that simulated a natural wetting-drying process, in which trees were subjected to one week of high soil water (-0.3 MPa), and three subsequent weeks of drier soil conditions (-1.5 MPa). Ramets underwent these treatments for a 12-week period from June 15, 2013 to September 7, 2013. Three wetting-drying cycles of the alternating water treatment were completed during the course of the experiment.

Soil moisture treatments were maintained by controlling soil volumetric water content using the technique of Nemali and van Iersel (2006). Soil volumetric water content was measured by the average output of two soil moisture sensors for each clone x treatment x replicate combination (EC-5 sensor, Decagon Devices, Pullman, WA, USA), which were randomly placed in two separate pots, in a horizontal orientation, 8.5 cm up from the base of the pot. Data were recorded on a data logger (CR23X, Campbell Scientific, Logan, UT, USA) at five-minute intervals. Soil water content values coinciding with -1.5 MPa and -0.3 MPa water potentials were predetermined by developing moisture release curves using a dew point hydrometer (Model WP4C, Decagon Devices, Inc, Pullman, WA, USA). Irrigation for each of the 27 clone x treatment x replicate plots was provided by a dedicated solenoid (Model 57100, Orbit, North Salt Lake, UT, USA) controlled by the data logger as follows: When measured soil moisture in a plot decreased below a specific set point coinciding with the moisture treatment (-1.5 MPa or –0.3 MPa), the data logger was programed to give a 15 second irrigation pulse resulting in a small increase in soil moisture. This sequence was repeated at five minute intervals, resulting in a series of small moisture pulses that maintained the soil volumetric content near the set point at all times. Water was delivered via a drip irrigation system to each pot in the plot. A precision drip emitter supplied each pot with a metered amount of water and a drip ring was used to distribute the water evenly to the soil surface.

Measurements

Biomass accumulation measurements were made by sequential harvests. Harvests were conducted at the start of the experiment and then at four-week intervals. At each harvest, four individuals from each clone x treatment x replicate combination were harvested and their roots and shoots were separated. All tissue was dried at 65°C to a constant mass. Allometric analyses were conducted on dry mass to determine carbon allocation to roots and shoots. Height and diameter were measured on all individuals in the entire experiment prior to each harvest.

Morning (9:00-11:00) and afternoon (14:00-16:00) gas exchange measurements were made approximately biweekly in all plots. Our measurement schedule insured that both high and low water conditions were equally represented in gas exchange measurements in the alternating water treatment. To control for time of day, gas exchange measurements were blocked by day over a three-consecutive-day period. A single replicate (block) was measured on each day, within which a single ramet in each clone x treatment combination (9 per block) was randomly selected for measurement. The order in which the replicates were measured over a three-day period was chosen at random. Gas exchange measurements, including net assimilation (A_{net}, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration (E, mol H_2O m^{-2} s^{-1}) and intercellular CO_2 (C_i, µmol CO_2 mol^{-1} air) were made with a portable photosynthesis system (LI-6400, Li-Cor Biosciences, Lincoln, NE, USA) fitted with a standard leaf chamber. Instantaneous water use efficiency (WUE_i) was calculated by dividing A_{net} by E. Conditions in the cuvette were set to 1000 µmol m^{-2} s^{-1} PAR and 30 °C block temperature, while cuvette humidity was adjusted to approximately match greenhouse humidity at the time of measurement. Three needles of a single fascicle were spread an equal distance apart and were placed parallel to one another in the cuvette. Gas exchange measurements were expressed on the basis of the total needle surface area enclosed in the cuvette. Needle surface area of the three needles enclosed in the cuvette was calculated as:

[1] Needle surface area in cuvette = $l\,(2\pi r+2Nr)$

Where l= length of cuvette (3cm), r=average radius of needles (cm), and N=number of needles per fascicle (three needles for all measurements). Needle radius was measured to 0.1 mm with a scaled 10x hand lens (PEAK Model 1983, SPI Supplies, West Chester PA, USA).

Dark respiration (R_d, μmol CO_2 $m^{-2}s^{-1}$) was measured with the same portable photosynthesis system. Measurements were conducted at approximately four-week intervals throughout the experiment on a needles of a single dark-acclimated individual in each clone x treatment x replicate combination on. Trees were dark-acclimated by placing them in a dark environmental chamber for >6 hours under temperature and humidity conditions that approximately matched ambient. Dark respiration was measured between 16:00 and 20:00.

Needle xylem pressure potential (Ψ, MPa), which was interpreted as needle water potential, was measured using a pressure chamber (Model 600, PMS Instruments Co., Corvallis, OR, USA). Measurements of Ψ were taken in conjunction with gas exchange measurements on a single ramet in every plot (27 total). Individuals used for pre-dawn Ψ measurements were used later the same day for morning gas exchange measurements. Afternoon Ψ measurements were likewise conducted on the same individuals used for midday gas exchange measurements. On each ramet, both predawn and afternoon Ψ measurements were conducted on two needles from a single fascicle taken from the most recent fully-expanded flush.

The ratio of carbon discrimination ($\delta^{13}C$) of needle tissue was determined on four individuals in each clone x treatment x replicate combination during Week 12 of the experiment. To ensure that tissues for analysis developed under treatment, a bulk sample of developing needles was taken from each ramet. Tissue was dried at 65°C to a constant mass, then finely ground and analyzed for $\delta^{13}C$ by mass spectrometry at the Stable Isotope and Soil Biology Laboratory, Odum School of Ecology, University of Georgia, Athens, GA, USA.

Biomass calculations and statistical analyses

Statistical differences in biomass accumulation among treatments were assessed with a repeated measures analysis of variance (RMANOVA) using PROC MIXED (Littell et al., 1996) with plot as the experimental unit. Prior to statistical analyses, biomass data were first tested for homogeneity and log transformed to satisfy Shapiro-Wilks normality. Treatment means were evaluated using Fisher's Least Significant Differences (LSD) test. Simple main effects were tested using the SLICE option in the LSMEANS statement of PROC MIXED (Littell et al., 1996; Schabenberger et al., 2000). Clone 2 was initially larger than Clones 1 and 3 at the start of the experiment. To account for initial size differences, size from the preceding harvest was used as a covariate.

Allometric relationships were utilized to explore how moisture regimes affected carbon allocation to the roots (fine and coarse root) and shoots (stem, branch, and needle). Allometric relationships were assessed using the model

[2] $\ln y = a + k\ln x$

(Ledig et al., 1970; Hunt, 1979; Coleman et al., 2004; Coyle et al., 2008): where x and y represent tissue components, a is the y-intercept and k is the allometric coefficient. Equivalent k values among treatments indicate that differences in allocation were due to development and not treatment conditions (Hunt, 1979).

Carbon isotope discrimination ($\delta^{13}C$) was analyzed using PROC MIXED (Littell et al., 1996) to assess clonal effects, treatment effects, and the relationship between $\delta^{13}C$ and biomass accumulation. Analyses of covariance were conducted using the natural log of biomass for linearization. Regression parameters were further determined using PROC REG.

Needle-level physiological measurements were statistically tested using a repeated measures analysis of variance (RMANOVA) using PROC MIXED (Littell et al., 1996). Here clone x treatment means were compared over six separate time intervals, with each interval coinciding with the wet or dry portion of the three wetting-drying cycles in the alternating water treatment. Measurements were taken biweekly; however, different sets of measurements (i.e. morning and afternoon gas exchange) were taken on different schedules. Different covariate structures were used for different sets of measurements to account for this difference in periodicity. We used the Akaike Information Criterion (AIC) (Akaike, 1974) to determine the optimal covariate structure for each of the models. A compound symmetry (CS) structure was used to assess pre-dawn Ψ and R_d as well as morning A_{net}, g_s, E and C_i. Afternoon A_{net}, g_s, E, C_i and Ψ were evaluated using the power spatial covariate structure (SP). Treatment means were compared using Fisher's Least Significant Differences (LSD) test. The SLICE tool in the

LSMEANS statement of PROC MIXED (Littell et al., 1996; Schabenberger et al., 2000) was used to test simple main effects.

All statistical analyses were conducted using SAS (version 9.3.1; SAS Institute Inc., Cary, NC, USA). Denominator degrees of freedom (DDFM) were calculated using the Kenward-Roger (Kenward and Roger, 1997) method. We used a Type-1 error rate of 0.05 for all analyses.

Results

Biomass accumulation

Total biomass accumulation differed among clones and their rank order depended on soil moisture treatment and date harvested, resulting in a significant ($p>0.0001$) clone-by-treatment-by-date interaction. Biomass accumulation was greatest between Week 8 and Week 12 and increased most rapidly in the high water treatment (Figure 2.1). Average biomass accumulation across clones at the end of the experiment was 15.95 (SE 0.96) g in the low water treatment and 65.15 (SE 2.33) g in the high water treatment. The alternating treatment had an average biomass accumulation of 26.93 (SE 1.22) g, which was intermediate between the low and high water treatments. Differences in growth among treatments, and clones within treatments, between Week 8 and Week 12 resulted in a G x E interaction (Figure 2.2). Within the low water treatment, Clone 1 grew more than Clones 2 or 3. In contrast, within the high water treatment, Clone 1 grew less than Clones 2 or 3. Biomass accumulation of Clones 2 and 3 did not differ within water treatments. All three clones had similar biomass accumulation in the alternating water treatment. Change in height (Figure 2.3) had a similar pattern as biomass accumulation, however, there was a significant interaction among clone, treatment, and initial height, preventing a statistical test of the main effects. Although tree diameter increased throughout the experiment there were no significant differences among clones and treatments (data not shown).

Allometric analysis

The three clones exhibited similar patterns of root and shoot allocation under the low and alternating soil moisture treatments (Table 2.1, Figure 2.4). Differences among clones were only detected in the high water treatment (p=0.007). Clone 1 allocated relatively less carbon to roots than the other two clones. Carbon allocation among treatments within clone only varied within Clone 1 (p=0.013), where there was significantly more carbon allocation to roots in the alternating water treatment compared to the low and high water treatments.

Carbon isotope discrimination and analysis

Carbon isotope discrimination differed among treatments and among clones within treatment (Figure 2.5). The $\delta^{13}C$ value was highest in the low water treatment, lowest in the high water treatment, and intermediate in the alternating water treatment. Clone 2 had a higher $\delta^{13}C$ value than Clones 1 and 3 in the low and high water treatments, and was higher than Clone 1, but not Clone 3, in the alternating water treatment. Carbon isotope discrimination in Clones 1 and 3 was not significantly different in any of the treatments.

Significant negative correlations were found between $\delta^{13}C$ and biomass accumulation when clones were analyzed across treatments (Figure 2.6, Table 2.2). The slopes of the relationships differed between Clones 1 and 2, but not between Clones 1 and

3 or between Clones 2 and 3. When the δ^{13}C data was examined within a water treatment (data from the three clones combined) there was a positive correlation between δ^{13}C and biomass accumulation in the high water treatment and a negative correlation between δ^{13}C and biomass accumulation in the low water treatment (Figure 2.7). Although the regression slopes between treatments were significantly different, R^2 values were low (Table 2.2). The relationship between δ^{13}C and biomass accumulation was positive in the alternating water treatment and significantly differed from the low water treatment but not the high water treatment.

Needle level physiology (A_{net}, g_s, E, C_i, WUE_i, R_d and Ψ):

Needle level physiology differed among treatments (Table 2.3). Among treatments, mean morning A_{net}, g_{s}, E, and predawn Ψ values were lowest in the low water treatment, highest in the high water treatment and intermediate in the alternating water treatment. Instantaneous water use efficiency and C_{i}, were highest in the high water treatment, and lowest in the low water treatment.

Significant differences in some gas exchange measurements were detected among clones (Table 2.3). Morning A_{net} and E were lowest in Clone 1, intermediate in Clone 2 and highest in Clone 3. A similar trend was noted in morning g_{s} and C_{i} although these differences were not significant ($p=0.0562$ and $p=0.0582$). Mean pre-dawn Ψ was -0.67 MPa in Clone 1, compared to -0.81 MPa in Clone 2 and -0.82 MPa in clone 3, but the difference between Clone 1 and the other clones was not significant ($p=0.0517$).

Similar to the pattern in morning gas exchange measurements, among treatments, mean afternoon A_{net}, g_{s}, E, and Ψ values, were lowest in the low water treatment and highest in the high water treatment (Table 2.4). All afternoon A_{net}, g_{s}, E, and Ψ values were significantly different among the three treatments. Instantaneous water use efficiency was highest in the low water treatment and the lowest in the high water treatment. That pattern can also be seen in the morning values, but it was not statistically significant. Afternoon WUE_i in the alternating treatment did not differ from afternoon WUE_i in the other two treatments. Afternoon C_i did not differ among the three treatments. Dark respiration also did not differ among the treatments.

Afternoon Ψ was significantly lower in Clone 1 than in Clones 2 or 3 (Table 2.4). However, afternoon needle gas exchange differed very little among clones. Similar gas exchange physiology was observed among all clones with no significant differences or discernable patterns in mean A_{net}, g_s, or E. No differences in R_d were observed among clones. No significant clone by treatment interactions were detected in any needle physiological measurements.

Discussion

Our study found some differences and some similarities in physiological traits of the three clones in response to soil moisture availability. Our first hypothesis was supported by the change in rank order of biomass accumulation among clones between the low and high water treatments, which resulted in a G × E interaction, demonstrating that closely related clones of loblolly pine can respond differently to moisture stress. Although previous work has demonstrated variation in growth among loblolly pine full-sibling families (Paul et al., 1997), to the best of our knowledge this is the first G × E interaction found among loblolly pine clones in response to moisture stress. However, other studies have found G x E interactions among loblolly pine clones in response to other environmental factors. For example, field studies using multiple clones of loblolly pine with and without fertilization have observed G × E interactions in leaf area (Tyree et al., 2009b), growth (King et al., 2008; Stovall et al., 2013), gas exchange (King et al., 2008) and carbon allocation (Stovall et al., 2013). In addition, a greenhouse experiment on loblolly pine clones demonstrated that clone × fertilizer interactions affected growth, allocation, and gas exchange (Tyree et al., 2009a). Silvicultural treatments have also resulted in G × E interactions (Roth et al., 2007). The significant differences in biomass accumulation among treatments in our study, consistent with other loblolly pine moisture studies (Wertin et al., 2012; Bauweraerts et al., 2014), demonstrated the importance of moisture in loblolly pine biomass production and the potential of future drought to have deleterious effects on loblolly pine production. Breeders may be able to exploit variation in drought response to develop genotypes and varieties that will be able to grow better on drier sites and in drier regions as well as to better withstand the expected increases in drought in the future.

Contrary to our second hypothesis, there were no differences in biomass accumulation among clones in the alternating water treatment. The increase in average biomass accumulation in the alternating water treatment, relative to the low water treatment, was approximately 22% of the increase in the high water treatment relative to the low water treatment. The 22% increase in biomass accumulation roughly coincides with the amount of time (25%) that the ramets in the alternating treatment were growing under high water conditions. A possible interpretation of these findings is that all clones in this study performed equally well with the moderate amount of water (between the low and high water treatment) collectively received in the alternating treatment. These findings could be further interpreted to suggest that trees in the alternating water treatment quickly recovered from the moisture stress they experienced under the low water conditions. Another possible explanation for the similarity in biomass accumulation among clones in the alternating water treatment may be the difference among clones in the rate of biomass accumulation in the low and high water treatments. In the alternating water treatment, Clone 1 may have had more biomass accumulation than Clones 2 and 3 in low water conditions but less biomass accumulation than Clones 2 and 3 in the high water conditions. Taken together, biomass accumulation among clones in the alternating treatment may have been comparable because each clone was exposed to a moisture regime where it had relatively higher or lower rates of accumulation, which generally offset one another when combined.

Consistent with our third hypothesis, there were some differences between root and shoot carbon allocation, however it is unclear how and if these differences affected drought resistance. When carbon allocation comparisons were made among clones within treatments, differences in root and shoot carbon allocation were found in the high water treatment. The genetic variation in carbon allocation found in this study is consistent with other studies which have found variation in carbon allocation among loblolly pine provinces (Bongarten and Teskey, 1987; Retzlaff et al., 2001), genotypes (Aspinwall et al., 2011b), families (Retzlaff et al., 2001; Coyle et al., 2008) and clones (Aspinwall et al., 2011b; Stovall et al., 2013). Our results are particularly interesting as they suggest the individuals with more carbon allocation to roots, relative to shoots, actually have more biomass accumulation under high water conditions than those with less carbon allocation to roots. A possible explanation for these findings is that the increased carbon allocation to roots allowed for better acquisition of nutrients under high water conditions. In regards to the low water and alternating water treatments, our findings are similar to those of Stovall et al. (2013), which also demonstrated significant differences in root and shoot carbon allocation among loblolly pine clones, but were unable to link these differences in allocation to differences in biomass accumulation. The data, both from Stovall et al. (2013) and the low water and alternating water treatments in our study, indicate that carbon allocation was not a major factor, relative to other physiological factors, in determining rates of biomass accumulation of the clones used in these studies.

Within each clone, carbon allocation did not differ between the low, alternating and high water treatments except in Clone 1, where there was greater carbon allocation to the roots in the alternating water treatment. A similar greenhouse study on seedlings from three half-sibling loblolly pine families (Seiler and Johnson, 1988) found a reduction in the root/shoot ratio in response to more moisture. A reduction in the ratio of root to shoot biomass in response to irrigation, and irrigation in combination with other treatments, has also been observed in a field study on loblolly pine (Samuelson et al., 2004). Our study, in contrast, indicated that some, but not all clones respond to moisture stress by altering their root/shoot carbon allocation. No differences in root or shoot allocation in response to different moisture treatments, as observed in Clones 2 and 3, were observed in a loblolly pine field study which examined the effects of irrigation and fertilization on carbon allocation (Coyle et al., 2008). The greater root carbon allocation in the alternating water treatment in Clone 1 did not result in greater biomass accumulation when compared to the other two clones, but could suggest that this clone may have greater plasticity in its capacity to respond to changing moisture regimes.

When analyzed across moisture treatments, strong negative correlations between $\delta^{13}C$ and biomass accumulation were found in all three of the clones. If consistent across the species, these strong correlations between $\delta^{13}C$ and biomass accumulation indicate that $\delta^{13}C$ could be used to assist in the selection of more productive genotypes and clones (Farquhar and Richards, 1984; Condon et al., 1987). Our findings indicate that selection for clones and genotypes with lower $\delta^{13}C$ would result in trees with greater rates of biomass accumulation. These results highlight the potential usefulness of $\delta^{13}C$ in future tree improvement efforts, especially in a common garden experiment where measurements could be broadly applied to different clones and genotypes. However, the different relationships between $\delta^{13}C$ and biomass accumulation among the clones in this study indicated that selection for $\delta^{13}C$ may result in greater genetic gain in some genotypes compared to others.

The overall relationship between $\delta^{13}C$ and biomass accumulation across soil moisture treatments was strong and clearly biologically significant, however, we also found additional weaker relationships between $\delta^{13}C$ and biomass accumulation within our treatments. In the high water treatment there was a weak, but statistically significant, positive relationship between $\delta^{13}C$ and biomass while in the low water treatment there was a weak but also statistically significant negative correlation between $\delta^{13}C$ and biomass. We interpret these results to suggest that the relationship between $\delta^{13}C$ and biomass growth differs between high and low moisture conditions. Under high moisture conditions, trees may avoid moisture stress by closing their stomata when needle water potentials begin to drop, resulting in a lower water use efficiency. Avoiding moisture stress in this manner may allow for trees under high moisture stress to photosynthesize more throughout the day and thus increase biomass accumulation. Under low moisture conditions, trees may tolerate lower needle water potentials and continue to photosynthesize for longer to increase biomass accumulation, which would produce a higher water use efficiency. Although negative (Bond and Stock, 1990; Prasolova et al., 2000) and positive (Aitken et al., 1995; Flanagan and Johnsen, 1995; Johnsen et al., 1999; Prasolova et al., 2001) correlations between biomass accumulation and $\delta^{13}C$ have been previously reported, this is the first study to report both negative and positive correlations between biomass accumulation and $\delta^{13}C$ under different moisture regimes in a tree species. In wheat, a positive correlation was found between $\delta^{13}C$ and above ground biomass at the driest site and a negative correlation between above ground biomass and $\delta^{13}C$ was found at wetter, higher yielding sites (Condon et al., 1987). Similar to Condon et al. (1987), our study demonstrated contrasting correlations between biomass accumulation and $\delta^{13}C$ under low and high water conditions, however, in contrast to Condon et al. (1987), we found a negative correlation in the low water regime and a positive correlation in the high water regime. Differences in $\delta^{13}C$ were consistent with the change in rank order in biomass accumulation in Clone 1 and Clone 2 between high and low water treatments. Clone 1, compared to Clone 2, had a higher $\delta^{13}C$ across treatments, which coincided with less biomass accumulation in the high water treatment and more biomass accumulation in the low water treatments.

Supporting the third hypothesis, differences were detected in needle physiological measurements among the clones. Similarly, in a study of the response of loblolly pine clones to fertilization, it was observed that some clones had lower g_s and E than other clones (Tyree et al., 2009a). Also, differences in light-saturated net photosynthesis were observed between two clones after fertilization (King et al., 2008; Tyree et al., 2009b). In contrast, in a field study, differences in gas exchange among three different loblolly pine clones were largely insignificant (Aspinwall et al., 2011c), which may have resulted from the inherent variation present in gas exchange measurements in the field.

The higher afternoon needle water potential in Clone 1 indicates that Clone 1 was significantly less moisture stressed than Clones 2 or 3, which was also reflected in the greater biomass accumulation in Clone 1 under the low moisture treatment. Equivalent C_i values indicated that stomatal limitations were similar among clones. Dark respiration was not affected by clone or treatment; nor was dark respiration correlated with biomass accumulation contrary to what was found in a previous study (Aspinwall et al., 2011c). However, differences in R_d in our study may have been too minute to detect with our equipment and measurement procedures.

Conclusion

Biomass accumulation differed by clone, as well as treatment, with a significant interaction between clone and treatment. Clone and treatment also affected root and shoot biomass allocation and needle physiology but, in contrast to biomass accumulation, no interactions between clone and treatment were detected. Differences in root and shoot carbon allocation were observed among clones, but were not correlated to differences in total biomass accumulation. A strong relationship between $\delta^{13}C$ and biomass accumulation was found when data were analyzed across treatments, suggesting that using δ^{13}C as a selection criterion may be useful in future efforts to improve biomass accumulation. The δ^{13}C results also indicated that water use efficiency diffed under different moisture conditions. Clonal differences in needle gas exchange were detected in morning, but not afternoon, measurements and may account for the differences in biomass accumulation under high water conditions.

References

Aitken, S.N., Kavanagh, K.L., and Yoder, B.J. (1995). Genetic variation in seedling water-use efficiency as estimated by carbon isotope ratios and its relationship to sapling growth in Douglas-fir. *Forest Genetics* 2**,** 199-206.

Akaike, H. (1974). A new look at the statistical model identification. *Automatic Control, IEEE Transactions* 19**,** 716-723.

Al-Rabab'ah, M.A., and Williams, C.G. (2004). An ancient bottleneck in the Lost Pines of central Texas. *Molecular Ecology* 13**,** 1075-1084.

Aspinwall, M.J., King, J.S., Domec, J.C., McKeand, S.E., and Isik, F. (2011a). Genetic effects on transpiration, canopy conductance, stomatal sensitivity to vapour pressure deficit, and cavitation resistance in loblolly pine. *Ecohydrology* 4**,** 168-182.

Aspinwall, M.J., King, J.S., McKeand, S.E., and Bullock, B.P. (2011b). Genetic effects on stand-level uniformity and above-and belowground dry mass production in juvenile loblolly pine. *Forest Ecology and Management* 262**,** 609-619.

Aspinwall, M.J., King, J.S., McKeand, S.E., and Domec, J.-C. (2011c). Leaf-level gas-exchange uniformity and photosynthetic capacity among loblolly pine (*Pinus taeda* L.) genotypes of contrasting inherent genetic variation. *Tree Physiology* 31**,** 78-91.

Baltunis, B.S., Huber, D.A., White, T.L., Goldfarb, B., and Stelzer, H.E. (2006). Genetic analysis of early field growth of loblolly pine clones and seedlings from the same full-sib families. *Canadian Journal of Forest Research* 37**,** 195-205.

Bauweraerts, I., Ameye, M., Wertin, T.M., McGuire, M.A., Teskey, R.O., and Steppe, K. (2014). Water availability is the decisive factor for the growth of two tree species in the occurrence of consecutive heat waves. *Agricultural and Forest Meteorology* 189**,** 19-29.

Bilan, M.V., Hogan, C.T., and Carter, H.B. (1977). Stomatal opening, transpiration, and needle moisture in loblolly pine seedlings from two Texas seed sources. *Forest Science* 23**,** 457-462.

Bond, W., and Stock, W. (1990). Preliminary assessment of the grading of *Eucalyptus* clones using carbon isotope discrimination. *South African Forestry Journal* 154**,** 51-55.

Bongarten, B.C., and Teskey, R.O. (1986a). Water relations of loblolly pine seedlings from diverse geographic origins. *Tree Physiology* 1**,** 265-276.

Bongarten, B.C., and Teskey, R.O. (1986b). Water relations of loblolly pine seedlings from diverse geographic origins. *Tree Physiology* 1**,** 265-276.

Bongarten, B.C., and Teskey, R.O. (1987). Dry weight partitioning and its relationship to productivity in loblolly pine seedlings from seven sources. *Forest Science* 33**,** 255-267.

Cannell, M., Bridgwater, F., and Greenwood, M. (1978). Seedling growth rates, water stress responses and root-shoot relationships related to eight-year volumes among families of *Pinus taeda* L. *Silvae Genetica* 27, 237-248.

Coleman, M.D., Friend, A.L., and Kern, C.C. (2004). Carbon allocation and nitrogen acquisition in a developing *Populus deltoides* plantation. *Tree Physiology* 24**,** 1347-1357.

Condon, A., Richards, R., and Farquhar, G. (1987). Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. *Crop Science* 27**,** 996-1001.

Cook, B.I., Ault, T.R., and Smerdon, J.E. (2015). Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Science Advances* 1**,** e1400082.

Coyle, D.R., Coleman, M.D., and Aubrey, D.P. (2008). Above-and below-ground biomass accumulation, production, and distribution of sweetgum and loblolly pine grown with irrigation and fertilization. *Canadian Journal of Forest Research* 38**,** 1335-1348.

Eckert, A.J., van Heerwaarden, J., Wegrzyn, J.L., Nelson, C.D., Ross-Ibarra, J., González-Martínez, S.C., and Neale, D.B. (2010). Patterns of population structure and environmental associations to aridity across the range of loblolly pine (*Pinus taeda* L., Pinaceae). *Genetics* 185**,** 969-982.

Farquhar, G., and Richards, R. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Functional Plant Biology* 11**,** 539-552.

Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T. (1989). Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Biology* 40**,** 503-537.

Farquhar, G.D., O'leary, M., and Berry, J. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Functional Plant Biology* 9**,** 121-137.

Flanagan, L.B., and Johnsen, K.H. (1995). Genetic variation in carbon isotope discrimination and its relationship to growth under field conditions in full-sib families of *Picea mariana*. *Canadian Journal of Forest Research* 25**,** 39-47.

Funk, J.L., Jones, C.G., and Lerdau, M.T. (2007). Leaf-and shoot-level plasticity in response to different nutrient and water availabilities. *Tree Physiology* 27**,** 1731-1739.

Goddard, R.E., and Brown, C.L. (1959). Growth of Drought Resistant Loblolly Pines. Texas Forest Service Research Note 23. Texas Forest Service, College Station, Texas.

Hunt, R. (1978). Plant growth analysis: Edward Arnold Ltd., London.

Isik, F., Li, B., and Frampton, J. (2003). Estimates of additive, dominance and epistatic genetic variances from a clonally replicated test of loblolly pine. *Forest Science* 49**,** 77-88.

Johnsen, K.H., Flanagan, L.B., Huber, D.A., and Major, J.E. (1999). Genetic variation in growth, carbon isotope discrimination, and foliar N concentration in *Picea mariana*: analyses from a half-diallel mating design using field-grown trees. *Canadian Journal of Forest Research* 29**,** 1727-1735.

Kenward, M.G., and Roger, J.H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53**,** 983-997.

King, N., Seiler, J., Fox, T., and Johnsen, K. (2008). Post-fertilization physiology and growth performance of loblolly pine clones. *Tree Physiology* 28**,** 703-711.

Kissee, K., Newton, R., and Carroll, L. (1985). "Loblolly seedling genotypic xylem pressure potential responses to cutting practices in the nursery". *In* The International Symposium on Nursery Management Practices for the Southern Pines: Auburn University, AL, pp. 311-316.

Ledig, F.T., Bormann, F.H., and Wenger, K.F. (1970). The distribution of dry matter growth between shoot and roots in loblolly pine. *Botanical Gazette* 13**,** 349-359.

Li, Y., Xue, J., Clinton, P.W., and Dungey, H.S. (2015). Genetic parameters and clone by environment interactions for growth and foliar nutrient concentrations in radiata pine on 14 widely diverse New Zealand sites. *Tree Genetics & Genomes* 11**,** 1-16.

Littell, R., Milliken, G., Stroup, W., Wolfinger, R., and Schabenberber, O. (1996). SAS system for linear mixed models. Cary, NC: SAS Institute.

Martin, B., and Thorstenson, Y.R. (1988). Stable carbon isotope composition (δ13C), water use efficiency, and biomass productivity of Lycopersicon esculentum, *Lycopersicon pennellii*, and the F1 hybrid. *Plant Physiology* 88**,** 213-217.

McDowell, N.G., Bond, B.J., Dickman, L.T., Ryan, M.G., and Whitehead, D. (2011). "Relationships between tree height and carbon isotope discrimination. *In* Size-and age-related changes in tree structure and function. Springer. pp. 255-286.

McKeand, S., Mullin, T., Byram, T., and White, T. (2003). Deployment of genetically improved loblolly and slash pines in the south. *Journal of Forestry* 101**,** 32-37.

McKeand, S.E., Jokela, E.J., Huber, D.A., Byram, T.D., Allen, H.L., Li, B., and Mullin, T.J. (2006). Performance of improved genotypes of loblolly pine across different soils, climates, and silvicultural inputs. *Forest Ecology and Management* 227**,** 178-184.

Meier, C., Newton, R., Puryear, J., and Sen, S. (1992). Physiological responses of loblolly pine (*Pinus taeda* L.) seedlings to drought stress: osmotic adjustment and tissue elasticity. *Journal of Plant Physiology* 140**,** 754-760.

Nemali, K.S., and van Iersel, M.W. (2006). An automated system for controlling drought stress and irrigation in potted plants. *Scientia Horticulturae* 110**,** 292-297.

Newton, R., Funkhouser, E., Fong, F., and Tauer, C. (1991). Molecular and physiological genetics of drought tolerance in forest species. *Forest Ecology and Management* 43**,** 225-250.

Oppenheimer, H., and Shomer-Ilan, A. (1963). A contribution to the knowledge of drought resistance of Mediterranean pine trees. *Mitt Florist-soziolog Arveit* 10**,** 42-55.

Pallardy, S. (1981). "Water deficits and Plant Growth" vol 6, Woody Plant Communities, *In* Closely Related Woody Plant, Academic Press, Inc., New York, pp. 511-548

Paul, A., Foster, G.S., Caldwell, T., and McRae, J. (1997). Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine. *Forest Science* 43**,** 87-98.

Prasolova, N., Xu, Z., Farquhar, G.D., Saffigna, P.G., and Dieters, M.J. (2000). Variation in branchlet δ^{13}C in relation to branchlet nitrogen concentration and growth in 8-year-old hoop pine families (*Araucaria cunninghamii*) in subtropical Australia. *Tree Physiology* 20**,** 1049-1055.

Prasolova, N.V., Xu, Z., Farquhar, G.D., Saffigna, P.G., and Dieters, M.J. (2001). Canopy carbon and oxygen isotope composition of 9-year-old hoop pine families in relation to seedling carbon isotope composition, growth, field growth performance, and canopy nitrogen concentration. *Canadian Journal of Forest Research* 31**,** 673-681.

Retzlaff, W., Handest, J., O'Malley, D., McKeand, S., and Topa, M. (2001). Whole-tree biomass and carbon allocation of juvenile trees of loblolly pine (*Pinus taeda*): influence of genetics and fertilization. *Canadian Journal of Forest Research* 31**,** 960-970.

Roth, B.E., Jokela, E.J., Martin, T.A., Huber, D.A., and White, T.L. (2007). Genotype× environment interactions in selected loblolly and slash pine plantations in the southeastern United States. *Forest Ecology and Management* 238**,** 175-188.

Samuelson, L.J., Johnsen, K., and Stokes, T. (2004). Production, allocation, and stemwood growth efficiency of *Pinus taeda* L. stands in response to 6 years of intensive management. *Forest Ecology and Management* 192**,** 59-70.

Schabenberger, O., Gregoire, T.G., and Kong, F. (2000). Collections of simple effects and their relationship to main effects and interactions in factorials. *The American Statistician* 54**,** 210-214.

Schultz, R.P. (1997). Loblolly pine: the ecology and culture of loblolly pine (*Pinus taeda* L.). *Agriculture Handbook (Washington)*.

Seiler, J.R., and Johnson, J.D. (1985). Photosynthesis and transpiration of loblolly pine seedlings as influenced by moisture-stress conditioning. *Forest Science* 31**,** 742-749.

Seiler, J.R., and Johnson, J.D. (1988). Physiological and morphological responses of three half-sib families of loblolly pine to water-stress conditioning. *Forest Science* 34**,** 487-495.

Stovall, J.P., Fox, T.R., and Seiler, J.R. (2013). Allometry varies among 6-year-old *Pinus taeda* (L.) clones in the Virginia Piedmont. *Forest Science* 59**,** 50-62.

Teskey, R., Bongarten, B., Cregg, B., Dougherty, P., and Hennessey, T. (1987a). Physiology and genetics of tree growth response to moisture and temperature stress: an examination of the characteristics of loblolly pine (*Pinus taeda* L.). *Tree Physiology* 3**,** 41-61.

Teskey, R.O., Bongarten, B.C., Cregg, B.M., Dougherty, P.M., and Hennessey, T.C. (1987b). Physiology and genetics of tree growth response to moisture and temperature stress: an examination of the characteristics of loblolly pine (*Pinus taeda* L.). *Tree Physiology* 3**,** 41-61.

Tyree, M.C., Seiler, J.R., and Maier, C.A. (2009a). Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting 2-year-old *Pinus taeda* clones during seedling establishment. *Forest Ecology and Management* 257**,** 1847-1858.

Tyree, M.C., Seiler, J.R., Maier, C.A., and Johnsen, K.H. (2009b). *Pinus taeda* clones and soil nutrient availability: effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology. *Tree Physiology* 29**,** 1117-1131.

Van Buijtenen, J. (1966). Testing loblolly pines for drought resistance. Texas Forest Service Research Note 13. Texas Forest Service, College Station, Texas.

Van Buijtenen, J., Bilan, M.V., and Zimmerman, R.H. (1976). Morpho physiological characteristics, related to drought resistance in *Pinus taeda*. *In* Tree Physiology and Yield Improvement. M.G.R. Canell and F.T. Last. Acedemic Press, London. pp. 349-359.

Wells, O.O., and Wakeley, P.C. (1966). Geographic variation in survival, growth, and fusiform-rust infection of planted loblolly pine. *Forest Science Mongraph* 11**.**

Wertin, T.M., McGuire, M.A., and Teskey, R.O. (2012). Effects of predicted future and current atmospheric temperature and [CO2] and high and low soil moisture on gas exchange and growth of *Pinus taeda* seedlings at cool and warm sites in the species range. *Tree physiology* 32**,** 847-858.

Xu, Z., Saffigna, P., Farquhar, G., Simpson, J., Haines, R., Walker, S., Osborne, D., and Guinto, D. (2000). Carbon isotope discrimination and oxygen isotope composition in clones of the F1 hybrid between slash pine and Caribbean pine in relation to tree growth, water-use efficiency and foliar nutrient concentration. *Tree Physiology* 20**,** 1209-1217.

Zobel, B.J., and Goddard, R.E. (1955). Preliminary results of tests of drought hardy strains of loblolly pine (*Pinus taeda L.).* Texas Forest Service Research Note 14. Texas Forest Service, College Station, Texas.

Table 2.1. Allometric coefficient, k, which is the slope of the relationship between log below-ground biomass and log above-ground biomass, compared within clones between treatments and within treatments between clones.

Analysis	**Clone**	**Low water**	**Alternate water**	**High water**	
Clone effect within treatment	1	0.17	1.47	1.00^{a}	
	2	0.74	0.79	1.47^{b}	
	3	0.50	0.81	1.68^{b}	
		p=0.581	*p*=0.600	**_p_=0.007**	
Treatment effect within clone	1	0.17^{a}	1.47^{b}	1.00^{ab}	**_p_=0.013**
	2	0.74	0.79	1.47	*p*=0.346
	3	0.50	0.81	1.68	*p*=0.283

Table 2.2. Parameter estimates (SE) for the relationship between δ^{13}C and the natural log of biomass accumulation. Parameters and SE are the results of a regression analysis. Different letters represent significant differences among slopes determined by mixed model analysis where α=0.05. Relationships are in the form $\ln(y)=mx + b$ where y is estimated biomass accumulation, *m* is the slope, *x* is δ^{13}C, and *b* is the intercept.

Analysis	**Subject**	**Slope (SE)**	**Intercept (SE)**	R^2	**MSE**	***p*-value**
Among treatments	Low water	-1.971(0.63)^{a}	-22.339(2.10)	0.231	0.722	0.0035
	Alternating water	1.077(0.51)^{b}	-33.972(1.86)	0.115	0.344	0.0433
	High Water	1.067(0.33)^{b}	-36.601(1.42)	0.231	0.152	0.0026
Among clones	Clone 1	-2.473(0.42)^{a}	-21.378(1.58)	0.502	0.978	<0.0001
	Clone 2	-2.723(0.24)^{b}	-19.460(0.94)	0.815	0.473	<0.0001
	Clone 3	-2.730(0.23)^{ab}	-20.268(1.42)	0.806	0.412	<0.0001

Table 2.3. Mean (SE) morning gas exchange values and needle water potential among clones and treatments. Different letters within each group indicate significant differences at α=0.05.

Analysis	**Subject**	**Net assimilation (µmol CO^2 m^{-2} s^{-1})**	**Stomatal conductance (mol H_2O m^{-2} s^{-1})**	**Intercellular CO_2 (µmol mol^{-1})**	**Transpiration (mmol H_2O m^{-2} s^{-1})**	**Instantaneous water use efficiency (µmol CO^2 $m^{-2}s^{-1}$/ mmol H_2O $m^{-2}s^{-1}$)**	**Pre-dawn needle water potential (MPa)**
Among treatments	Low water	3.74(0.40)^{a}	0.043(0.007)^{a}	208.8(16.4)^{a}	1.00(0.11)^{a}	3.98(0.35)^{a}	-1.10(0.09)^{a}
	Alternating water	4.94(0.38)^{b}	0.063(0.007)^{b}	229.2(13.3)^{ab}	1.46(0.12)^{b}	3.46(0.23)^{ab}	-0.80(0.06)^{b}
	High water	7.18(0.30)^{c}	0.108(0.007)^{c}	253.7(8.5)^{b}	2.59(0.18)^{c}	3.12(0.14)^{b}	-0.43(0.03)^{c}
	p-value	***p*<0.0001**	***p*<0.0001**	***p*=0.0038**	***p*<0.0001**	***p*=0.0103**	***p*<0.0001**
Among clones	Clone 1	4.82(0.34)^{a}	0.057(0.006)	215.5(14.4)	1.41(0.13)^{a}	3.84(0.26)	-0.67(0.05)
	Clone 2	5.36(0.44)^{ab}	0.074(0.008)	233.4(15.7)	1.78(0.19)^{ab}	3.45(0.32)	-0.81(0.09)
	Clone 3	5.85(0.45)^{b}	0.086(0.009)	245.6(9.6)	1.94(0.18)^{b}	3.21(0.16)	-0.82(0.07)
	p-value	***p*=0.0177**	p=0.0562	*p*=0.0586	***p*=0.0107**	*p*=0.0756	*p*=0.0517

Table 2.4. Mean (SE) afternoon gas exchange values and needle water potential and mean dark respiration among clones and treatments. Different letters within each group indicate significant differences at α=0.05.

Analysis	**Subject**	**Net assimilation (μmol CO^2 m^{-2} s^{-1})**	**Stomatal conductance (mol H_2O m^{-2} s^{-1})**	**Intercellular CO_2 (μmol mol^{-1})**	**Transpiration (mmol H_2O m^{-2} s^{-1})**	**Instantaneous water use efficiency (μmol CO^2 / mmol H_2O)**	**Afternoon needle water potential (MPa)**	**Respiration (μmol CO^2 m^{-2} s^{-1})**
Among treatments	Low water	3.00(0.33)^a	0.037(0.005)^a	328.5(54.0)	0.86(0.11)^a	3.98(0.35)a	-1.65(0.08)^a	-0.40(0.07)
	Alternating water	4.11(0.39)^b	0.067(0.007)^b	280.4(11.59)	1.62(0.19)^b	3.46(0.23)ab	-1.26(0.06)^b	-0.41(0.03)
	High water	7.41(0.29)^c	0.125(0.008)^c	265.4(6.7)	3.07(0.22)^c	3.13(0.14)b	-0.94(0.05)^c	-0.55(0.09)
	p-value	**$p<0.0001$**	**$p<0.001$**	$p=0.4667$	**$p<0.0001$**	**$p=0.0103$**	**$p<0.0001$**	$p=0.4038$
Among Clones	Clone 1	4.99(0.36)	0.073(0.007)	252.0(15.3)	1.79(0.18)	3.84(0.26)	-1.15(0.06)^a	-0.52(0.05)
	Clone 2	4.93(0.44)	0.080(0.009)	293.3(20.4)	1.96(0.23)	3.45(0.32)	-1.36(0.10)^b	-0.39(0.06)
	Clone 3	4.81(0.42)	0.081(0.009)	325.2(43.7)	1.92(0.23)	3.21(0.16)	-1.31(0.06)^b	-0.45(0.08)
	p-value	$p=0.8416$	$p=0.5118$	$p=0.1035$	$p=0.7188$	$p=0.0756$	**$p=0.0041$**	$p=0.2504$

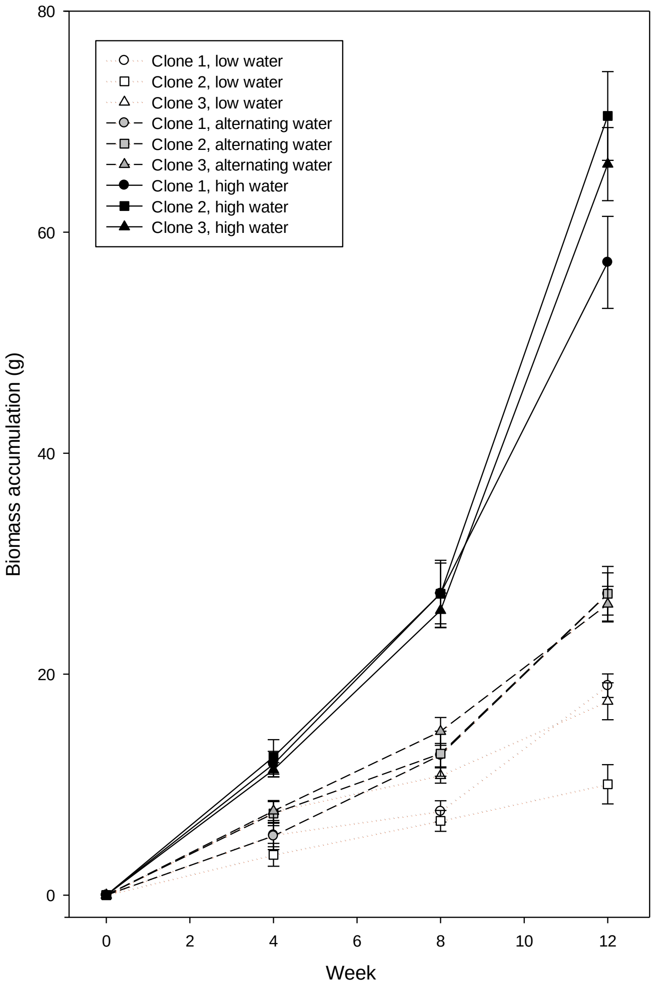

Figure 2.1. Mean biomass accumulation (SE) at three harvest dates. Harvests occurred at 4-weeks intervals. Solid lines and filled black symbols represent the high water treatment (-0.3 MPa), dotted lines with unfilled symbols represent the low water treatment (-1.5 MPa), and dashed lines and grey symbols represent the alternating water treatment. Circles, squares and triangles represent Clone 1, Clone 2, and Clone 3.

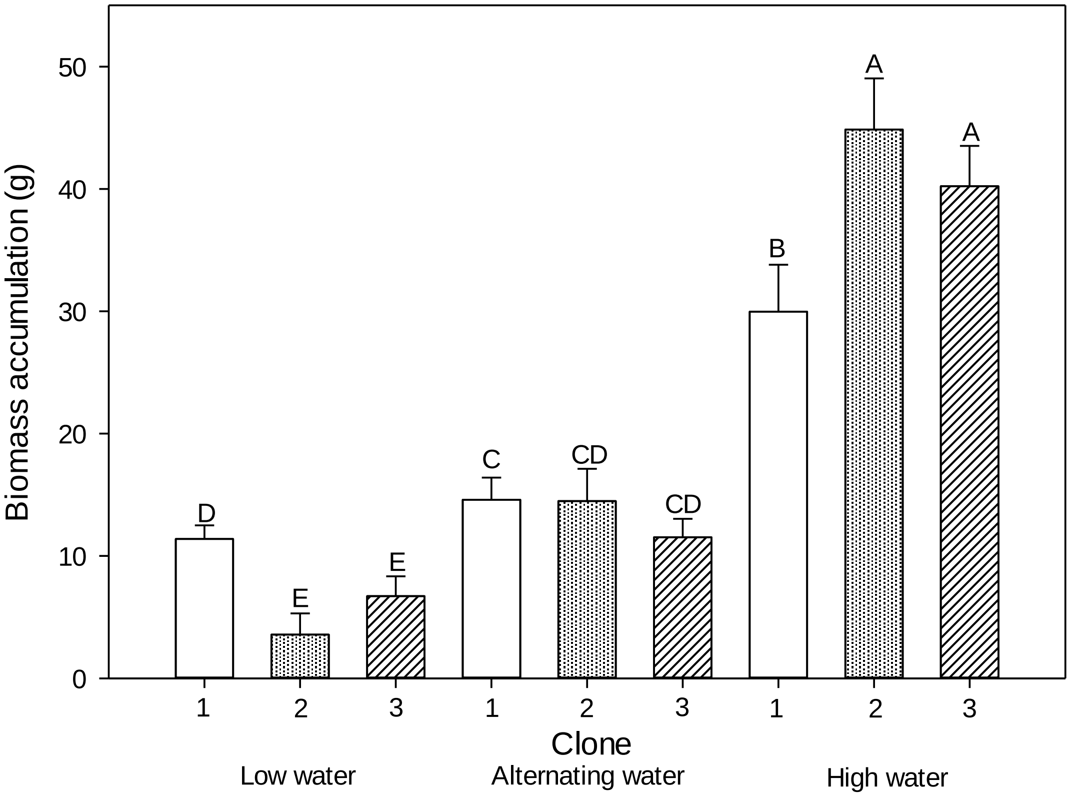

Figure 2.2. Mean biomass accumulation between week 8 and 12. Different letters indicate significant differences among clones within and among treatments (α=0.05). Unfilled bars, filled bars, and slashed bars represent low water, alternating water and high water treatments, respectively. Standard error represented by thin vertical bars.

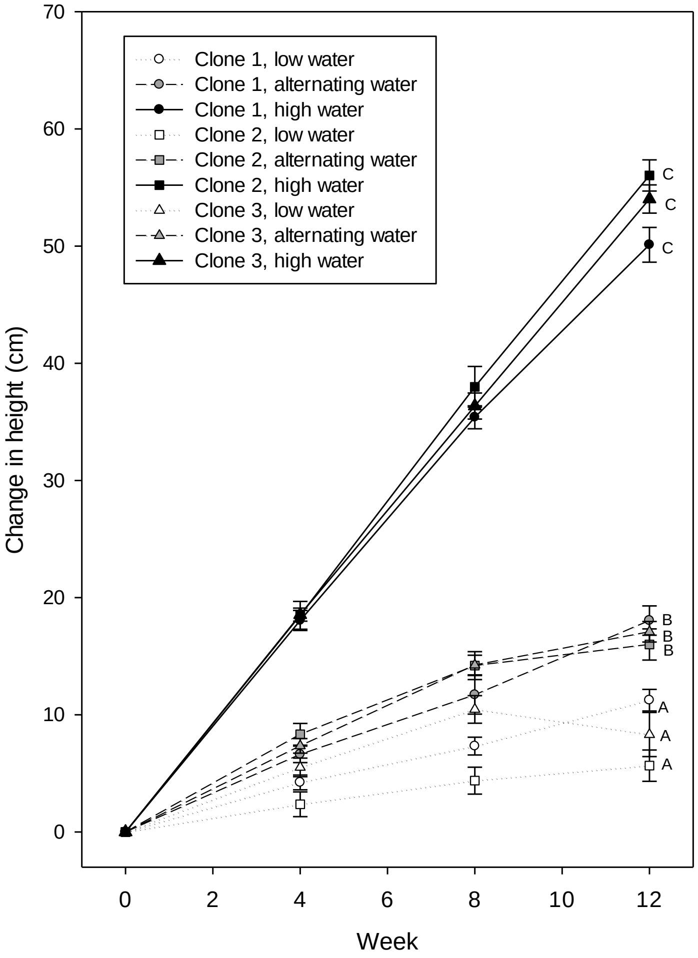

Figure 2.3. Mean height growth measured every 4 weeks over the course of the experiment. Solid lines and filled black symbols represent the high water treatment (-0.3 MPa) the, dotted lines with unfilled symbols represent the low water treatment (-1.5 MPa), and dashed lines and grey symbols represent the alternating water treatment. Circles, squares and triangles indicate Clone 1, Clone 2, and Clone 3, respectively. Standard error represented by thin vertical bars on symbols. Letters denote significant differences at α=0.05.

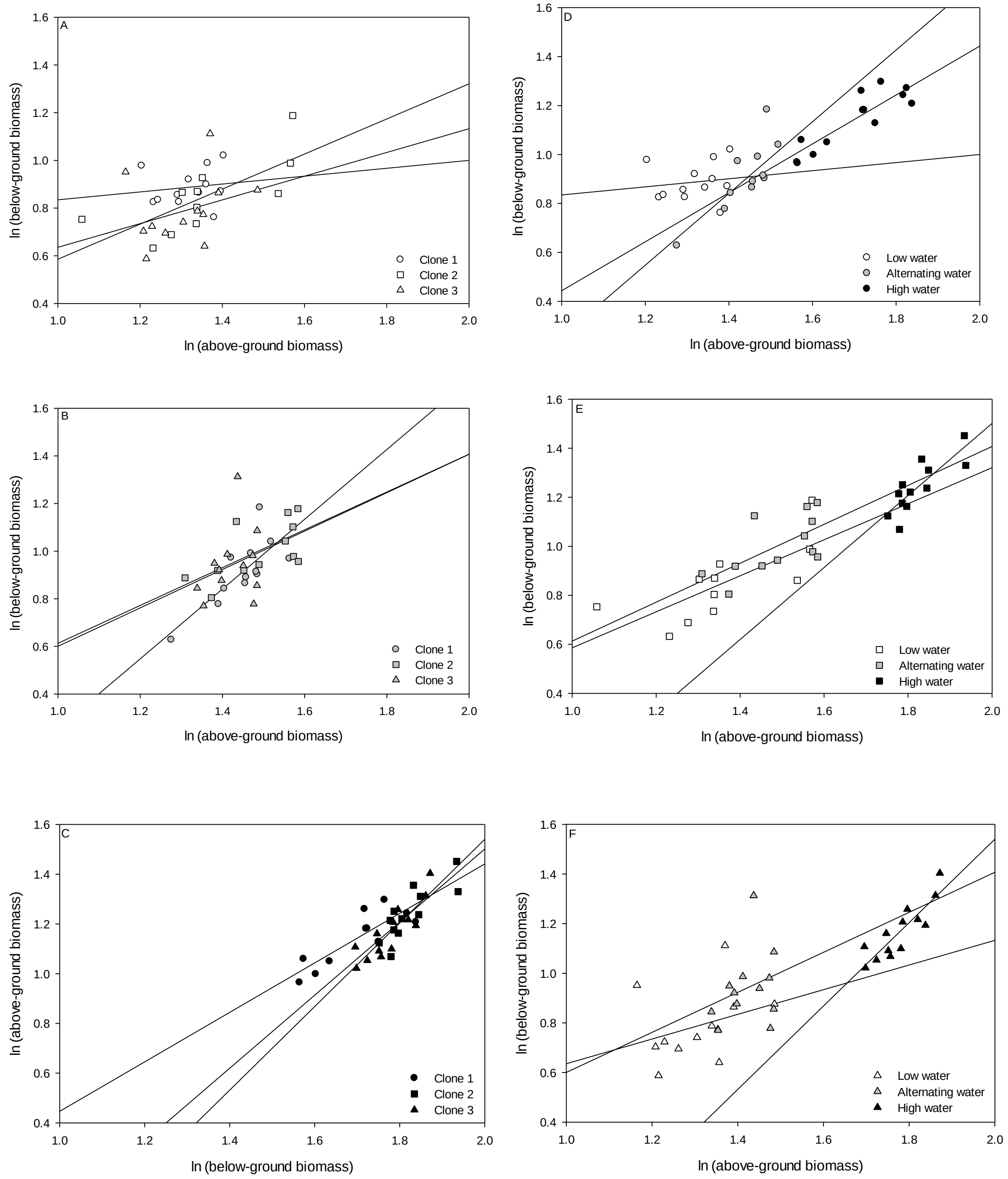
A
ln (below-ground biomass)
ln (above-ground biomass)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
1.0
1.2
1.4
1.6
1.8
2.0
○ Clone 1
□ Clone 2
△ Clone 3
D
ln (below-ground biomass)
ln (above-ground biomass)
○ Low water
● Alternating water
● High water
B
ln (below-ground biomass)
ln (above-ground biomass)
● Clone 1
■ Clone 2
▲ Clone 3
E
ln (below-ground biomass)
ln (above-ground biomass)
□ Low water
■ Alternating water
■ High water
C
ln (above-ground biomass)
ln (below-ground biomass)
● Clone 1
■ Clone 2
▲ Clone 3
F
ln (below-ground biomass)
ln (above-ground biomass)
△ Low water
▲ Alternating water
▲ High water

Figure 2.4. Relationship between final root and shoot biomass among clones in low water treatment (A), alternating water treatment (B), and high water treatment (C); and among water treatments within Clone 1 (D), Clone 2 (E), and Clone 3 (F). Significant differences were found among clones in the high water treatment (C) and among treatments in Clone 1 (D). Allometric coefficients (k) are shown in table 1.

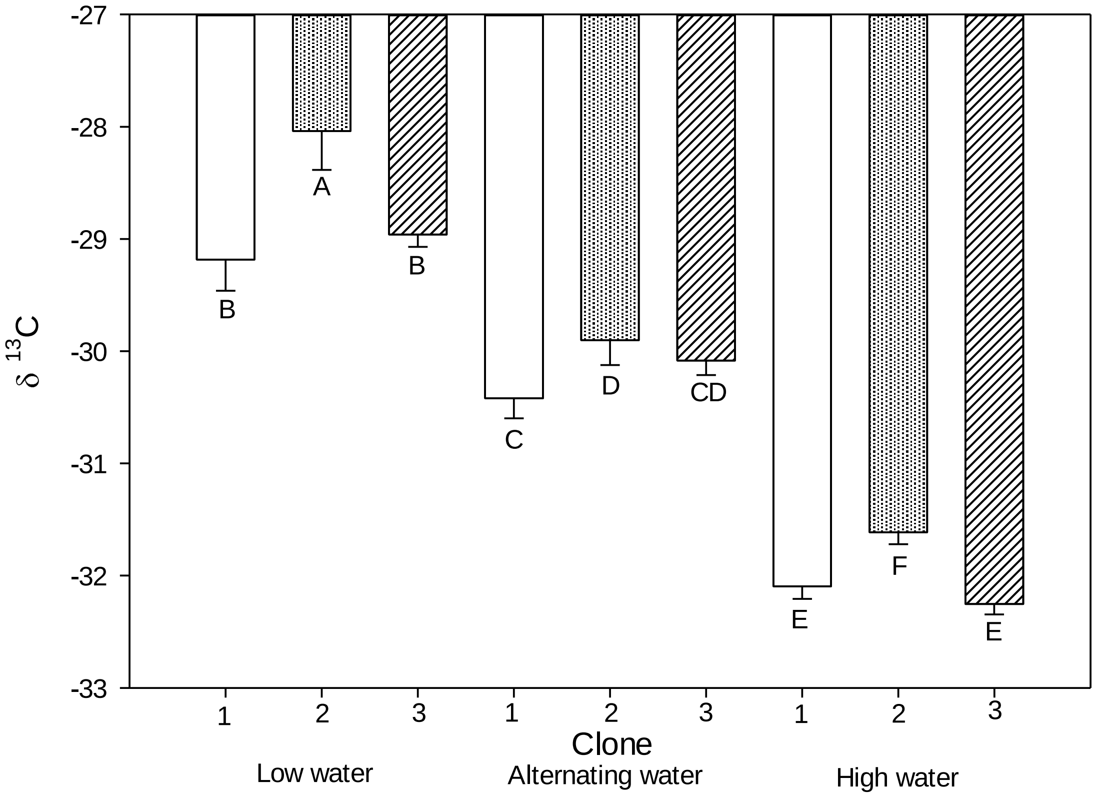

Figure 2.5. Mean carbon isotope discrimination ($\delta^{13}C$) among clones and treatments at week 12 of the experiment. Different letters indicate significant differences among clones within and among treatments ($\alpha=0.05$). Unfilled bars, filled bars, and slashed bars represent Clone 1, Clone 2 and Clone, respectively. Standard error represented by thin vertical bars.

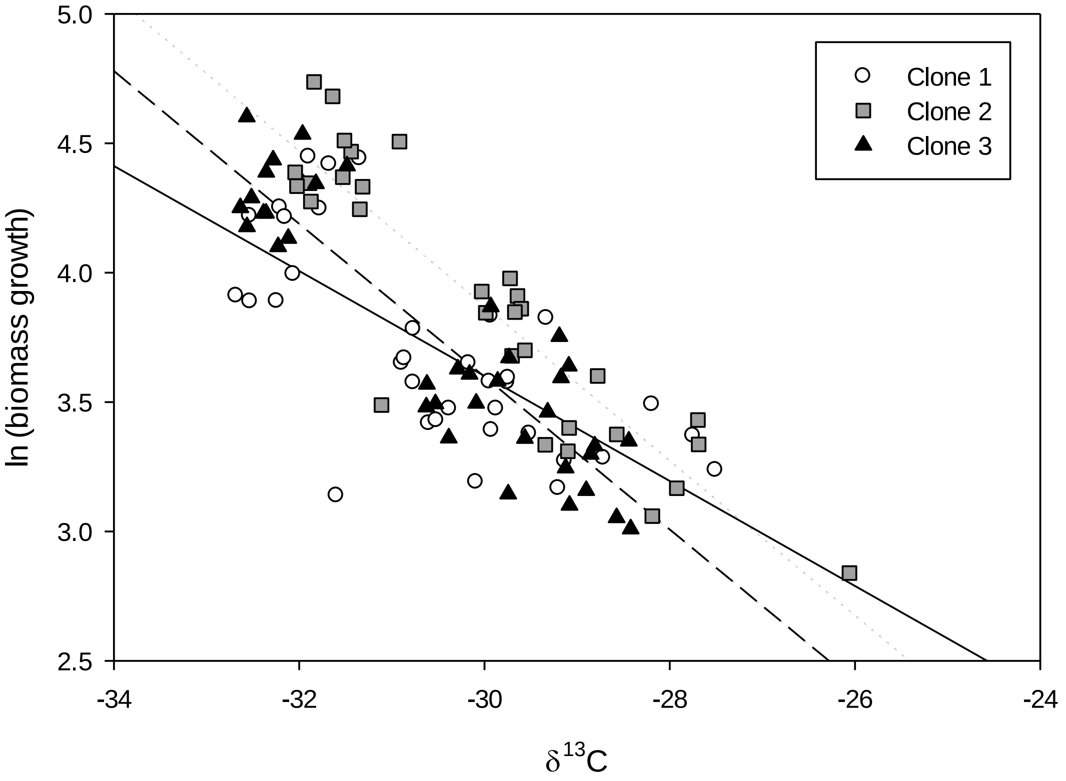

Figure 2.6. The relationship between biomass accumulation and $\delta^{13}C$ in the three clones. Circles, squares and triangles indicate Clone 1, Clone 2 and Clone 3. Solid, dotted and dashed lines indicate Clone 1, Clone 2 and Clone 3. Parameters for the slopes are shown in Table 2.

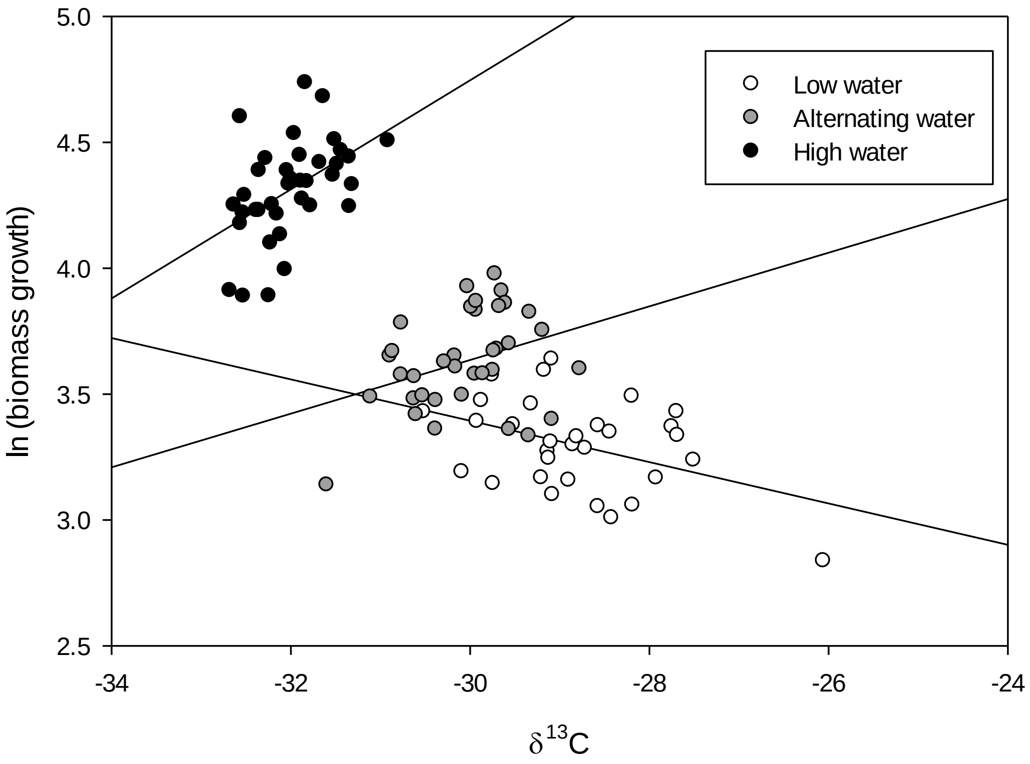

Figure 2.7. The relationship between biomass accumulation and δ^{13}C and treatment. Unfilled, grey and black symbols indicate the low water, alternating water and high water treatments. Parameters for the slopes are shown in Table 2.

CHAPTER 3[2]

PHYSIOLOGICAL ATTRIBUTES OF THREE- AND FOUR-NEEDLE FASCICLES OF LOBLOLLY PINE (*PINUS TAEDA* L.)

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[2] Ingwers MW, Urban J, McGuire MA, Bhuiyan, RA and Teskey RO. Submitted to *Trees Structure and Function*.

Abstract

In *Pinus* species, fascicles can develop with a different number of needles than what is typical. For example, *Pinus taeda* fascicles typically have three needles, but sometimes have two or four. Although differing fascicle morphology could be a response to changes in the environment designed to optimize carbon gain or minimize water loss, we are unaware of any work comparing physiological differences between fascicles with different numbers of needles. We compared the physiological and morphological characteristics of three- and four-needle fascicles of a loblolly pine clone with an abnormally high abundance of four-needle fascicles to better understand whether differences in needle morphology affected photosynthetic capacity or transpiration. Three- and four-needle fascicles had equal length, diameter, and volume, but four-needle fascicles had significantly greater surface area, mass, and tissue density. Equal fascicle total volume resulted in smaller per-needle volume in four-needle fascicles compared to three-needle fascicles. On a unit surface area basis, light saturated net assimilation, stomatal conductance and transpiration were similar between the three- and four-needle fascicles although the maximum rate of carboxylation (Vc_{max}) was significantly greater in four-needle fascicles. On a per-fascicle basis, four-needle fascicles had greater transpiration, stomatal conductance, and maximum rate of light saturated net assimilation. Our results suggest that several factors, including increased tissue density and stomatal density, offset the reduction in needle volume in four-needle fascicles, resulting in similar levels of gas exchange per unit surface area in three- and four-needle fascicles.

Introduction

Loblolly pine (*Pinus taeda*) is a tree species of major economic importance, with an estimated 13.4 million hectares planted in the Southeastern United States (Schultz, 1997). Approximately 95% of all currently planted loblolly pine seedlings are genetically improved, and full-sib families, half-sib families, and clonal varieties are widely distributed across the region (McKeand et al., 2003). For this reason, there is interest in determining physiological, morphological, or anatomical features that could be used to select genotypes or clones with improved growth rates. Loblolly pine typically has three needles per fascicle (Schultz, 1997); however, two-, four-, and five-needle fascicles (hereafter referred to as different fascicle types) have been observed (Zutter et al., 1986). For loblolly pine, little is known about fascicle type distribution, the relative abundance of different fascicle types per tree, environmental effects on fascicle type abundance, or whether fascicle types differ in physiological traits such as net assimilation and stomatal conductance.

The adaptive importance of needle number and morphology has been demonstrated in Ponderosa pine (*Pinus ponderosa*) populations, which exhibit regional differences in the proportions of two-needle, three-needle, and five-needle fascicles (Shaw, 1914). In Ponderosa pine, a higher abundance of two-needle compared with three-needle fascicles has been observed in arid areas (Haller, 1965), and more three-needle compared with five-needle fascicles are found in various taxa of Ponderosa pine found at higher elevations (Epperson et al., 2001). Needles of Ponderosa pine populations in more arid areas have also been reported to be shorter, with smaller surface area and lower stomatal density relative to populations in wetter areas (Cregg, 1994). Studies on one- and two-needled pinyon pines (*Pinus* subsection *Cembriodes*) indicated that one-needle fascicles were more prevalent than two-needle fascicles in populations in arid environments, consistent with the observations for Ponderosa pine (Tausch and West, 1987; Cole et al., 2008). While it has been inferred that the lower surface area-to-volume ratio associated with fewer needles per fascicle may be an important adaptation to low moisture environments (Haller, 1965; Tausch and West, 1987; Cregg, 1994; Cole et al., 2008), direct physiological evidence for this relationship is lacking.

Here, we investigate the photosynthetic capacity of four-needle compared with three-needle fascicles in *Pinus taeda*, focusing primarily on a clone with a high proportion of four-needle fascicles. This clone also had slower growth than similar clones with an abundance of three-needle fascicles. We hypothesized that four-needle fascicles, compared with three-needle fascicles, would have greater mass, surface area, and/or tissue density and that these differences would affect light saturated net assimilation, stomatal conductance, and transpiration.

Methods

Plant material and growth conditions

One-year-old container-grown loblolly pine ramets were obtained from a production nursery (Arborgen Inc., Summerville, SC, USA) in March, 2013. These ramets had been propagated by tissue culture from three half-sibling clones, representing individuals from the Atlantic coastal plain. The three clones shared the same female parent, but each resulted from a controlled cross with a different male parent. Clone 1 had a large number of four-needle fascicles, while three-needle fascicles were dominant on Clones 2 and 3. Ramets were transplanted into 4.3 L tree pots (model CP612R, Steuwe and Son, Inc., Tangent, OR, USA) in a soilless mixture of fritted clay granules (Turface Field and Fairway, Profile Products LLC, Buffalo, IL, USA) and fine sand (3:1, v:v) in March 2013. Prior to initiation of the experiment, ramets were grown for one year in a greenhouse at Whitehall Forest, a research unit of the University of Georgia in Athens, GA, USA. During that time period, temperature in the greenhouse fluctuated with and approximately tracked outdoor ambient temperature. The mean annual temperature was 15.5 ^{o}C (www.usclimatedata.com). Ramets were manually watered to field capacity daily and fertilized three times with 40 g of 15-9-12 extended release fertilizer (Osmocote Plus #903286, Scotts-Sierra Horticultural Products, Marysville, OH, USA) and 0.2 g of chelated iron (Sprint 138, Becker Underwood, Ames, IA, USA).

Comparisons among clones: Fascicle type distribution, biomass accumulation and gas exchange

To verify our observation of an unusual fascicle type distribution in Clone 1, we compared it with two closely related clones (Clones 2 and Clone 3) grown under the same conditions. Total percent fascicle type per tree and the density of fascicle type per unit branch length and unit branch area were estimated for six ramets of each of the three clones. Each individual tree was divided into four to seven segments based on the number of growth whorls present. Within each of these segments, a smaller 5 cm segment was randomly selected for determination of three- and four-needle fascicle abundance and density. The value for each 5 cm segment was then scaled to its larger segment, and the larger segment values were summed to obtain whole-tree estimates. Fascicle surface area (SA_f) and volume (V_f) were calculated for ten fascicles in each of the 5 cm segments. Fascicle diameter was measured to 0.1 mm with a scaled 10x hand lens (PEAK Model 1983, SPI Supplies, West Chester PA, USA). Whole loblolly pine fascicles have a circular cross sectional area which is divided radially into multiple needles, with each needle having 2 straight sides (the radii of the circle) and one curved side (the partial circumference of the circle). Fascicle surface area and volume were therefore calculated as:

$$SA_f = l\,(2\pi r+2Nr) \qquad (1)$$

$$V_f = \pi r^2 l \qquad (2)$$

Where l = length of fascicle, r = average radius of needles, and N = number of needles per fascicle. Taper at the distal end of the fascicle was not accounted for because it was minuscule in length and assumed to be equivalent among all fascicles. We estimated fascicle surface area for each branch segment by multiplying the estimated number of fascicles by the average surface area per fascicle in the segment. The ratio of fascicle surface area to branch length and branch surface area were calculated by dividing the estimated fascicle surface area by branch length and branch surface area (SA_b). Branch surface area was estimated by:

$$SA_B = L2\pi R \qquad (3)$$

Where L= length of branch segment and D=diameter of branch segment.

Biomass accumulation for each of the three clones was determined from harvests at the beginning and end of a 12-week period from June 15, 2013 to September 7, 2013. Twelve ramets of each clone were harvested at each date, dried at 65 °C to constant mass, and weighed on a balance. Biomass accumulation over the twelve weeks was calculated for each clone by subtracting mean biomass at the first harvest from mean biomass at the second harvest.

To determine differences in net assimilation (A_{net}, μmol CO_2 $m^{-2}s^{-1}$) among clones, we measured midday (9:00-11:00) net assimilation on 3 saplings of each clone in weeks 1, 8 and 12 of the previously described 12 week growing period. Net assimilation was measured with a portable photosynthesis system (Model LI-6400; Li-Cor, Lincoln, NE, USA) equipped with a 2x3 cm broadleaf cuvette. Cuvette conditions were set to 1000 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR), 400 μmol mol^{-1} CO_2, and 30 °C block temperature. Cuvette humidity was adjusted to match that of the greenhouse (i.e. 50%). All measurements were made on three-needle fascicles for comparison among clones.

The analyses described in this section were the only analyses conducted among clones. All other analyses described in this study were conducted between three and four-needle fascicles of ramets of Clone 1.

Comparisons between fascicle types within Clone 1: *Physiological and anatomical measurements*

Gas exchange and water potential measurements were made on pairs of adjacent three- and four-needle fascicles on six saplings of Clone 1 from March 3 to 17, 2014. The six saplings were approximately the same size with an average basal diameter of 1.4 (SE±0.1) cm and height of 84.0 (SE±1.6) cm. Five days prior to the start of measurements, the plants were transported to walk-in growth chambers (EGC 36, Environmental Growth Chambers, Chagrin Falls, OH, USA) located ~ 7 km from the greenhouse. Growth chamber conditions were: relative humidity = 45%, day/night temperature = 30/25 °C, and light = 520 µmol m^{-2} s^{-1} PAR measured at the top of the saplings, with a 12-hour light/dark cycle.

Gas exchange parameters were measured on pairs of three and four-needle fascicles selected from the most recently developed fully expanded flush of each sapling (n=6 trees) with a portable photosynthesis system and 2x3 cm broadleaf cuvette. For each measurement, one fascicle was arranged in the cuvette on a flat plane with ~5 mm between needles to maximize light interception of each needle. All gas exchange results were calculated based on the surface area of the fascicle (either three or four needles) enclosed in the cuvette (e.g. µmol m^{-2} s^{-1}). Measurements were repeated on a second pair of fascicles per tree and results were averaged per fascicle type per tree prior to statistical analysis.

To investigate how changes in needle morphology could theoretically impact whole-fascicle physiology, measurements were also scaled to the whole fascicle (e.g. µmol fascicle^{-1} s^{-}

^{1}) by multiplying gas exchange per unit surface area by the total length of the fascicle. This measurement estimates theoretical differences in gas exchange capacity between three- and four-needle fascicles, as it does not account for mutual shading of needles that occurs in field conditions.

Assimilation (A) to intercellular [CO_2] (C_i) curves (A/C_i) were generated from gas exchange measurements as described above. Cuvette conditions were set to 1800 μmol m^{-2} s^{-1} PAR and 30^{o}C block temperature. Cuvette humidity was adjusted to match that of the growth chamber (i.e. 45 %). Fascicles were allowed to acclimate to cuvette conditions for approximately 20 minutes (until A stabilized) prior to measurements. Thirty-six measurement steps were used to construct A/C_i curves. Measurements began at ambient CO_2 concentration (400 μmol mol^{-1}), which was then decreased to 100 μmol mol^{-1} in 100 μmol mol^{-1} steps; then decreased to 50 μmol mol^{-1}; then increased from 100 μmol mol^{-1} to 1600 μmol mol^{-1} in 100 μmol mol^{-1} steps; and finally decreased again to 400 μmol mol^{-1}. Maximum rates of carboxylation (Vc_{max}), photosynthetic electron transport (J_{max}) and triose-phosphate utilization (V_{TPU}) were calculated using the A/C_i Curve Fitting Utility version 1.1 (Long and Bernacchi, 2003)**.** Measured parameters were maximum light-saturated (i.e. PAR=1800) net assimilation (A_{sat}, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration ($C_{i,}$ μmol CO_2 mol air^{-1}). Instantaneous water use efficiency (WUE_i) was calculated by dividing A_{sat} by E. These measurements of light-saturated gas exchange (PAR=1800) were scaled to the whole fascicle and compared between three-and four-needle fascicles under conditions that approximated ambient CO_2 concentration (400 μmol mol^{-1}) and CO_2 saturation (1600 μmol mol^{-1}).

Light response curves were also measured at a CO_2 concentration of 400 µmol mol^{-1} as described above. Fascicles were allowed to equilibrate in the cuvette at 1800 µmol m^{-2} s^{-1} PAR for approximately 20 minutes prior to measurements. Measurements were taken at 1800, 1500, 1200, 900, 800, 700, 600, 500, 400 300, 200, 100, 50 and 0 µmol m^{-2} s^{-1} PAR. A light response curve-fitting tool (Lobo et al., 2013) was used to calculate maximum photosynthetic rate (A_{max}), apparent quantum yield (ϕ), dark respiration (R_d) and light compensation point. The shape of the responses was approximated by rectangular hyperbolas.

When gas exchange measurements were complete, fascicle length and diameter were measured as described above to determine total fascicle surface area and volume, as well as fascicle surface area enclosed in the cuvette. Fascicles were weighed on a balance to determine fresh mass, dried at 65 °C for 72 hours, and weighed again to determine fascicle dry mass. Fascicle water content (%) was calculated as the difference between fresh and dry mass. Fascicle tissue density was calculated by dividing fascicle fresh volume (calculated from measurements of needle dimensions) by fascicle dry mass. Specific leaf area (SLA) was calculated by dividing fascicle fresh surface area by fascicle dry mass. Dried samples were then ground to a powder and analyzed for $^{13}C/^{12}C$ ratio, percent nitrogen, and carbon:nitrogen (C:N) ratio at the Stable Isotope and Soil Biology Laboratory, Odum School of Ecology, University of Georgia, Athens, GA, USA.

Needle xylem pressure potential, which was interpreted as needle water potential, was measured both pre-light and in the middle of the light period (midday, when plants were photosynthesizing and gas exchange measurements were underway) with a pressure chamber (Model 600, PMS Instruments, Corvallis, Oregon, USA).

To determine stomatal density, needle surface casts were made using a cyanoacrylate-based resin (Loctite Super Glue Liquid, Henkel, Westlake OH, USA) and viewed and photographed at 40x magnification under a light microscope (BX 51, Olympus, Tokyo, Japan) equipped with a digital camera (DP70, Olympus, Tokyo, Japan). The number of stomata was counted using an image-processing program (Image J v. 1.48u4, Wayne Rasband, National Institutes of Health, USA) to determine stomatal density (stomata cm^{-2}). Light microscopy was also used to view cross sections of needles from adjacent three- and four-needle fascicles of each sapling. Cross sections were prepared by first dehydrating needles in increasing concentrations of 35, 50, 60, 75 and 85 % ethanol and then fixing them using an embedding kit (JB-4, Polysciences, Warrington, PA, USA). Cross-sections (6µm thick) were cut from polymerized samples and stained using 1% acid fuchsin and 0.02% toluidine blue and viewed and photographed as described above.

Statistical Analysis

Differences in biomass accumulation and fascicle type distribution among clones were analyzed using an ANOVA in SAS (version 9.3.1; SAS Institute Inc., Cary, NC, USA). Differences in A_{net} among clones were assessed using a repeated measures analysis of covariance using PROC MIXED (Littell et al., 1996). Denominator degrees of freedom (DDFM) were calculated using the Kenward-Roger adjustment (Kenward and Roger, 1997). Paired t-tests were used for comparisons between three and four-needle fascicles (n=6 trees per comparison) (Sigma Plot 12.5, Systat Software, San Jose, CA, USA). All data passed a test for normality. A type-I error rate of 0.05 was used for all analyses.

Results

Differences among clones

Clone 1 averaged 42.8 % four-needle fascicles, while Clone 2 and Clone 3 averaged only 1.1 and 1.6 % four-needle fascicles, respectively (Table 3.1). Total number of fascicles and total fascicle surface area did not differ among the three clones per unit branch length. However, Clone 1 had fewer fascicles than Clone 2 (p=0.013) per unit branch surface area. The ratio of fascicle surface area to branch surface area was similar among all three clones. Biomass accumulation was lower in Clone 1 than in the other two clones. Net assimilation of three needle fascicles did not differ among clones.

Comparison of morphological and anatomical attributes between fascicle types of Clone 1.

Fascicle surface area, dry mass, and tissue density were significantly higher for four-needle fascicles than for three-needle fascicles (Table 3.2). No significant differences were observed in fascicle length, diameter, specific leaf area, or percent water content. Fascicle volume also did not differ between fascicle types, resulting in smaller volume of individual needles of four-needle fascicles compared with needles of three-needle fascicles. Greater dry mass of the four-needle fascicles resulted in no difference in specific leaf area between three- and four- needle fascicles despite greater fascicle surface area of four-needle fascicles. Stomatal density on the inside (adaxial) and the outside (abaxial) needle surfaces did not differ between three- and four-needle fascicles (Table 3.2). For three-needle fascicles, stomatal density was significantly higher on the abaxial than on the adaxial needle surface (p=0.004); however, no difference was observed between the abaxial and adaxial needle surfaces for the four-needle fascicles (p=0.918). The total number of stomata per needle did not differ between the three- and four-needle fascicles (p=0.270), which resulted in a greater total number of stomata for the four-needle fascicles due to additional surface area provided by the extra needle. The smaller volume of individual needles of the four-needle fascicles also resulted in higher stomatal density per unit volume compared with three-needle fascicles ($p<0.001$). Percent nitrogen and C:N were similar between fascicle types, however four-needle fascicles had a higher concentration of nitrogen per unit volume compared with three-needle fascicles (Table 3.3).

Microscopy was used to determine if there were major morphological differences between needles of three- and four-needle fascicles and to determine how the addition of the fourth needle of four-needle fascicles affected needle cross-sectional area. Needle cross sections revealed that needles of four-needle fascicles, like needles of three-needle fascicles, had two vascular bundles and three resin ducts and similar overall morphology (Figure 3.1). However, while individual needles of three-needle fascicles tended to be similar in cross-sectional area and shape (Figure 3.1A), cross-sectional area of individual needles of four-needle fascicles tended to be more variable. Some four-needle fascicles contained a single needle with a cross-sectional area that was greater than or equal to the other needles in the fascicle (Figure 3.1B), while other four-needle fascicles contained a single needle with a cross sectional area that was much less than the other needles in the fascicle (Figure 3.1C).

Comparison of physiological characteristics between fascicle types of Clone 1

A comparison of the light response curves of three- and four-needle fascicles indicated that there were no differences in A_{max}, ϕ, R_d, or light compensation point on a surface area basis between the fascicle types (Figure 3.2, Table 3.2). A comparison of the A/C_i curves measured on needles of three- and four needle fascicles indicted that there were no significant differences in J_{max} or V_{TPU} (Table 3.3, Figure 3.3). Internal CO_2 concentration (C_i) also did not differ between fascicle types (Table 3.4). However, Vc_{max} was significantly higher in needles of four-needle fascicles compared with needles of three-needle fascicles.

Other physiological attributes, including pre-light and midday xylem water potential, nitrogen concentration per unit mass, and carbon isotope ratio, were not significantly different between needles of three- and four-needle fascicles (Table 3.3). However, equivalent nitrogen concentration per unit mass resulted in significantly greater nitrogen concentration per unit volume in four-needle fascicles due to greater needle tissue density in four-needle fascicles compared with three-needle fascicles.

Comparison of whole-fascicle gas exchange between fascicle types of Clone 1

When scaled to a whole-fascicle basis, four-needle fascicles had significantly higher A_{sat} and E and marginally higher g_s (p=0.054) compared with three-needle fascicles when measured under CO_2- saturated conditions ($[CO_2]$ =1600) (Table 3.4). Under ambient $[CO_2]$ conditions ($[CO_2]$=400), E was significantly higher in the four-needle fascicles, while A_{sat} and g_s were only marginally higher (p=0.062 and p=0.054).

Discussion

Comparison of gas exchange per unit surface area and morphological attributes between fascicle types of Clone 1

We hypothesized that mass, surface area, and tissue density would differ between three- and four-needle fascicles and that these differences would affect photosynthetic capacity. While mass, surface area, and tissue density differed between the fascicle types, these differences did not significantly affect A_{sat} or most aspects of photosynthetic capacity on a surface area basis. The increase in tissue density in the four-needle fascicles did not translate to a decrease in SLA, contrary to observations across many species (Niinemets, 1999), due to a concomitant increase in fascicle surface area resulting from the presence of the additional needle. Assimilation per unit surface area was within the range of assimilation values previously reported in two and three year-old loblolly pine ramets (Tyree et al., 2009a; Tyree et al., 2009b; Aspinwall et al., 2011), seedlings (Tissue et al., 1993; Will and Teskey, 1997), and trees (Samuelson et al., 2008).

In our study, when the thinner needles of the four-needle fascicle were compared to the thicker needles of the three-needle fascicles, the thinner needles of the four-needle fascicles did not display the increase in R_d or ϕ or decrease in A_{sat} that might be expected. A possible explanation for the lack of increase in ϕ and decrease in A_{sat} is that the increase in four-needle tissue density offset the reduction in needle thickness, resulting in similar light transmission through needles of three and four-needle fascicles. The lack of increase in R_d may also be explained by the increase in tissue density, which may have resulted in a higher concentration of respiring cells per unit volume. There could be several explanations for differences in tissue density between fascicle types. For example, components of the epidermis, which appear to occupy a greater proportion of the needle cross sectional area in the four-needle fascicles, may have greater mass than other tissues which could result in a greater tissue density in the four-needle fascicles. In addition, cells of the mesophyll may be more closely packed with less air and water space between them (Roderick et al., 1999a; Roderick et al., 1999b). Individual needles of four-needle fascicles had smaller volume but the same SLA compared with needles of three-needle fascicles. Smaller and thinner needles in conifers have been considered an adaptation to lower light or shade-acclimated conditions (Richardson et al., 2000; Richardson et al., 2001; Niinemets et al., 2002; Cescatti and Zorer, 2003; Ishii et al., 2003; Niinemets et al., 2007; Poorter et al., 2009). When needles at different canopy levels in Scots pine (*Pinus sylvestris*, L.) were compared, thinner needles in the lower canopy had lower R_d (Palmroth and Hari, 2001). These findings, however, may be due to differences in light acclimation not present in our study.

A study in American sweetgum (*Liquidambar styraciflua*) also found a higher A_{sat}, R_d and ϕ in the thicker light-adapted sun leaves when compared to the thinner shade leaves (Herrick and Thomas, 1999).

Nitrogen concentration was similar on a mass basis in the two fascicle types. Therefore, higher tissue density in the four-needle fascicles resulted in an increase in nitrogen per unit volume. In a meta-analysis of numerous species, a positive correlation between nitrogen per unit mass and leaf tissue density was established (Niinemets, 1999). The higher concentration of nitrogen on a unit volume basis could explain the higher Vc_{max} in four- needle fascicles. Although a positive correlation between Vc_{max} and nitrogen concentration per unit volume has been previously shown in Japanese red pine (*Pinus densiflora*) (Han, 2011) and many other species, the increase in Vc_{max} we observed was unexpected, because increases in leaf tissue density can increase the resistance to diffusion of CO_2 to RuBisCO carboxylation sites (Niinemets, 1999). However, if leaves with higher density have greater foliar nitrogen concentration per unit volume, the increase in diffusive resistance might be offset by a greater concentration of RuBisCO. In addition, the average length of the diffusion pathway for CO_2 to reach RuBisCO may have decreased in four-needle fascicles due to smaller needle volume and higher stomatal density per unit volume.

Comparison of whole-fascicle gas exchange between fascicle types of Clone 1

Gas exchange on a surface area basis was scaled to the whole fascicle to investigate how changes in needle morphology could theoretically affect gas exchange capacity. Notably, these measurements reflect ideal gas exchange conditions that are not achieved in nature due to mutual shading of needles within and among fascicles. This study did not investigate how mutual shading of needles differs between fascicle types or how the presence of four-needle fascicles affects intercepted radiation. When gas exchange was scaled to the whole fascicle, four-needle fascicles had substantial increases in A_{sat} and E compared with three-needle fascicles, consistent with the finding that the ratio of leaf surface area to volume is correlated with photosynthetic capacity (Roderick et al., 1999a). Theoretically, assuming no difference in mutual shading between fascicle types, if a tree were to replace all of its three-needle fascicles with four-needle fascicles it would have a higher capacity for assimilation, essentially due to greater needle surface area. Comparisons among hybrids with different leaf types in the willow genus (*Salix*), found a strong correlation between individual leaf area and biomass productivity, suggesting that leaf traits may be useful in predicting productivity (Robinson et al., 2004). In that study, the hybrid with thinner leaves and greater individual leaf area was higher yielding, despite having lower J_{max} and Vc_{max}. However, when analyses were conducted on data from juvenile trees of 271 different species, there was only a weak positive correlation between SLA and relative growth (Paine et al., 2015).

Fascicle size, morphology, and type distribution among clones

In our study, despite greater A_{sat} on a per-fascicle basis, growth was lower in the clone with an abundance of four-needle fascicles compared with clones with an abundance of three-needle fascicles. Whole-plant biomass growth in loblolly pine has been strongly correlated with total intercepted radiation (Will et al., 2005; Akers et al., 2013). It is possible that mutual shading of needles was greater in saplings with an abundance four-needle fascicles, resulting in reduced intercepted radiation and lower growth compared with trees with an abundance of three-needle fascicles. Clone 1, with abundant four-needle fascicles, appeared to maintain a similar amount of total needle surface area compared with Clone 2 and Clone 3, because Clone 1 had greater mean surface area per fascicle, but fewer fascicles overall. It is conceivable that fewer fascicles, with higher surface area per fascicle, would result in more light penetration through the tree canopy and less self-shading, but this concept is not supported by our growth results. It is also conceivable that an abundance of four-needle fascicles might increase growth due to increased A_{sat}. Although the abundance of four-needle fascicles in Clone 1 was associated with lower biomass accumulation under the growth conditions in this study, four-needle fascicles could prove advantageous in a different environment.

Saplings of Clone 1 had significantly more four-needle fascicles than saplings of Clones 2 and 3 and a higher proportion of four-needle fascicles than previously reported in other studies (Shelton and Switzer, 1984; Zutter et al., 1986). The genetic contributions of the parent trees, or perhaps genetics in combination with the specific environmental conditions in this study, likely influenced the abundance of four-needle fascicles observed in Clone 1. Genetic influences on fascicle type distribution have been demonstrated in common garden experiments comparing Ponderosa pine seedlings from different geographic origins (Weidman, 1939) and different taxa in *Ponderosae*, a subsection of the genus *Pinus* (Rehfeldt, 1999). Both of those studies found that the location of the seed source influenced fascicle type distribution, indicating a heritable genetic component for fascicle needle number and suggesting that if fascicle type distribution proves advantageous, it could be selected for in breeding programs.

Conclusions

Results of this study showed that different fascicle types had different total fascicle surface area and needle tissue density, but similar specific leaf area and fascicle volume. Photosynthetic capacity on a unit surface area basis was similar between fascicle types, however, when scaled to the whole fascicle, four-needle fascicles had greater A_{sat} and E compared with three-needle fascicles. In addition, Vc_{max} was higher in four-needle fascicles, which could be advantageous under well-watered and high light conditions that are conducive to high rates of A_{sat}. Greater A_{sat} on a per-fascicle basis in four-needle fascicles did not translate to greater growth, in fact the clone with a large abundance of four-needle fascicles had less biomass accumulation than clones with a normal abundance of four-needle fascicles. The reasons for these relationships are unclear, but they illustrate that growth rate can be affected by many factors in addition to leaf-level carbon gain.

References

Akers, M.K., Kane, M., Zhao, D., Teskey, R.O., and Daniels, R.F. (2013). Effects of planting density and cultural intensity on stand and crown attributes of mid-rotation loblolly pine plantations. *Forest Ecology and Management* 310**,** 468-475.

Aspinwall, M.J., King, J.S., McKeand, S.E., and Domec, J.-C. (2011). Leaf-level gas-exchange uniformity and photosynthetic capacity among loblolly pine (*Pinus taeda* L.) genotypes of contrasting inherent genetic variation. *Tree physiology* 31**,** 78-91.

Cescatti, A., and Zorer, R. (2003). Structural acclimation and radiation regime of silver fir (*Abies alba* Mill.) shoots along a light gradient. *Plant, Cell & Environment* 26**,** 429-442.

Cole, K.L., Fisher, J., Arundel, S.T., Cannella, J., and Swift, S. (2008). Geographical and climatic limits of needle types of one- and two-needled pinyon pines. *Journal of Biogeography* 35**,** 257-269.

Cregg, B. (1994). Carbon allocation, gas exchange, and needle morphology of *Pinus ponderosa* genotypes known to differ in growth and survival under imposed drought. *Tree Physiology* 14**,** 883-898.

Epperson, B.K., Telewski, F.W., Plovanich-Jones, A.E., and Grimes, J.E. (2001). Clinal differentiation and putative hybridization in a contact zone of *Pinus ponderosa* and *P. arizonica* (Pinaceae). *American Journal of Botany* 88**,** 1052-1057.

Haller, J.R. (1965). The role of 2-needle fascicles in the adaptation and evolution of Ponderosa pine. *Brittonia* 17**,** 354-382.

Han, Q. (2011). Height-related decreases in mesophyll conductance, leaf photosynthesis and compensating adjustments associated with leaf nitrogen concentrations in *Pinus densiflora*. *Tree physiology* 31**,** 976-984.

Herrick, J.D., and Thomas, R.B. (1999). Effects of CO_2 enrichment on the photosynthetic light response of sun and shade leaves of canopy sweetgum trees (*Liquidambar styraciflua*) in a forest ecosystem. *Tree Physiology* 19**,** 779-786.

Ishii, H., Ooishi, M., Maruyama, Y., and Koike, T. (2003). Acclimation of shoot and needle morphology and photosynthesis of two *Picea* species to differences in soil nutrient availability. *Tree physiology* 23**,** 453-461.

Kenward, M.G., and Roger, J.H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53**,** 983-997.

Littell, R., Milliken, G., Stroup, W., Wolfinger, R., and Schabenberber, O. (1996). SAS system for linear mixed models. Cary, NC: SAS Institute.

Lobo, F.D., de Barros, M.P., Dalmagro, H.J., Dalmolin, A.C., Pereira, W.E., de Souza, E.C., Vourlitis, G.L., and Ortiz, C.E.R. (2013). Fitting net photosynthetic light-response curves with Microsoft Excel - a critical look at the models. *Photosynthetica* 51, 445-456.

Long, S., and Bernacchi, C. (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany* 54, 2393-2401.

McKeand, S., Mullin, T., Byram, T., and White, T. (2003). Deployment of genetically improved loblolly and slash pines in the south. *Journal of Forestry* 101, 32-37.

Niinemets, Ü. (1999). Research review. Components of leaf dry mass per area – thickness and density – alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist* 144, 35-47.

Niinemets, Ü., Ellsworth, D.S., Lukjanova, A., and Tobias, M. (2002). Dependence of needle architecture and chemical composition on canopy light availability in three North American *Pinus* species with contrasting needle length. *Tree Physiology* 22, 747-761.

Niinemets, Ü., Lukjanova, A., Turnbull, M.H., and Sparrow, A.D. (2007). Plasticity in mesophyll volume fraction modulates light-acclimation in needle photosynthesis in two pines. *Tree Physiology* 27, 1137-1151.

Paine, C., Amissah, L., Auge, H., Baraloto, C., Baruffol, M., Bourland, N., Bruelheide, H., Daïnou, K., Gouvenain, R.C., and Doucet, J.L. (2015). Globally, functional traits are weak predictors of juvenile tree growth, and we do not know why. *Journal of Ecology* 103, 978-989.

Palmroth, S., and Hari, P. (2001). Evaluation of the importance of acclimation of needle structure, photosynthesis, and respiration to available photosynthetically active radiation in a Scots pine canopy. *Canadian Journal of Forest Research* 31**,** 1235-1243.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J., and Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* 182**,** 565-588.

Rehfeldt, G.E. (1999). Systematics and genetic structure of *Ponderosae* taxa (Pinaceae) inhabiting the mountain islands of the Southwest. *American Journal of Botany* 86**,** 741-752.

Richardson, A., Ashton, P., Berlyn, G., McGroddy, M., and Cameron, I. (2001). Within-crown foliar plasticity of western hemlock, *Tsuga heterophylla*, in relation to stand age. *Annals of Botany* 88**,** 1007-1015.

Richardson, A., Berlyn, G., Ashton, P.M., Thadani, R., and Cameron, I. (2000). Foliar plasticity of hybrid spruce in relation to crown position and stand age. *Canadian Journal of Botany* 78**,** 305-317.

Robinson, K., Karp, A., and Taylor, G. (2004). Defining leaf traits linked to yield in short-rotation coppice *Salix*. *Biomass and Bioenergy* 26**,** 417-431.

Roderick, M., Berry, S., Saunders, A., and Noble, I. (1999a). On the relationship between the composition, morphology and function of leaves. *Functional Ecology* 13**,** 696-710.

Roderick, M., Berry, S.L., Noble, I., and Farquhar, G. (1999b). A theoretical approach to linking the composition and morphology with the function of leaves. *Functional Ecology* 13**,** 683-695.

Samuelson, L.J., Butnor, J., Maier, C., Stokes, T.A., Johnsen, K., and Kane, M. (2008). Growth and physiology of loblolly pine in response to long-term resource management: defining growth potential in the southern United States. *Canadian Journal of Forest Research* 38**,** 721-732.

Schultz, R.P. (1997). Loblolly pine: the ecology and culture of loblolly pine (*Pinus taeda* L.). *Agriculture Handbook (Washington).*

Shaw, G.R. (1914). *The Genus Pinus.* Mass. Riverside Press: Cambridge.

Shelton, M., and Switzer, G. (1984). Variation in the surface area relationships of loblolly pine fascicles. *Forest Science* 30**,** 355-363.

Tausch, R.J., and West, N.E. (1987). Morphological variation/precipitation relationships of Great Basin single-needled pinyon. *Proceedings of the Pinyon-juniper Conference, USDA, Forest Service, Intermountain Research Station, Ogden, UT***,** 86-91.

Tissue, D., Thomas, R., and Strain, B. (1993). Long-term effects of elevated CO_2 and nutrients on photosynthesis and rubisco in loblolly pine seedlings. *Plant Cell Environment* 16**,** 859-859.

Tyree, M.C., Seiler, J.R., and Maier, C.A. (2009a). Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting 2-year-old *Pinus taeda* clones during seedling establishment. *Forest Ecology and Management* 257**,** 1847-1858.

Tyree, M.C., Seiler, J.R., Maier, C.A., and Johnsen, K.H. (2009b). *Pinus taeda* clones and soil nutrient availability: effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology. *Tree physiology* 29**,** 1117-1131.

Weidman, R.H. (1939). Evidences of racial influence in a 25-year test of Ponderosa pine. *Journal of Agricultural Research* 59**,** 855-887.

Will, R., and Teskey, R. (1997). Effect of elevated carbon dioxide concentration and root restriction on net photosynthesis, water relations and foliar carbohydrate status of loblolly pine seedlings. *Tree Physiology* 17**,** 655-661.

Will, R.E., Narahari, N.V., Shiver, B.D., and Teskey, R.O. (2005). Effects of planting density on canopy dynamics and stem growth for intensively managed loblolly pine stands. *Forest Ecology and Management* 205**,** 29-41.

Zutter, B.R., Gjerstad, D.H., and Glover, G.R. (1986). Effects of interfering vegetation on biomass, fascicle morphology and leaf area of loblolly pine seedlings. *Forest Science* 32**,** 1016-1031.

Table 3.1.

Mean (standard error) characteristics of loblolly pine clones. Biomass accumulation measured on 12 saplings per clone (n=12) after 12 weeks of growth. Net assimilation measured on 3 saplings per clone (n=3) on weeks 1, 8, and 12 of the measured growth period and averaged across the three measurement dates. Mean fascicle abundance per sapling and fascicle number and surface area per unit branch length and unit branch surface area measured on 6 saplings per clone (n=6). Significant p-values shown in bold. Letters denote significant differences at α=0.05.

Parameter	**Clone 1**	**Clone 2**	**Clone 3**	**p-value**
Biomass accumulation (g)	55.6 (±4.2) a	70.5 (±4.0) b	66.2 (±3.3) b	**0.032**
Net assimilation (μmol CO_2 $m^{-2}s^{-1}$)	6.9 (±1.7)	6.7 (±1.6)	7.5 (±2.6)	0.727
Four-needle fascicle abundance (%)	42.8 (±7.2) a	1.1 (±0.5) b	1.6 (±0.7) b	**0.003**
Four-needle fascicle surface area abundance (%)	43.6 (±7.1) a	0.8 (±0.5) b	1.6 (±0.8) b	**0.002**
Fascicle number per branch length (fascicle cm^{-1})	3.3 (±0.2)	3.6 (±0.2)	3.2 (±0.2)	0.373
Fascicle surface area per branch length (cm^2 cm^{-1})	40.6 (±2.2)	35.9 (±2.8)	41.2 (±3.2)	0.345
Fascicle number per branch area (fascicle cm^{-2})	0.2 (±0.0) a	0.3 (±0.0) b	0.2 (±0.0) ab	**0.013**
Fascicle surface area per branch area (cm^2 cm^{-2})	3.0 (±0.3)	3.5 (±0.3)	2.8 (±0.2)	0.189

Table 3.2.

Mean (standard error) morphological characteristics of adjacent three- and four-needle fascicles of ramets (n=6) of a loblolly pine clone with a large abundance of four-needle fascicles (Clone 1). Significant differences at α=0.05 shown in bold.

Parameter	**Three-needle**	**Four-needle**	**p- Value**
Fascicle dry mass (g)	0.08 (±0.005)	0.09 (±0.005)	**0.01**
Fascicle diameter (cm)	0.13 (±0.006)	0.13 (±0.050)	0.60
Fascicle length (cm)	18.2 (±0.5)	18.2 (±0.5)	0.95
Tissue density (kg cm^{-3})	0.003 (±0.00)	0.004 (±0.00)	**0.03**
Specific leaf area (SLA) (cm^{-2} g^{-1})	184.5 (±4.1)	186.8 (±6.7)	0.68
Stomatal density (abaxial) (stomata mm^{-2})	64 (±4)	74 (±5)	0.13
Stomatal density (adaxial) (stomata mm^{-2})	79 (±5)	74 (±6)	0.44
Stomatal density (volume) (stomata cm^{-3})	1707 (±75)	2086 (±69)	**<0.01**
Surface area (cm^2)	14.3 (±0.9)	16.9 (±0.8)	**0.01**
Volume (cm^3)	59.5 (±1.8)	59.5 (±1.5)	0.97
Water content (%)	63.4 (±0.8)	64.7 (±0.3)	0.16

Table 3.3.

Mean (standard error) physiological characteristics of adjacent three and four-needle fascicles of ramets (n=6) of a loblolly pine clone with a large abundance of four-needle fascicles (Clone 1). Significant differences at α=0.05 shown in bold.

Parameter	**Three-needle**	**Four-needle**	**p- Value**
A/Ci analysis			
J_{max} (mmol m^{-2} s^{-1})	70.99 (±4.77)	77.35 (±4.15)	0.11
Vc_{max} (mmol m^{-2} s^{-1})	56.87 (±6.19)	69.06 (±4.13)	**0.05**
V_{TPU} (mmol m^{-2} s^{-1})	5.11 (±0.3)	5.50 (±0.3)	0.20
WUE_i (µmol CO_2 mmol H_2O^{-1})	3.49 (±0.21)	3.32 (±0.13)	0.14
Light response analysis			
A_{max} (µmol m^{-2} s^{-1})	6.35 (±0.38)	6.35 (±0.4)	0.43
Light compensation point (µmol m^{-2} s^{-1})	25.82 (±3.26)	19.49 (±1.81)	0.13
ϕ (umol CO_2)(umol $photons^{-1}$)	0.04 (±0.0)	0.04 (±0.0)	0.94
R_d (µmol m^{-2} s^{-1})	0.93 (±0.11)	0.74 (±0.06)	0.16
Water potential			
Midday (MPa)	0.76 (±0.05)	0.78 (±0.04)	0.89
Pre-Dawn (MPa)	0.45 (±0.01)	0.44 (±0.02)	0.34
Elemental composition			
Carbon isotope ratio ($\delta^{13}C$)	-29.59 (±0.63)	-28.51 (±1.14)	0.36
C:N	28.02 (±0.79)	26.33 (±1.58)	0.38
Nitrogen content (dry mass) (%)	1.63 (±0.07)	1.88 (±0.13)	0.41
Nitrogen content (volume) (g cm^{-3})	0.0023 (±0.00)	0.0028 (±0.00)	**0.02**

Table 3.4.

Mean (standard error) whole-fascicle light saturated net assimilation, intercellular [CO_2] (Ci), and transpiration at 1600 µmol mol^{-1} and 400 µmol mol^{-1} [CO_2] (PAR=1800) of adjacent three-needle and four-needle fascicles of ramets (n=6) of a loblolly pine clone with a large abundance of four-needle fascicles (Clone 1). Significant differences at α=0.05 shown in bold.

Parameter	400 µmol mol^{-1}			1600 µmol mol^{-1}		
	Three-needle	Four-needle	p-value	Three-needle	Four-needle	p-value
Light saturated net assimilation (µmol CO_2 $fascicle^{-1}$ s^{-1})	6.27E-03 (±7.53E-04)	7.16E-03 (±8.37E-04)	0.062	2.26E-02 (±1.72E-03)	2.56E-02 (±1.14E-03)	**0.048**
Stomatal conductance (mol $fascicle^{-1}$ s^{-1})	5.33E-05 (±9.08E-06)	6.35E-05 (±9.16E-06)	**0.041**	4.99E-05 (±5.03E-06)	6.36E-05 (±9.06E-06)	0.054
Intercellular [CO_2] (µmol CO_2 mol air $^{-1}$)	1.83E+02 (±8.91E+00)	1.95E+02 (±5.16E+00)	0.137	7.56E+02 (±5.74E+01)	8.00E+02 (±8.06E+01)	0.394
Transpiration (mmol fascicle $^{-1}$ s^{-1})	1.86E-03 (±2.90E-04)	2.20E-03 (±2.71E-04)	**0.043**	1.75E-03 (±1.92E-04)	2.23E-03 (±2.79E-04)	**0.041**

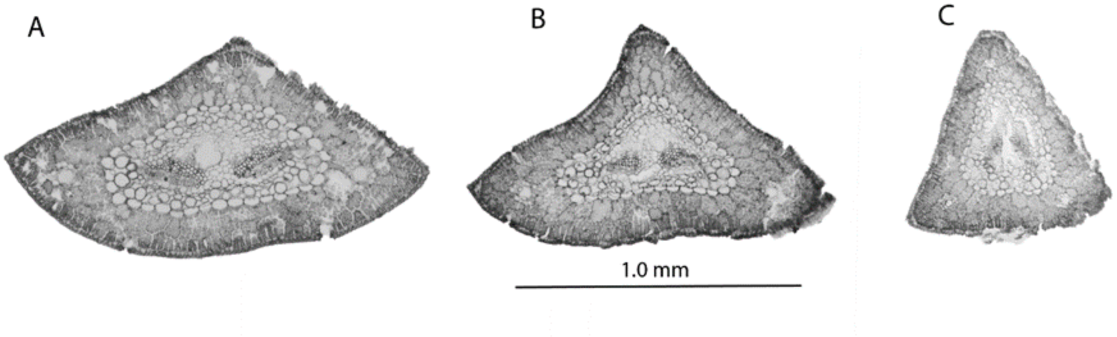

Figure 3.1.

Cross sections of individual needles of three- and four-needle fascicles showing a typical needle from a three-needle fascicle (A), a typical needle from a four-needle fascicle (B) and a small needle from a four-needle fascicle (C).

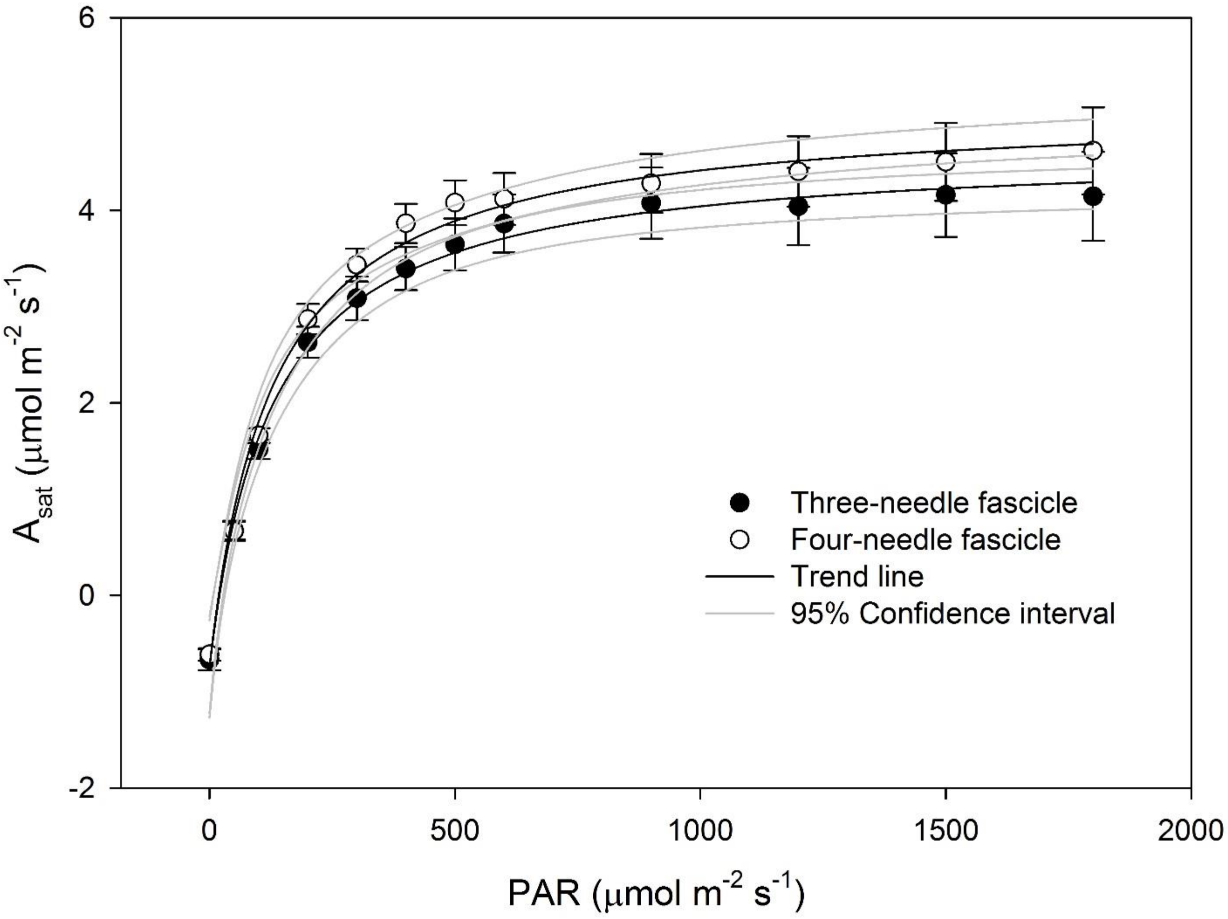

Figure 3.2.

Mean photosynthetic light response of three-needle (filled symbols) and four-needle (open symbols) fascicles (n=6). The shape of the responses was approximated by rectangular hyperbolas (black lines) (Lobo et al., 2013). Error bars represent standard error of mean; grey lines indicate 95% confidence intervals.

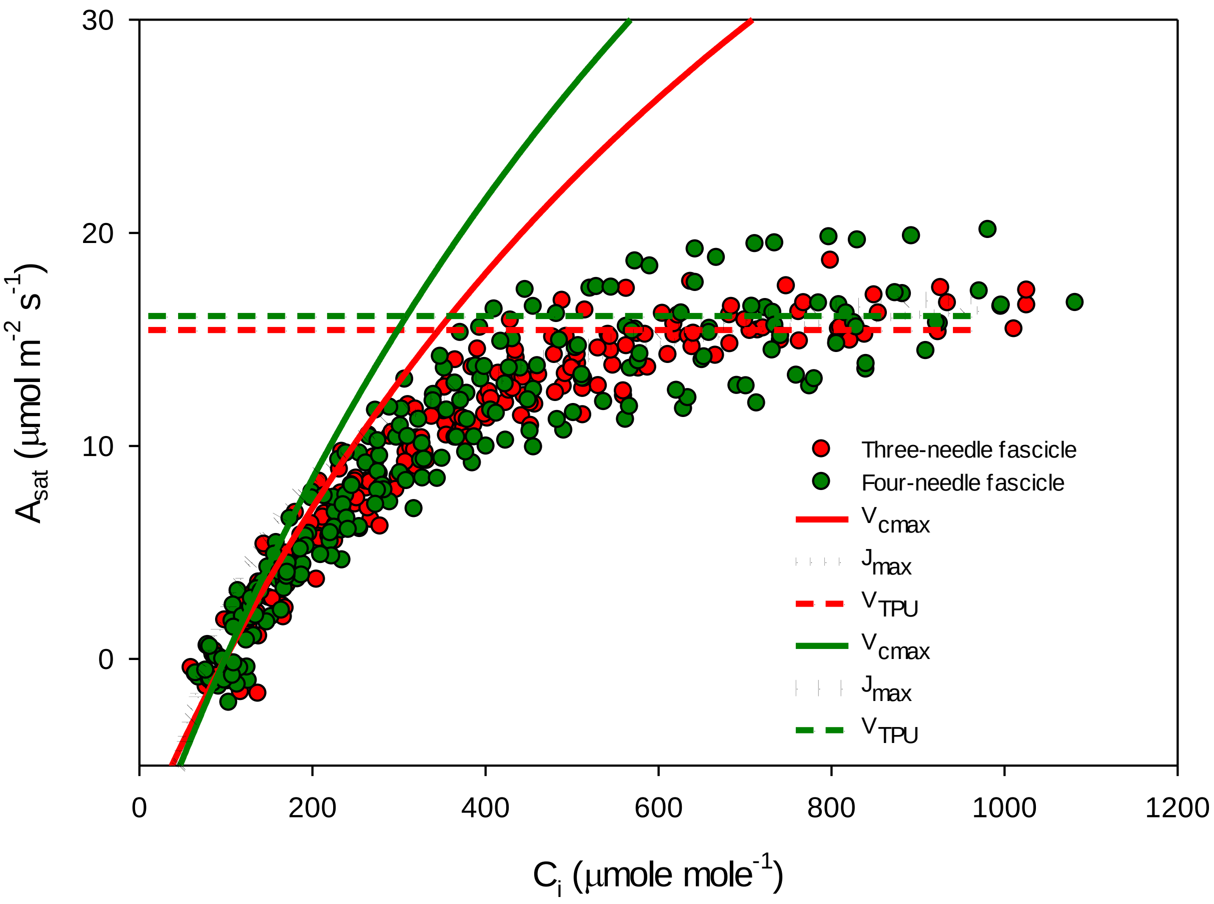

Figure 3.3.

Mean A/Ci relationships for the two fascicle types (n=6). Assimilation measurements were conducted under light-saturated conditions. Red circles represent three-needle fascicles; green circles represent four-needle fascicles. Solid lines indicate Vc_{max}-limited photosynthesis; dashed lines indicate V_{TPU}; the small dotted line indicates three-needle J_{max}; the large dotted line indicates four-needle J_{max} (Long and Bernacchi, 2003). Red lines indicate three-needle fascicles; green lines indicate four-needle fascicles.

CHAPTER 4

CONCLUSIONS

Summary of Previous Chapters

Chapters 2 and 3 utilized loblolly pine ramets to explore physiological and morphological differences among clones in response to different moisture regimes (Chapter 2) and between two different fascicle types on the same clone (Chapter 3). The findings in Chapter 2 demonstrate that there is intraspecific variation in productivity and drought resistance among half sibling clones. When the rank order of biomass accumulation was compared between the low and high water soil moisture treatments, a change in rank order was observed, i.e. a G x E interaction. One of the clones, relative to the other two clones, had more biomass accumulation in the low water treatment, but less biomass accumulation in the high water treatment. Differences in physiological and morphological traits among the clones may have accounted for the observed differences in biomass accumulation. For example, rates of morning gas exchange were significantly greater in the high water treatment in the two clones that had the most biomass accumulation. Likewise, there was more below-ground carbon allocation in the two clones that had the most biomass accumulation in the high water treatment. Perhaps most intriguing were the differences in carbon isotope discrimination, which can be used as a surrogate for time-integrated water use efficiency (Farquhar and Richards, 1984). Carbon isotope discrimination was consistently higher in Clone 2 compared to the other two clones in all three of the treatments. Clone 2 also had the most biomass accumulation in the high soil moisture treatments. When analyzed across all three soil moisture treatment., the relationship between carbon isotope discrimination and biomass accumulation was strongly negative for all three clones. When analyzed between treatments, the relationship between carbon isotope discrimination and biomass accumulation was different in high and low soil moisture conditions. Higher levels of carbon isotope discrimination coincided with greater amounts of biomass accumulation in the high water treatment, but lower amounts of biomass accumulation in the low water treatment. A strong relationship between carbon isotope discrimination and biomass accumulation suggests that carbon isotope discrimination could potentially be used as a breeding marker, however, this approach would be nuanced by the contrasting relationships observed under different soil moisture conditions. The results in this study also suggest some value in selective deployment of loblolly pine clones. For example, Clone 1, relative to Clone 2 appears to be suited for regions with lower soil moisture. In contrast, Clone 2, relative to Clone 1, appears to be better suited for regions of higher soil moisture. Clone 3, relative to Clones 1 and 2, appears to be moderately well suited for regions of either low or high soil moisture.

The clone with the most biomass accumulation under low soil moisture and the least biomass growth under high soil moisture had an abnormal abundance of four-needle fascicles. The physiology and morphology of three- and four-needle fascicles were the subjects of Chapter 3. Needle characteristics such as biomass, distribution, longevity and leaf area have been linked to growth in previous studies (McCrady and Jokela, 1996; 1998) and may have resulted in the different amounts of biomass accumulation observed in Chapter 2. The findings in Chapter 3 revealed that the four-needle fascicles, compared to the three-needle fascicles, were anatomically similar with equal volumes and leaf areas. Four-needle fascicles did have a greater surface area and mass and also greater needle tissue density. Total needle area was not different among clones. Light response and A/Ci curves were also similar between the two fascicle types. The similarity in gas exchange on a surface area basis between the two fascicle types suggests that photosynthetic capacity is largely conserved.

Although photosynthetic capacity was similar between the two fascicle types, four-needle fascicles, compared to three-needle fascicles, had a significantly higher rate of RuBisCo-mediated carboxylation. A higher rate of carboxylation may have been beneficial under low water conditions and may partially account for the greater biomass accumulation in Clone 1 in the low moisture treatment compared to the other clones. It is conceivable that under conditions of partial stomatal closure, which would occur when plants are moderately moisture stressed (i.e. afternoon, low water treatment), the high rate of carboxylation may have resulted in greater amounts of CO_2 assimilation, which could be reflected in greater amounts of biomass accumulation. Different rates of gas exchange were not, however, observed in afternoon measurements. However, differences among clones were found in carbon isotope measurements, which integrate differences in gas exchange throughout the growth period. Collectively, this study demonstrated that intraspecific variation in drought resistance exists in loblolly pine even among closely related clones.

References

Farquhar, G., and Richards, R. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Functional Plant Biology* 11**,** 539-552.

McCrady, R., and Jokela, E. (1996). Growth phenology and crown structure of selected loblolly pine families planted at two spacings. *Forest Science* 42**,** 46-57.

McCrady, R., and Jokela, E. (1998). Canopy dynamics, light interception, and radiation use efficiency of selected loblolly pine families. *Forest Science* 44**,** 64-72.