



Comparison of lignin and polysaccharide sugar contents for slash, longleaf, and loblolly pine growth rings formed during periods of soil moisture extremes

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Abstract

Lignin contents of mature wood growth rings formed under soil moisture extremes were determined to investigate the possibility of drought-caused reductions in lignin deposition for the southern pines. A well-defined set of slash (*Pinus elliottii* Engelm.), longleaf (*Pinus palustris* Mill.) and loblolly pine (*Pinus taeda* L.) increment cores were processed to excise growth rings formed during multiyear periods of above- (wet) or below-normal (drought) soil moisture. The average acid-insoluble (Klason) lignin content of the resultant drought-formed slash pine wood samples was significantly lower (31.4% vs. 34.1%; $P=0.0005$) than that for the corresponding wood samples from growth rings formed under conditions of ample soil moisture; respectively higher glucan (40.6% vs. 36.9%; $P=0.0010$) and mannan (11.7 vs. 10.4%; $P=0.0010$) values were also observed. Small, but significant differences for other hemicellulose derived sugars (xylan, arabinan) were determined for the longleaf and loblolly pine wood samples. Altogether, these data represent the first wet chemical results showing differences in lignin and polysaccharide sugar contents for mature wood formed in pine trees during annual droughts. Among the southern pines evaluated here, slash pine is the least drought tolerant; the lower lignin content observed for the slash pine drought-formed wood is particularly intriguing since it occurred with the species most susceptible to water stress.

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Introduction

The pine forests of the southeastern USA have changed in species composition from the predominance of longleaf pine (*Pinus palustris* Mill.) at the time of colonial settlement, to the highly productive pine plantations of loblolly (*Pinus taeda* L.) and slash (*Pinus elliottii* Engelm.) pines that are now widespread across the region (Fox et al. 2007). The current longleaf pine forest only occupies about 3% of its pre-settlement range (Frost 1993; Outcalt and Sheffield 1996); however, longleaf pine restoration efforts, coupled with a nearly 50% decrease in shortleaf pine (*Pinus echinata* Mill.) forestland from 1992 to 2012 (South and Harper 2016), have allowed longleaf pine to regain some of its prior stature, now ranking as the third most abundant southern pine species in terms of land area. Following a nearly 18-fold increase in pine plantation forestland over a timeframe of 50 years, the volume of planted southern pine timber was estimated to be at 677 million m³ at the close of the twentieth century (Wear and Greis 2002; Fox et al. 2007). Accordingly, the southern pine forests are the dominant resource for wood production in the USA (Schultz 1999).

Wood features and variability

Southern (yellow) pine wood has characteristically wide latewood bands that sets it apart among the hard pines (Kubler 1980; Panshin and de Zeeuw 1980). These wide latewood bands can comprise more than 60% of the ring width in the mature wood zone (Eberhardt and Samuelson 2015). As the growing season commences in the spring, temperatures increase and the cambium resumes producing wood cells in radial files (Creber and Chaloner 1984; Samuels et al. 2006; Rathgeber et al. 2016). Earlywood (springwood) is formed as the development of new tracheids is favored over secondary wall thickening (Larson 1969). As temperatures rise and moisture availability declines into the summer months, latewood (summerwood) is formed with tracheids having smaller lumina, thicker cell walls, and smaller cell sizes (Plomion et al. 2001; Cuny et al. 2014). The smaller lumina of the latewood have been suggested to reduce xylem vulnerability to embolism under water stress (Plomion et al. 2001); however, an exception has been shown for Douglas-fir (*Pseudotsuga menziesii*) where vulnerability to embolism was lower in the earlywood than in the latewood under normal moisture conditions (Domec and Gartner 2002). For loblolly pine growing on the southernmost sites of its range, the growing season is longer, resulting in greater proportions of latewood in the growth rings (Jokela et al. 2004). Cell wall thickening has been shown to continue into winter dormancy for both loblolly and slash pines in the upper Piedmont region in South Carolina (Nix and Villiers 1985).

Although the initiation of latewood formation coincides with changing climate conditions of reduced water availability and increased temperatures, the signals produced are presumably linked with an array of metabolic processes including the transport of photosynthates and changes in auxin availability (Larson 1969; Ugglà et al. 2001). These metabolic processes not only influence within-growth-ring

variability, but also between growth-ring variability, progressing from the pith outward toward the bark (Lachenbruch et al. 2011). The wood in the center of a pine tree, comprising the juvenile wood zone, shows the greatest progression of anatomical and physical property changes, differentiating it from the mature wood (Larson et al. 2001). The characteristics of juvenile wood (or corewood) in the southern pines include a lower proportion of latewood, lower specific gravity (SG), and higher occurrence of abnormal features such as compression wood (Larson et al. 2001). The juvenile wood core in pines is generally viewed to extend from the base of the tree to the crown (Zobel and Sprague 1998); however, some assessments indicate that the wood in the tree crown has properties with greater similarity to the mature wood at the tree base (Kibblewhite 1999; Burdon et al. 2004; Eberhardt et al. 2019).

Water stress and the southern pines

Longleaf pine is particularly useful for dendroclimatic and dendroecological (Manzanedo and Pederson 2019) studies in the southeastern USA because it has the longest lifespan among the southern pines and can be found as well-preserved resinous relicts (Devall et al. 1991; Bhuta et al. 2009; Henderson and Grissino-Mayer 2009; Kush et al. 2004). Its wide distribution before colonial settlement can be attributed to greater suitability to drier sites (Lantz 1987; Landers 1991) and lower susceptibility to fire (Stanturf et al. 2002) compared to loblolly and slash pines, with these latter two species being relegated to wet sites. Resistance to water stress does not appear to carry through all tissues, with leaf physiology measurements for longleaf pine showing greater susceptibility to water stress than for loblolly and slash pines in both mature trees (Samuelson et al. 2012) and seedlings (Hart et al. 2020). Comparing slash and loblolly pines, the former performs better on the wettest sites (Borders and Harrison 1989).

Studies on the effect of drought (or water availability) on wood properties in the southern pines have focused on loblolly pine (Cregg et al. 1988; Albaugh et al. 2004; Hennessey et al. 2004; González-Martínez et al. 2007; Gonzalez-Benecke et al. 2010, 2015). Aside from false rings (Gonzalez-Benecke et al. 2015), drought resulted in less latewood and lower ring SG (Hennessey et al. 2004). Ample supplies of water, via irrigation, can result in opposite trends (Cregg et al. 1988; Gonzalez-Benecke et al. 2010) or no impact (Albaugh et al. 2004). It should be noted that the above-mentioned growth patterns are by no means confined to the southern pines, with similar observations being reported in the literature of various pine and spruce species (Bouriaud et al. 2005; Zubizarreta-Gerendiain et al. 2012; Camarero et al. 2017; Candel-Pérez et al. 2018).

Water stress and cell wall chemistry

Currently, there is only limited evidence for moisture stress impacting the chemistry of wood cell walls. For loblolly pine stem wood, glycome profiling was indicative of changes in cell wall ultrastructure, leading to the author's stated need for wet

chemical analyses (Pattathil et al. 2016). Determinations of lignin contents of stems from longleaf pine saplings exposed to normal and elevated CO₂ levels showed an instance of reduced lignin contents when the saplings also were under water stress (Runion et al. 1999). Although the most accurate wet chemical method (Klason) for softwood lignin content (Brunow et al. 1999) was used for that study, intact stem tissue (bark and wood together) was analyzed. Unfortunately, bark is known to give inflated values for lignin content (Eberhardt 2012), and the results were likely impacted by the bark quantities in the samples.

Lignin distributions/amounts in tracheids have also been assessed by various microscopies (UV, UV fluorescence, Raman) used to observe false rings (Gindl 2001; Mayer et al. 2020) and abnormal tracheids showing collapse (Donaldson 2002; Rosner et al. 2018). In a recent study by Mayer et al. (2020), a decrease in the cellulose to lignin ratio for a false ring could be attributed to either a decrease in cellulose content or an increase in lignin content; assuming that an increase in lignin content would coincide with increased resistance to tracheid collapse led to the tentative conclusion that water stress resulted in increased lignification. Under conditions of severe drought, *Pinus radiata* has been shown to have abnormal/collapsed tracheids having decreased lignin contents (Donaldson 2002); however, the collapse of earlywood tracheids in *Picea abies* under conditions of extreme drought may be a product of tree genetics predisposing them to this wood defect (Rosner et al. 2018).

In a recent study by Frelin et al. (2017), drought stress in loblolly pine was shown to increase transcript abundance for the gene encoding the enzyme phenylalanine hydroxylase (PtPheH) involved in the hydroxylation of phenylalanine into tyrosine. Aside from being a key building block in proteins, phenylalanine is metabolized into a wide variety of phenylpropanoids, with lignin being the dominant product in woody plants (Vogt 2010; Pascual et al. 2016; Deng and Lu 2017; Maeda 2019). While tyrosine is consumed for lignification in some plants, especially grasses (Schenck and Maeda 2018; Xie et al. 2018), the shunting of phenylalanine to tyrosine, as suggested for loblolly pine under moisture stress (Frelin et al. 2017), could have the potential to reduce phenylalanine availability for monolignol biosynthesis. Note that the transcriptional control of monolignol biosynthesis involves many genes that can be activated by stress (Anterola et al. 2002; Wang et al. 2013). Frelin et al. (2017) acknowledged that the pathways leading up to phenylalanine are also likely regulated; however, their observations with loblolly pine under water stress may reflect that reduced growth lowers the demand for lignification, and thus phenylalanine would be better utilized through catabolic metabolism.

To date, studies have not used an established wet chemical method to compare the lignin contents for mature wood formed within the same tree during periods of ample and below normal (i.e., drought) annual moisture availability. The intriguing results reported by Frelin et al. (2017) prompted us to glean additional data from a set of southern pine increment cores (Eberhardt and Samuelson 2015) to give the first direct comparisons of lignin content in mature wood growth rings using annual Palmer Drought Severity Index (PDSI) values as a guide for moisture availability extremes; as a meteorological index, the PDSI classifies drought conditions using precipitation and temperature data along with soil type (Palmer 1965). In addition, since these three southern pines have different tolerances to moisture stress, it

remained to be determined if this manifests in between-species differences in wood chemistry.

Materials and methods

Study trees and increment core collection

Slash, longleaf, and loblolly pine trees sampled for this study were from the USDA Forest Service's Harrison Experimental Forest (30.65 N, 89.04 W) within the DeSoto National Forest, near Saucier, Mississippi, USA. Soils are well-drained, low-nutrient, fine-sandy loams of the Poarch series and the Saucier-Susquehanna complex. Average soil moisture for all three species was reported to range from 18.4 (July) to 21.4% (September), and to not differ significantly between species in any month (Samuelson et al. 2012). The climate at the study site is temperate-humid subtropical (Adams et al. 2004). Temperature, precipitation, and PDSI data (Figs. 1 and 2) from a nearby weather station were obtained from the National Oceanic and Atmospheric Administration (<https://www.ncdc.noaa.gov/cdo-web/>). From the year the trees were planted, until the year the increment cores were collected, the average annual temperature varied within a two-degree range (18.6–20.8 °C). No trend was apparent between the values for average annual temperature and annual precipitation, the latter varying to the extent that the highest precipitation year (1979) had twice the amount of rainfall (231.2 cm vs. 113.6 cm) than the lowest precipitation year (1968).

Information on the study design and history of the trees that were sampled are thoroughly described in Samuelson et al. (2012). Briefly, the plantations making

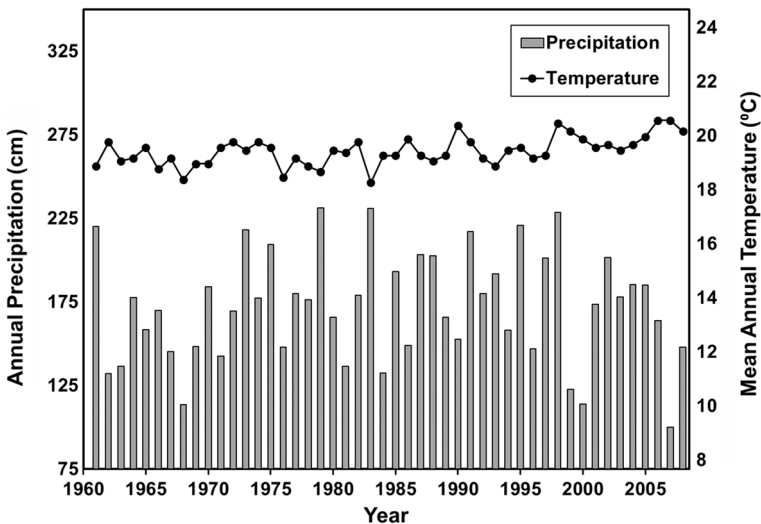


Fig. 1 Annual precipitation and mean annual temperature at the study site over the years assigned for the increment cores

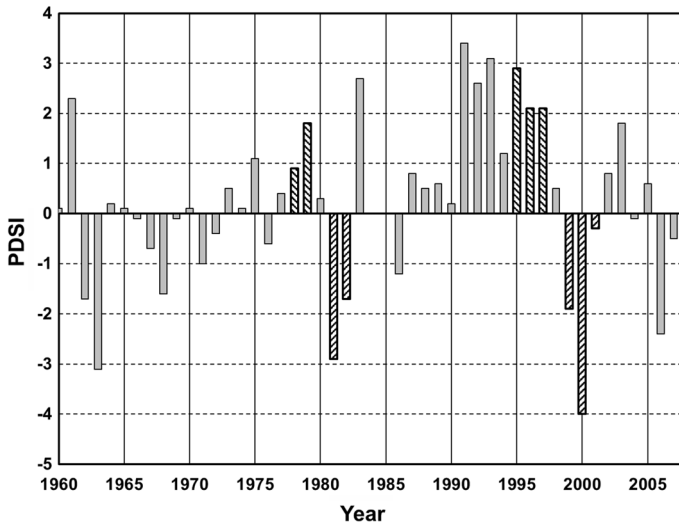


Fig. 2 Palmer Drought Severity Index (PDSI) at the study site over the years assigned for the increment cores. Bars with diagonal lines show selected +PDSI and -PDSI years corresponding to growth rings excised from increment cores for chemical analysis

up this replicated field experiment were established in 1960 to study the genetics and growth of slash, longleaf, and loblolly pines (Schmidtling 1973). In each of 3 blocks, 3 trees of each species were selected for physiological and chemical analyses of the leaves (Samuelson et al. 2012). Increment cores (12 mm diameter) were removed from each tree with an increment borer at breast height (1.4 m) in 2010 and processed into X-ray densitometry specimens as described in Eberhardt and Samuelson (2015); thus, there were 9 specimens per species available for the current study. The breast-height diameter ranges for the trees were as follows: slash pine, 30.5–35.4 cm; longleaf pine, 31.1–39.1 cm; loblolly pine, 31.5–40.2 cm.

Excision of growth rings from increment cores

Processing of the increment cores into X-ray densitometry specimens involves gluing them into wood core holders and the resulting dried assemblies being sawn to obtain a constant thickness (2.3 mm) for the X-ray beam to penetrate. The adhering wood strips remaining from the core holders after sawing serve several functions, not limited to providing mechanical support to keep the cut increment core intact, prevent warping, and to facilitate its positioning with the X-ray densitometer sample holder. For the present study, the specimens had already been subjected to exhaustive (i.e., Soxhlet) extraction with acetone (Eberhardt and Samuelson 2015), negating the need for organic solvent extraction prior to the chemical analyses described below.

Using the plotted PDSI data (Fig. 2), years were selected as representing periods of above-normal (+PDSI) and below-normal (drought, -PDSI) soil

moisture conditions at the study site. These periods of time constituted 2 (or 3) years with +PDSI values being paired with the same number of years with –PDSI values. Selecting individual years was deemed impractical for collecting enough material for wet chemical analysis. There was also the potential for tree growth/physiology for a given year being influenced by environmental conditions carrying over from the preceding year, such as high soil moisture from a +PDSI year still being available early in the growing season of a subsequent –PDSI year. Given that longleaf pine needles are retained for a couple of years, Meldahl et al. (1999) suggested that climatic conditions as far back as 2 years prior could impact current growth. Fortunately, a single low positive (i.e., near normal) PDSI year was present between the growth ring groupings selected for having high positive and low negative PDSIs, ameliorating some of the concern of moisture carryover from a year with above-normal moisture into a year of moderate to extreme drought.

Excluding the wood in the juvenile zone, two sampling zones were selected at two designated ages (at 20 and 38 years) showing patterns of noticeably +PDSI years followed by –PDSI (i.e., drought) years. For the sampling zone centered on the age of 20 years, there were 2 +PDSI years (1978, 1979) followed by 2 –PDSI years (1981, 1982). The growth ring for 1980 was excluded, having normal soil moisture. Likewise, at the sampling zone centered on the age of 38 years, +PDSI years (1995, 1996, 1997) and –PDSI years (1999, 2000, 2001) were taken, excluding the growth ring for 1998. Since growth ring widths decline from pith to bark, including one more ring was rationalized to compensate for the narrower ring widths in the sampling zone furthest from the pith. Although the PDSI in 2001 was barely negative, it was nevertheless included with the other 2 years (1999, 2000), providing necessary mass for the analysis of that sampling zone; it also followed a particularly negative PDSI year with a value of –4 (extreme drought). A challenge to this study was the ability to collect enough material for duplicate wood chemistry analyses representing +PDSI and –PDSI timeframes for each tree.

Working from the outermost growth ring (including the cambium), the growth rings for each core were carefully counted to assign a year to each growth ring in each sampling zone (centered at either an age of 20 or 38 years). Care was taken to ensure that false rings were not counted as growth rings. Note that in the southern pines, the latewood to earlywood transition between growth rings is very abrupt in the mature wood zone (Eberhardt and Samuelson 2015); false rings are usually quite narrow and have gradual transitions into and out of the false ring. Year assignments were carefully marked with colored pencils to keep the excision process as straightforward as possible.

Dissection tools were selected depending upon the cutting operation being performed; cutting/chopping operations were carried out on a polyethylene cutting board. The preferred cutting tool was an X-ACTO knife set (Model X5285), using a #19 blade for splitting and chopping operations, and a #11 blade for fine detailed shaving operations. Frequent use of a Flexcut dual-sided paddle strop (Model PW16) was used to maintain knife sharpness of the #19 blade. Wood fragments were collected into pre-weighed vials using forceps or by brushing onto glassine paper. A Zerostat 3 anti-static device proved to be quite useful for the collection of smaller wood particles having a static charge.

Processing of an increment core proceeded by carefully removing one of the support strips of wood by working along the glue line with the #19 blade. Each sampling zone was then excised by splitting the increment core into 2- or 3-ring segments. Any glue adhering to the growth rings was removed by cutting with the #19 blade. A hand lens (10×) was used to carefully inspect each wood fragment to ensure that the glue was completely removed. In cases where a small amount of wood from an excluded growth ring needed to be removed, or a small amount of a selected growth ring needed to be collected, the #11 blade was used. All wood samples from each growth ring grouping were weighed and then pooled to give a single +PDSI wood sample and a single –PDSI wood sample for each increment core; all pooled wood samples were finely chopped (approximately 20 mesh) for chemical analysis.

Lignin and polysaccharide sugar determinations

Lignin contents and polysaccharide sugar contents of the dry extractive-free wood particles were determined by established methods (Effland 1977; Davis 1998). Briefly, the wood particles were hydrolyzed in 72% sulfuric acid (w/w, 1 h, 30 °C) with the mixture then diluted with distilled water to obtain a sulfuric acid concentration of 4% (w/w). After autoclaving (1 h, 120 °C), and cooling, the acid-insoluble (Klason) lignin was collected using a glass fiber filter (Whatman 934-AH) in a Gooch crucible, dried (103 ± 2 °C), and weighed to obtain the value for lignin content. An aliquot of the filtrate was filtered through a 0.45 μm Teflon syringe filter and analyzed directly for polysaccharide sugars (arabinose, galactose, glucose, mannose, xylose) by anion-exchange high performance liquid chromatography using the method by Davis (1998). Paired *t*-tests were carried out in SAS using the MEANS procedure and the power of those tests determined using the POWER procedure (SAS version 9.1; SAS Institute Inc., Cary, N.C.). Regression analyses were carried out using MS Excel.

Results and discussion

Sample weights from growth ring excisions

Calculations based on previously reported wood SG values (Eberhardt and Samuelson 2015), and rough estimates of the volumes of wood in the selected growth rings of representative increment cores, indicated that sufficient wood mass could be obtained to represent +PDSI and –PDSI wood from each increment core for wet chemical analysis. The weights of recovered material for each growth ring grouping and species are shown in Table 1. Paired *t*-tests showed no significant differences for comparisons of the +PDSI and –PDSI mass values within any of the sampling zones (e.g., years 1978–1979 vs. 1981–1982). The ratio of +PDSI material (by mass) over –PDSI material was the highest for slash pine (1.31) and the lowest for loblolly pine (1.08) for the less mature wood (years 1978–1979 and 1981–1982). The higher weight of wood for the +PDSI samples was expected given the likelihood

Table 1 Mass and corresponding mass ratios for selected growth ring groupings representing +PDSI and -PDSI wood excised from slash, longleaf, and loblolly pine increment cores

| Pine species | Mass (mg) | Mass ratio (+ PDSI/- PDSI) | | Mass (mg) | | Mass ratio (+ PDSI/- PDSI) | | Mass ratio (+ PDSI/- PDSI) | |
|--------------|-------------|----------------------------|--------------------|--------------------|--------------------|----------------------------|--------------------|----------------------------|--------------------|
| | | 1978–1979 (+ PDSI) | 1981–1982 (- PDSI) | 1978–1979 (+ PDSI) | 1981–1982 (- PDSI) | 1995–1997 (+ PDSI) | 1999–2001 (- PDSI) | 1995–1997 (+ PDSI) | 1999–2001 (- PDSI) |
| Slash | 64.4 ± 29.1 | 47.9 ± 9.1 | 1.31 ± 0.44 | 90.0 ± 29.5 | 91.1 ± 21.4 | 0.99 ± 0.28 | 1.12 ± 0.27 | 0.99 ± 0.28 | 1.12 ± 0.27 |
| Longleaf | 95.8 ± 32.9 | 77.5 ± 17.9 | 1.24 ± 0.30 | 90.3 ± 16.5 | 87.8 ± 27.2 | 1.08 ± 0.26 | 1.20 ± 0.30 | 1.08 ± 0.26 | 1.20 ± 0.30 |
| Loiblolly | 56.3 ± 13.8 | 58.3 ± 19.6 | 1.08 ± 0.50 | 86.4 ± 25.1 | 71.7 ± 18.5 | 1.24 ± 0.35 | 1.12 ± 0.21 | 1.24 ± 0.35 | 1.12 ± 0.21 |

Data are means ± SD of both mass and corresponding mass ratio values determined for each increment core

of wider growth rings in years with ample precipitation relative to those from years of drought. For example, in mature loblolly pine, growth ring widths were shown to increase with growing-season precipitation over the most recent 40 years of growth (Graham et al. 2012). Curiously, a reversed trend by species was observed for the more mature wood (years 1995–1998 and 1999–2001), with the +PDSI/–PDSI ratio being the highest for loblolly pine (1.24) and the lowest for slash pine (0.99).

The total amounts of wood produced in +PDSI years were seemingly 20% higher for longleaf pine and 10% higher for slash and loblolly pines. Note again that paired *t*-tests of sample weights from each tree showed no significant differences between the +PDSI and –PDSI values for either of the two sampling timeframes (1978–1982, 1995–2001). Reduced ring widths from drought can be accompanied by lower wood densities for various hard pines (Hennessey et al. 2004; Candel-Pérez et al. 2018). Since such reductions in wood density are relatively small, it is unlikely that the absence of significant differences in mass between +PDSI and –PDSI sample weights resulted from offsetting reductions of mass from lower wood densities.

Determinations of lignin content

The results from the wet chemical analysis for the lignin content of +PDSI and –PDSI wood samples for each pine species are shown in Table 2. To account for the known tree-to-tree variability in lignin content (Via et al. 2007; Aspinwall et al. 2010), paired *t*-tests were used to test for significant differences between the +PDSI and –PDSI wood samples. For slash pine, the lignin content of the +PDSI wood (34.1%) was significantly higher ($P = 0.0005$) than for the –PDSI wood (31.4%). Despite the limited sample size per species, that being 9 increment cores each, the power of the hypothesis test was very high at 0.998, thus giving a high probability that the current assessment of a significant lignin content difference for the slash pine samples is correct. Significant differences were not observed for either longleaf pine (32.2% vs. 31.2%; $P = 0.0510$) or loblolly pine (30.0% vs. 30.0%; $P = 0.9672$). In the case of the former result for longleaf pine, the probability value barely exceeded the threshold for significance ($\alpha = 0.05$). Since the power of this test was determined to be 0.523, there is a nearly equal probability of falsely accepting that there is no lignin content difference when there actually is a difference. Unfortunately, the study was constrained by the number of trees sampled, and the limited amounts of recoverable wood from growth rings assigned to +PDSI and –PDSI years. Even if the two sampling zones (centered at ages of 20 and 38 years) had not been pooled by PDSI designation, the number of independent samples would not have changed for each species.

The lignin data reported here for slash pine support the findings of a limited number of studies in which lower levels of lignification seemingly occurring in hard pines grown under moisture stress. Prior studies with loblolly pine have provided indirect evidence for the lignin content to be altered by drought stress (Pattathil et al. 2016; Frelin et al. 2017); it should be noted that Pattathil et al. (2016) stated that wet chemical analyses were needed to support their findings by glycome profiling. To date, we have found only one other study (Runion et al. 1999) in which the effect of

Table 2 Differences in lignin and polysaccharide sugar contents for selected growth ring groupings representing +PDSI and –PDSI wood excised from slash, longleaf, and loblolly pine increment cores

| Pine species | Lignin (%) | Arabinan (%) | Galactan (%) | Glucan (%) | Mannan (%) | Xylan (%) | All sugars (%) | |
|--------------|----------------------|--------------|--------------|------------|------------|------------|----------------|------------|
| Slash | 1978–1979 | 34.1 ± 1.4 | 0.9 ± 0.1 | 2.7 ± 1.5 | 36.9 ± 1.4 | 10.4 ± 1.3 | 4.8 ± 0.2 | 55.8 ± 1.2 |
| | 1995–1997 (+PDSI) | | | | | | | |
| | 1981–1982 | 31.4 ± 2.2 | 1.0 ± 0.1 | 2.6 ± 1.7 | 40.6 ± 2.0 | 11.7 ± 1.6 | 5.0 ± 0.3 | 60.8 ± 2.2 |
| Longleaf | 1999–2001 (–PDSI) | | | | | | | |
| | Difference | 2.7 | 0.1 | 0.1 | 3.7 | 1.3 | 0.2 | 5.0 |
| | <i>P</i> value | 0.0005 | 0.3870 | 0.5014 | 0.0010 | 0.0010 | 0.2388 | 0.0003 |
| Loblolly | 1978–1979 | 31.2 ± 0.9 | 1.1 ± 0.1 | 2.4 ± 0.9 | 42.8 ± 1.5 | 11.3 ± 0.7 | 5.6 ± 0.7 | 63.2 ± 1.6 |
| | 1995–1997 (+PDSI) | | | | | | | |
| | 1981–1982 | 32.2 ± 1.2 | 0.9 ± 0.2 | 2.3 ± 1.3 | 41.4 ± 1.8 | 10.8 ± 0.9 | 5.1 ± 0.8 | 60.6 ± 1.2 |
| Loblolly | 1999–2001 (–PDSI) | | | | | | | |
| | Difference | 1.0 | 0.2 | 0.1 | 1.4 | 0.5 | 0.5 | 2.6 |
| | <i>P</i> value | 0.0510 | 0.0798 | 0.9691 | 0.0964 | 0.0494 | 0.0026 | 0.0139 |
| Loblolly | 1978–1979 | 30.0 ± 1.3 | 0.9 ± 0.0 | 1.5 ± 0.3 | 47.7 ± 1.5 | 11.4 ± 0.7 | 4.8 ± 0.3 | 66.2 ± 1.4 |
| | 1995–1997 (+PDSI) | | | | | | | |
| | 1981–1982 | 30.0 ± 1.1 | 1.0 ± 0.1 | 1.6 ± 0.8 | 46.0 ± 1.8 | 11.7 ± 0.9 | 4.8 ± 0.5 | 65.1 ± 1.8 |
| Loblolly | 1999–2001 (–PDSI) | | | | | | | |
| | Difference | 0.0 | 0.1 | 0.1 | 1.7 | 0.3 | 0.0 | 1.1 |
| | <i>P</i> value | 0.9672 | 0.0366 | 0.5880 | 0.0740 | 0.0992 | 0.9658 | 0.1841 |

Data are mean percentages of extractive-free dry matter ± SD. Significant differences ($P < 0.05$) from paired *t*-tests are in boldface

drought stress on lignin content was determined using the acid-insoluble (Klason) lignin method.

Results obtained by Runion et al. (1999) showed a significantly lower lignin content in longleaf pine seedlings grown under water stress and an elevated level of CO₂ (720 vs. 365 μmol CO₂ mol⁻¹); their results, reported as lignin carbon fractions (mg fraction g⁻¹ tissue), were 141.4 for the treatment (elevated CO₂ and water stress) and 182.6 for the control (elevated CO₂ and no water stress). Although the value under the conditions of water stress and an ambient level of CO₂ (166.8 mg fraction g⁻¹ tissue) was seemingly lower than the ambient CO₂ control without water stress (182.6 mg fraction g⁻¹ tissue), the difference was not statistically significant. Since the sapling stem tissue used by Runion et al. (1999) was not partitioned to remove the bark from the wood, their results do not provide a direct observation of reduced lignification in wood from trees growing under water stress. To the best of the authors' knowledge, the present results with slash pine represent the first direct (wet chemical) observation of different moisture conditions (+PDSI vs. -PDSI) resulting in wood formed with different lignin contents in the same trees, aside from reduced lignification for wood formed in a conifer during a period of drought.

Analysis of polysaccharide sugars

The acid hydrolysis of wood by the Klason method yields the lignin as acid-insoluble particles that are collected by filtration, dried, and quantified gravimetrically. The resultant hydrolysate contains the polysaccharide sugars that are analyzed by liquid chromatography. The hydrolysis of cellulose, the most abundant of the cell wall polysaccharides, affords glucan as the most abundant sugar in wood. Given that the determinations of lignin and the polysaccharide sugars are based on the weight of extractive-free wood, higher values for lignin content would be offset by lower values for glucan content. Recently, Mayer et al. (2020) used two Raman signals (380 cm⁻¹ and 1600 cm⁻¹) as proxies for cellulose and lignin to report a seemingly lower cellulose to lignin ratio in false rings of *Abies alba*. A relative increase in lignin content was assumed, as opposed to a relative decrease in cellulose content, using the rationale of false ring tracheids needing higher lignin contents to prevent collapse when under water stress.

The relationship we found for slash pine was the inverse, with the condition of drought (-PDSI) giving wood with a lower lignin content and a higher glucan content (40.6% vs. 36.9%; $P=0.0010$). Since efforts to modify the chemical composition of wood have largely focused on presumed plasticity for lignin biosynthesis (Anterola and Lewis 2002; Novaes et al. 2010; Deng and Lu 2017; Xie et al. 2018; Vanholme et al. 2019), a drought induced reduction in lignin content relative to static polysaccharide contents can be rationalized. Indeed, the findings of Frelin et al. (2017) with loblolly pine, showing the upregulation of a gene (PtPheH) involved in tyrosine biosynthesis, and in the homogentisate pathway, suggests plasticity in cell wall chemistry in response to drought rests with lignin biosynthesis; however, Hori et al. (2020) recently employed an alternative strategy to improve the enzymatic saccharification of poplar wood, not by downregulating lignin genes, but

upregulating cellulose synthase genes. While concurrent plasticity in the biosynthesis of the cell wall polysaccharides in response to drought is plausible, we have not found any studies in the literature to support this as an alternative explanation for the present results.

Simple regression analyses were carried out to screen for any data trends between the +PDSI and –PDSI lignin contents for a given species, or between the lignin and sugar contents (or between two different sugar contents) within a PDSI grouping for a given species. None of the lignin content plots gave a coefficient of determination indicating that the +PDSI lignin content values for a species increased (or decreased) with the –PDSI lignin content values. Plotting the lignin contents against the glucan contents gave a negative relationship ($R^2=0.81$) for the –PDSI samples from slash pine (Fig. 3). For the –PDSI samples from loblolly pine, lignin content increased with glucan content ($R^2=0.43$). By removing one data point

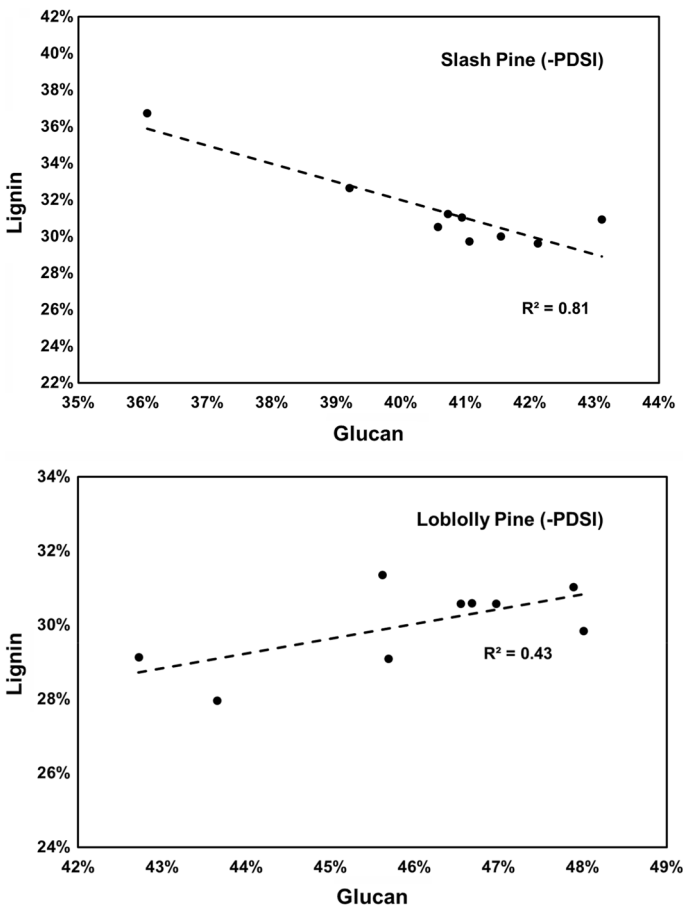


Fig. 3 Regression analyses of percentage lignin against percentage glucan for –PDSI wood excised from slash and loblolly pine increment cores

(lignin = 36.7%, glucan = 36.1%) that was obviously driving the stronger relationship for slash pine, the coefficient of determination was low ($R^2 = 0.36$). Altogether, the regression analyses suggest that for a given species and PDSI grouping, the lignin and glucan contents for individual samples are not directly offsetting values.

The hemicelluloses in the southern pines also contribute to the values for glucan, albeit to a minor level compared to the cellulose. Galactoglucomannans are the predominant hemicelluloses in softwoods, comprised of a backbone of glucan and mannan with branches containing galactan (Jones and Painter 1957, 1959; Rowell et al. 2013). The mannan content for slash pine was significantly higher (11.7% vs. 10.4%; $P = 0.0010$) for the –PDSI samples (Table 2). The other significant differences for individual sugars were observed for longleaf pine, having lower mannan (10.8% vs. 11.3%; $P = 0.0494$) and xylan (5.1% vs. 5.6%; $P = 0.0026$) for the –PDSI samples, the latter derived from another group of softwood hemicelluloses, the arabinoglucuronoxylans. Screening for relationships by simple linear regression showed

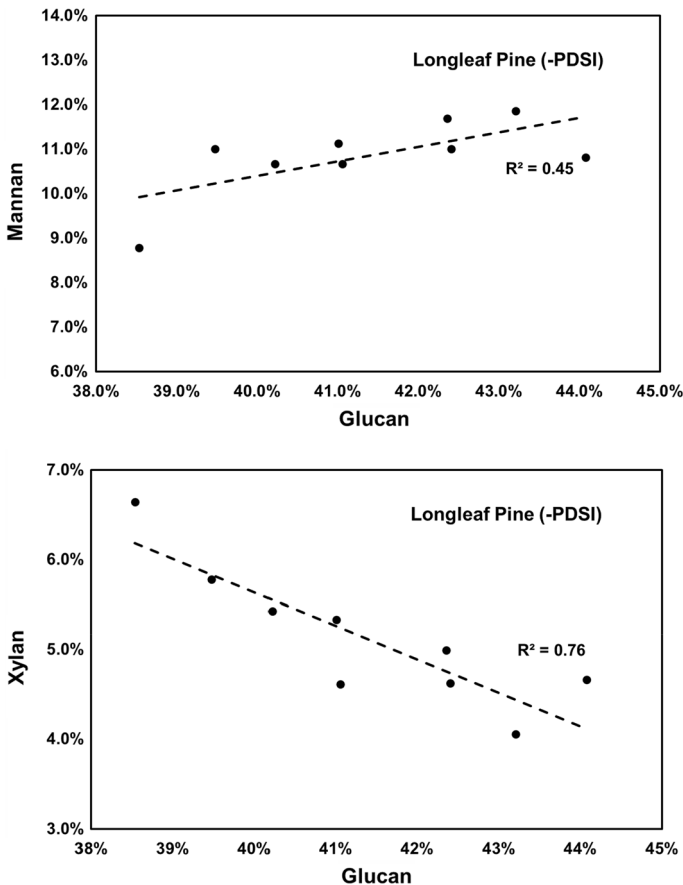


Fig. 4 Regression analyses of percentages of mannan or xylan against percentage glucan for –PDSI wood excised from longleaf pine increment cores

only a couple of trends with the corresponding longleaf pine – PDSI samples, those being mannan contents increasing with increasing glucan contents, and xylan contents decreasing with increasing glucan contents (Fig. 4). It is well beyond the scope of this study to unravel the potentially interconnected impacts of water stress on all cell wall polymer components; however, the regression analyses illustrate the potential for the biosyntheses of different hemicelluloses to respond differently to various growth conditions, among these being moisture stress, thus warranting further investigation.

While lignin and sugar analyses can be used to discern differences in wood chemistry between treatments (Tuskan et al. 1999; Jones et al. 2006; Rajan et al. 2020), it is acknowledged that they do not have the capacity to detect the upregulation of specific monomers or polymers, especially the hemicelluloses. Another caveat with the data presented here is that the cell wall chemistry is known to vary between earlywood and latewood within a growth ring, radially from pith to bark, and vertically in a tree at similar distances from the pith. Nevertheless, this study is the first of its kind to attempt the time-consuming task of collecting wood from specific growth rings of mature trees and comparing the actual polymeric (lignin) and monomeric (polysaccharide sugars) components to assess the impacts of moisture stress on the chemical composition of pine wood.

Southern pine drought resistance

Among the southern pines, longleaf pine is better suited to drier sites (Lantz 1987; Landers 1991) and is more drought resistant than slash and loblolly pines (Samuelson et al. 2012; Hart et al. 2020). Through fire suppression and widespread planting, loblolly and slash pines are no longer relegated to wetter sites, especially very wet sites where slash pine outperforms loblolly pine (Borders and Harrison 1989). The primary objective of the current study was to determine if any diversion of phenylalanine away from lignification during drought conditions, as suggested by the results of Frelin et al. (2017) for loblolly pine, could be detected by wet chemical analysis of the wood from any of the three southern pine species. It is particularly intriguing that the only difference in lignin content between periods of above- (+PDSI) and below-normal (–PDSI) soil moisture conditions was with slash pine, the species least suited for dry sites.

Prior leaf physiology assessments of the same trees used here did not support the contention of longleaf pine being better adapted to conserve water use (Samuelson et al. 2012). Longleaf pine drought tolerance was thereby attributed to aspects of its hydraulic conductivity (architecture/efficiency) that may be better suited to conditions of reduced moisture availability. By extension, it appears that slash pine does not have the same hydraulic conductivity attributes as longleaf pine and therefore may be subject to mechanisms to respond to moisture stress that extend back to the biosynthesis of lignin. Under normal (i.e., not drought) conditions, the mean lignin content of the slash pine samples was significantly higher (*t*-test, $P < 0.0001$) than that for the longleaf pine samples. It is plausible that the higher lignin content for slash pine could offset anatomical features imparting less efficient hydraulic conductivities. Moreover,

under drought conditions, the detected reduction in lignin content may be a product of reduced tree growth redirecting carbon away from lignin and toward the homogentisate pathway (Frelin et al. 2017), presumably as a reallocation of carbon resources to metabolic functions better served to endure a state of water stress.

Conclusion

Lower weights of wood recovered from $-$ PDSI growth rings than that for $+$ PDSI growth rings can be attributed to a relative reduction in growth during years with drought conditions. The higher lignin content (34.1%) of the $+$ PDSI slash pine wood, relative to that for the corresponding $-$ PDSI wood (31.4%), coincided with lower glucan and mannan contents. These results represent the first data for differences in lignin and polysaccharide sugars for mature wood formed during periods of ample moisture or drought. Among the three southern pines, slash pine is best suited to wet sites. The reduced lignin content for the $-$ PDSI wood is attributed to this species' higher susceptibility to drought. While these results suggest plasticity in cell wall chemistry in response to drought rests with lignin biosynthesis, concurrent plasticity in the biosynthesis of the cell wall polysaccharides cannot be excluded; the differences for the polysaccharide sugar contents illustrate the potential for the hemicellulosic biosynthetic processes to vary as a response to moisture stress.

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Declarations

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References

Adams MB, Loughry LH, Plaugher LL (2004) Revised March 2008, Experimental forests and ranges of the USDA Forest Service, Gen Tech Rep NE-321. USDA Forest Service, Northeastern Research Station, Newtown Square, PA

- Albaugh TJ, Allen HL, Dougherty PM, Johnsen KH (2004) Long term growth responses of loblolly pine to optimal nutrient and water resource availability. For Ecol Manage 192:3–19
- Anterola AM, Jeon J-H, Davin LB, Lewis NG (2002) Transcriptional control of monolignol biosynthesis in *Pinus taeda*. Factors affecting monolignol ratios and carbon allocation in phenylpropanoid metabolism. J Biol Chem 277(21):18272–18280
- Anterola AM, Lewis NG (2002) Trends in lignin modification: a comprehensive analysis of the effects of genetic manipulations/mutations on lignification and vascular integrity. Phytochemistry 61:221–294
- Aspinwall MJ, Li B, McKeand SE, Isik F, Gumpertz ML (2010) Prediction of whole-stem α -cellulose yield, lignin content, and wood density in juvenile and mature loblolly pine. South J Appl For 34(2):84–90
- Bhuta AAR, Kennedy LM, Pederson N (2009) Climate-radial growth relationships of Northern latitudinal range margin longleaf pine (*Pinus palustris* P. Mill.) in the Atlantic Coastal Plain of southeastern Virginia. Tree-Ring Res 65(2):105–115
- Borders BE, Harrison WM (1989) Comparison of slash pine and loblolly pine performance on cutover site-prepared sites in the Coastal Plain of Georgia and Florida. South J Appl For 13:204–207
- Bouriaud O, Leban J-M, Bert D, Deleuze C (2005) Intra-annual variations in climate influence growth and wood density of Norway spruce. Tree Physiol 25:651–660
- Brunow G, Lundquist K, Gellerstedt G (1999) Lignin. In: Sjöström E, Alén R (eds) Analytical methods in wood chemistry, pulping, and papermaking. Springer-Verlag, Berlin, pp 77–124
- Burdon RD, Kibblewhite RP, Walker JCF, Megraw RA, Evans R, Cown DJ (2004) Juvenile versus mature wood: a new concept, orthoganol to corewood versus outerwood, with special reference to *Pinus radiata* and *P. taeda*. For Sci 50(4):399–415
- Camarero JJ, Fernández-Pérez L, Kirilyanov AV, Shestakova TA, Knorre AA, Kukarskih VV, Voltas J (2017) Minimum wood density of conifers portrays changes in early season precipitation at dry and cold Eurasian regions. Trees Struct Funct 31:1423–1437
- Candel-Pérez D, Lo Y-H, Blanco JA, Chiu C-M, Camarero JJ, de Andrés EG, Imbert JB, Castillo FJ (2018) Drought-induced changes in wood density are not prevented by thinning in Scots pine stands. Forests 9:4
- Creber GT, Chaloner WG (1984) Influence of environmental factors on the wood structure of living and fossil trees. Bot Rev 50(4):357–448
- Cregg BM, Dougherty PM, Hennessey TC (1988) Growth and wood quality of young loblolly pine trees in relation to stand density and climatic factors. Can J For Res 18:851–858
- Cuny HE, Rathgeber CBK, Frank D, Fonti P, Fournier M (2014) Kinetics of tracheid development explain conifer tree-ring structure. New Phytol 203:1231–1241
- Davis MW (1998) A rapid modified method for compositional carbohydrate analysis of lignocellulosics by high pH anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD). J Wood Chem Tech 18(2):235–252
- Deng Y, Lu S (2017) Biosynthesis and regulation of phenylpropanoids in plants. Crit Rev Plant Sci 36(4):257–290
- Devall MS, Grender JM, Koretz J (1991) Dendroecological analysis of a longleaf pine *Pinus palustris* forest in Mississippi. Vegetatio 93:1–8
- Domec J-C, Gartner BL (2002) How do water transport and water storage differ in coniferous earlywood and latewood? J Exp Bot 53(379):2369–2379
- Donaldson LA (2002) Abnormal lignin distribution in wood from severely drought stressed *Pinus radiata* trees. IAWA J 23(2):161–178
- Eberhardt TL (2012) Impact of industrial source on the chemical composition of loblolly pine bark. For Prod J 62(7/8):516–519
- Eberhardt TL, Samuelson LJ (2015) Collection of wood quality data by X-ray densitometry: a case study with three southern pines. Wood Sci Technol 49:739–753
- Eberhardt TL, So C-L, Leduc DJ (2019) Wood property maps showing wood variability in mature longleaf pine: does getting old change juvenile tendencies? Wood Fib Sci 51(2):193–208
- Effland MJ (1977) Modified procedure to determine acid-insoluble lignin in wood and pulp. Tappi 60(10):143–144
- Fox TR, Jokela EJ, Allen HL (2007) The development of pine plantation silviculture in the southern United States. J For 105(7):337–347
- Frelin O, Dervinis C, Wegrzyn JL, Davis JM, Hanson AD (2017) Drought stress in *Pinus taeda* L. induces coordinated transcript accumulation of genes involved in the homogentisate pathway. Tree Genet Genom 13:27

- Frost CC (1993) Four centuries of changing landscape patterns in the longleaf pine ecosystem. In: Hermann SM (ed) Proceedings of the 18th Tall Timbers fire ecology conference. Tall Timbers Research Station, Tallahassee, FL, pp 14–43
- Gindl W (2001) Cell wall lignin content related to tracheid dimensions in drought-sensitive Austrian pine (*Pinus nigra*). IAWA J 22(2):113–120
- Gonzalez-Benecke CA, Martin TA, Clark A III, Peter GF (2010) Water availability and genetic effects on wood properties of loblolly pine (*Pinus taeda*). Can J For Res 40:2265–2277
- Gonzalez-Benecke CA, Riveros-Walker AJ, Martin TA, Peter GF (2015) Automated quantification of intra-annual density fluctuations using microdensity profiles of mature *Pinus taeda* in a replicated irrigation experiment. Trees Struct Funct 29:185–197
- González-Martínez SC, Wheeler NC, Ersoz E, Nelson CD, Neale DB (2007) Association genetics in *Pinus taeda* L. 1. Wood Property Traits Genet 175:399–409
- Graham JH, Duda JJ, Brown ML, Kitchen S, Emlen JM, Malol J, Bankstahl E, Krzysik AJ, Balbach H, Freeman DC (2012) The effects of drought and disturbance on the growth and developmental instability of loblolly pine (*Pinus taeda* L.). Ecol Indic 20:143–150
- Hart J, O’Keefe K, Augustine SP, McCulloh KA (2020) Physiological responses of germinant *Pinus palustris* and *P taeda* seedlings to water stress and the significance of the grass-stage. For Ecol Manage 458:117647
- Henderson JP, Grissino-Mayer HD (2009) Climate-tree growth relationships of longleaf pine (*Pinus palustris* Mill.) in the southeastern Coastal Plain, USA. Dendrochronologia 27:31–43
- Hennessey TC, Dougherty PM, Lynch TB, Wittwer RF, Lorenzi EM (2004) Long-term growth and eco-physiological responses of a southeastern Oklahoma loblolly pine plantation to early rotation thinning. For Ecol Manage 192:97–116
- Hori C, Takata N, Lam PY, Tobimatsu Y, Nagano S, Mortimer JC, Cullen D (2020) Identifying transcription factors that reduce wood recalcitrance and improve enzymatic degradation of xylem cell wall in *Populus*. Sci Rep 10:22043
- Jokela EJ, Dougherty PM, Martin TA (2004) Production dynamics of intensively managed loblolly pine stands in the southern United States: a synthesis of seven long-term experiments. For Ecol Manage 192:117–130
- Jones JKN, Painter TJ (1957) The hemicelluloses of loblolly (*Pinus taeda*) wood. Part 1. The isolation of five oligosaccharide fragments. J Chem Soc 669–673
- Jones JKN, Painter TJ (1959) The hemicelluloses of loblolly (*Pinus taeda*) wood. Part 2. The constitution of hexosan and pentosan components. J Chem Soc 573–580
- Jones PD, Schimleck LR, Peter GF, Daniels RF, Clark A III (2006) Nondestructive estimation of wood chemical composition of sections of radial strips by diffuse reflectance near infrared spectroscopy. Wood Sci Technol 40:709–720
- Kibblewhite RP (1999) Designer fibres for improved papers through exploiting genetic variation in wood microstructure. Appita J 52(6):429–435
- Kubler H (1980) Wood as building and hobby material. John Wiley and Sons, New York
- Kush JS, Meldahl RS, McMahan CK, Boyer WD (2004) Longleaf pine: a sustainable approach for increasing terrestrial carbon in the southern United States. Environ Manage 33(Supplement 1):S139–S147
- Lachenbruch B, Moore JR, Evans R (2011) Radial variation in wood structure and function in woody plants, and hypotheses for its occurrence. In: Meinzer FC, Lachenbruch B, Dawson TE (eds) Size- and age-related changes in tree structure and function. Springer, Berlin, pp 121–164
- Landers JL (1991) Disturbance influence on pine traits in the southeastern United States. In: Hermann SM (ed) Proceedings 17th Tall Timbers fire ecology conference. Tall Timbers Research Station, Tallahassee, FL, pp 61–98
- Lantz CW (1987) Which Southern pine species is best for your site? Forest Farmer 47(1):11–12
- Larson PR (1969) Wood formation and the concept of wood quality. Yale University, School of Forestry Bulletin No. 74. Yale University, New Haven, CT
- Larson PR, Kretschmann DE, Clark A III, Isebrands JG (2001) Formation and properties of juvenile wood in the Southern pines: a synopsis. Gen Tech Rep FPL-GTR-129. USDA Forest Service, Forest Products Laboratory, Madison, WI
- Maeda HA (2019) Harnessing evolutionary diversification of primary metabolism for plant synthetic biology. J Biol Chem 294(45):16549–16566
- Manzanedo RD, Pederson N (2019) Towards a more ecological dendroecology. Tree-Ring Res 75(2):152–159

- Mayer K, Grabner M, Rosner S, Felhofer M, Gierlinger N (2020) A synoptic view on intra-annual density fluctuations in *Abies alba*. *Dendrochronologia* 64:125781
- Meldahl RS, Pederson N, Kush JS, Varner JM III (1999) Dendrochronological investigations of climate and competitive effects on longleaf pine growth. In: Wimmer R, Vetter RE (eds) *Tree-ring analysis: biological, methodological, and environmental aspects*. CABI Publishing, London, pp 265–285
- Nix LE, Villiers K (1985) Tracheid differentiation in Southern pines during the dormant season. *Wood Fib Sci* 17(3):397–403
- Novaes E, Kirst M, Chiang V, Winter-Sederoff H, Sederoff R (2010) Lignin and biomass: a negative correlation for wood formation and lignin content in trees. *Plant Physiol* 154:555–561
- Outcalt KW, Sheffield RM (1996) *Tapping the trees: the naval stores industry in the American South*. Louisiana State University Press, Baton Rouge, LA
- Palmer WC (1965) *Meteorological drought*. US Department of Commerce Weather Bureau, Research Paper No. 45, Washington, DC
- Panshin AJ, de Zeeuw C (1980) *Textbook of wood technology*, 4th edn. McGraw-Hill, New York
- Pascual MB, El-Azaz J, de la Torre FN, Canas RA, Avila C, Cánovas FM (2016) Biosynthesis and metabolic fate of phenylalanine in conifers. *Front Plant Sci* 7:1030
- Pattathil S, Ingwers MW, Victoriano OL, Kandemkavil S, McGuire MA, Teskey RO, Aubrey DP (2016) Cell wall ultrastructure of stem wood, roots, and needles of a conifer varies in response to moisture availability. *Front Plant Sci* 7:882
- Plomion C, Leprovost G, Stokes A (2001) Wood formation in trees. *Plant Physiol* 127:1513–1523
- Rajan K, Djioleu A, Kandhola G, Labbé N, Sakon J, Carrier DJ, Kim J-W (2020) Investigating the effects of hemicellulose pre-extraction on the production and characterization of loblolly pine nanocellulose. *Cellulose* 27:3693–3706
- Rathgeber CBK, Cuny HE, Fonti P (2016) Biological basis of tree-ring formation: a crash course. *Front Plant Sci* 7:734
- Rosner S, Gierlinger N, Klepsch M, Karlsson B, Evans R, Lundqvist S-O, Svétlík J, Børja I, Dalsgaard L, Andreassen K, Solberg S, Jansen S (2018) Hydraulic and mechanical dysfunction of Norway spruce sapwood due to extreme summer drought in Scandinavia. *For Ecol Manage* 409:527–540
- Rowell RM, Pettersen R, Tshabalala MA (2013) Cell wall chemistry. In: Rowell RM (ed) *Handbook of wood chemistry and wood composites*, 2nd edn. CRC Press, Boca Raton, FL, pp 33–72
- Runion GB, Entry JA, Prior SA, Mitchell RJ, Rogers HH (1999) Tissue chemistry and carbon allocation in seedlings of *Pinus palustris* subjected to elevated atmospheric CO₂ and water stress. *Tree Physiol* 19:329–335
- Samuels AL, Kaneda M, Rensing KH (2006) The cell biology of wood formation: from cambial divisions to mature secondary xylem. *Can J Bot* 84:631–639
- Samuelson LJ, Stokes TA, Johnsen KH (2012) Ecophysiological comparison of 50-year-old longleaf pine, slash pine and loblolly pine. *For Ecol Manage* 274:108–115
- Schenck CA, Maeda HA (2018) Tyrosine biosynthesis, metabolism, and catabolism in plants. *Phytochemistry* 149:82–102
- Schmidting RC (1973) Intensive culture increases growth without affecting wood quality of young southern pines. *Can J For Res* 3:565–573
- Schultz RP (1999) Loblolly—the pine for the twenty-first century. *New For* 17:71–88
- South DB, Harper RA (2016) A decline in timberland continues for several Southern yellow pines. *J For* 114(2):116–124
- Stanturf JA, Wade DD, Waldrop TA, Kennard DK, Achtemeier GL (2002) Fire in Southern forest landscapes. In: Wear DM, Greis JG (eds) *Southern forest resource assessment*. Gen Tech Rep SRS-53. USDA Forest Service, Southern Research Station, Asheville, NC, pp 607–630
- Tuskan G, West D, Bradshaw HD, Neale D, Sewell M, Wheeler N, Megraw B, Jech K, Wiseloge A, Evans R, Elam C, Davis M, Dinus R (1999) Two high-throughput techniques for determining wood properties as part of a molecular genetics analysis of hybrid poplar and loblolly pine. *Appl Biochem Biotechnol* 77–79:55–65
- Ugla C, Magel E, Moritz T, Sundberg B (2001) Function and dynamics of auxin and carbohydrates during earlywood/latewood transition in Scots pine. *Plant Physiol* 125:2029–2039
- Vanholme R, De Meester B, Ralph J, Boerjan W (2019) Lignin biosynthesis and its integration into metabolism. *Curr Opin Biotech* 56:230–239
- Via BK, So C-L, Groom LH, Shupe TF, Stine M, Wikaira J (2007) Within tree variation of lignin, extractives, and microfibril angle coupled with the theoretical and near infrared modeling of microfibril angle. *IAWA J* 28(2):189–209

- Vogt T (2010) Phenylpropanoid biosynthesis. *Mol Plant* 3(1):2–20
- Wang Y, Chantreau M, Sibout R, Hawkins S (2013) Plant cell wall lignification and monolignol metabolism. *Front Plant Sci* 4:220
- Wear D, Greis J (2002) Southern forest resource assessment. Gen Tech Rep SRS-53. USDA Forest Service, Southern Research Station, Asheville, NC
- Xie M, Zhang J, Tschaplinski TJ, Tuskan GA, Chen J-G, Muchero W (2018) Regulation of lignin biosynthesis and its role in growth-defense tradeoffs. *Front Plant Sci* 9:1427
- Zobel BJ, Sprague JR (1998) Juvenile wood in forest trees. Springer-Verlag, Berlin
- Zubizarreta-Gerendiain A, Gort-Oromi J, Mehtätalo L, Peltola H, Venäläinen A, Pulkkinen P (2012) Effects of cambial age, clone and climatic factors on ring width and ring density in Norway spruce (*Picea abies*) in southeastern Finland. *For Ecol Manage* 263:9–16

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