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# Genetic effects on transpiration, canopy conductance, stomatal sensitivity to vapour pressure deficit, and cavitation resistance in loblolly pine

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## ABSTRACT

Physiological uniformity and genetic effects on canopy-level gas-exchange and hydraulic function could impact loblolly pine (*Pinus taeda* L.) plantation sustainability and ecosystem dynamics under projected changes in climate. Over a 1-year period, we examined genetic effects on mean and maximum mid-day canopy conductance ($G_s$, $G_{smax}$) and transpiration ($E$, max-$E$) within a juvenile loblolly pine plantation composed of 'genotypes' (e.g. different genetic entries) from each of the three different genetic groups (clones, full-sibs, open-pollinated). We also compared reference canopy conductance ($G_{s-ref}$ or $G_s$ at a vapour pressure deficit ($D$) = 1 kPa), maximum $E$ ($E_{max}$) in response to $D$, stomatal sensitivity to $D$, specific hydraulic conductivity ($k_s$), and cavitation resistance among genotypes. Based on genetic and physiological principles, we hypothesized that (1) within genotypes, physiological uniformity will increase as inherent genetic diversity decreases and (2) genotypes with greater $k_s$ and higher canopy-level gas-exchange rates will be more sensitive to increases in $D$, and more susceptible to loss of $k_s$. In our results, high- and low-genetic diversity genotypes showed no differences in $E$ and $G_s$ uniformity over time. However, $E$ and max-$E$ were significantly different among genotypes, and genotypes showed significant seasonal variability in $G_s$ and $G_{smax}$. Additionally, there were significant differences in $E_{max}$, $G_{s-ref}$, $G_s$ sensitivity to $D$, and the pressure at which 50% loss of $k_s$ occurs ($P_{50}$) among individual genotypes. We found no relationship between mean hydraulic conductivity parameters and overall $G_{s-ref}$ or $G_s$ sensitivity. However, the genotype full embolism point ($P_{88}$) and loss of $k_s$ rate ($LC_{rate}$) both showed a significant positive relationship with genotype $G_{s-ref}$ during the spring, indicating that genotypes with higher $G_s$ were less resistant to cavitation. Overall, genetic effects on canopy-level gas-exchange and cavitation resistance were significant, implying that physiological differences among genotypes might affect stand water use, carbon gain, drought tolerance, and hydrologic processes. Contrary to our expectations, uniformity in physiological process rates did not increase as inherent genetic diversity decreased, suggesting that clonal genotypes exhibit high physiological plasticity under plantation conditions. Lastly, our results imply that genotypes with higher spring-time gas-exchange rates may be more susceptible to catastrophic loss of $k_s$. With changes in climate expected to continue, physiological differences among genotypes may affect loblolly pine plantation carbon and water cycling. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS climate change; clone; drought resistance; hydraulic conductivity; water use

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## INTRODUCTION

Over the last 50 years, pine forest management in the southern US has shifted from natural stands, to intensively managed plantations (Prestemon and Abt, 2002; Wear and Greis, 2002), established with a variety of genetically improved, highly productive loblolly pine (*Pinus taeda* L.) genotypes (selected individuals with specific and known genetic ancestry) (McKeand *et al.*, 2003, 2006). Because of the work of loblolly pine genetic improvement programs, forest managers in the South currently have access to a wide range of half-sib (open-pollinated) and full-sib families, and a growing number of pine clonal varieties, each with varying amounts of inherent genetic variation. This range in genetic variation allows forest managers to weigh the risks of decreased genetic variation versus the gains of controlling genetic effects on growth (McKeand *et al.*, 2003). With greater control of genetic potential, the deployment of full-sib families and clones could result in greater stand-level uniformity and potentially enhanced productivity (Jansson and Li, 2004; Isik *et al.*, 2005). Alternatively, as more genetically homogeneous material is planted on more land, genotypes should be thoroughly tested and the potential gains and risks must be considered to maximize productivity and ensure sustainability (McKeand *et al.*, 2003; Bridgwater *et al.*, 2005), especially with changes in climate projected to continue (Trenberth *et al.*, 2007). Genetic effects on physiological traits such as hydraulic conductivity, gas exchange, stomatal sensitivity (Oren *et al.*, 1999), and cavitation resistance could impact stand productivity, sustainability, hydrology, and ecosystem function (Bond *et al.*, 2007) during periods of

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drought, high temperature and low humidity. Moreover, greater physiological uniformity over time within more genetically homogeneous loblolly pine genotypes could result in more uniform stand water use, evapotranspiration (ET), and streamflow. Conversely, more plasticity in these traits may help maintain stand growth and survival through periods of extreme environmental stress brought about by climate change (McNulty *et al*., 1997; Breshears *et al*., 2005; McDowell *et al*., 2008). Despite the potential importance of these physiological traits, research has provided insufficient information on physiological differences among highly productive, genetically improved loblolly pine. Considering the projected changes in climate, it is important that we evaluate how genetic differences in physiological process rates and hydraulic traits might impact forest sustainability and hydrological processes (Martin *et al*., 2005; McDowell *et al*., 2008).

Traits such as potential xylem permeability, quantified as the maximum specific conductivity of the xylem ($k_{s(max)}$) and cavitation resistance are traits that affect the ability of a tree to transport water and maintain leaf-water status (leaf-water potential, $\psi$) (Tyree and Ewers, 1991). Embolized conduits result in reduced hydraulic conductivity, ultimately limiting the ability of the plant to supply water to transpiring leaves, which can potentially result in desiccation or even plant death (Tyree and Sperry, 1989). Canopy conductance ($G_s$) and transpiration ($E$) govern the balance between carbon gain and whole-tree water use. $G_s$ is very sensitive and dynamically linked to hydraulic conductivity (Meinzer, 2002), so decreases in $k_s$(max) limit gas exchange (Comstock, 2002). Stomata control plant-water status and regulate the amount of water extracted by the plant from the soil by controlling the rate of water loss to the atmosphere such that the capacity of the soil–plant hydraulic system to supply water to leaves is matched (Oren *et al*., 1999; Franks *et al*., 2007; Domec *et al*., 2009a). Should stomata fail to sense and respond to a lower capacity of the soil–plant system to supply water, xylem would embolize rapidly, increasing the risk of hydraulic dysfunction and dehydration of leaves (Maseda and Fernández, 2006). Xylem dysfunction as a result of cavitation-induced embolism is the primary factor determining the large decreases in $k_s$ recorded in stems (Tyree and Ewers, 1991; Domec and Gartner, 2002), roots (Sperry and Ikeda, 1997; Domec *et al*., 2009a) and, thus, in whole plants (Meinzer, 2002; Domec *et al*., 2009a). The vapour pressure deficit ($D$) between the leaf and air is the driving force for $E$, so if $G_s$ is unchanged, transpiration increases with a rise in $D$ resulting from drying air. When the canopy is well coupled to the atmosphere, $D$ is a major factor governing stomatal response and depending on water status, trees typically respond to increasing $D$ by closing their stomata thus limiting water loss and reducing the likelihood for cavitation. The rate of stomatal closure or the response of stomata to changing $D$ is referred to as stomatal sensitivity (Oren *et al*., 1999; Domec *et al*., 2009a). Although guard cell stomata primarily respond to hydraulic supply and demand, stomata are heavily influenced by $D$ (Monteith, 1995; Buckley, 1997) and there are considerable differences in stomatal sensitivity to $D$ among species (Oren *et al*., 1999). As a whole, genetic variability in $G_s$ and its sensitivity to $D$ during different parts of the growing season may influence stand water use, carbon gain, drought resistance, and forest hydrology.

Numerous water relations studies in loblolly pine have focused on, among others, nutrient and water supply effects (Pataki *et al*., 1998a; Ewers *et al*., 2000), weed control effects (Samuelson and Stokes, 2006), soil porosity (Hacke *et al*., 2000), seed origin/provenance effects (Bilan *et al*., 1977; Bongarten and Teskey, 1986; Blazier *et al*., 2004), and elevated $CO_2$ (Pataki *et al*., 1998b and others). However, no studies have measured $G_s$ and stomatal sensitivity within highly selected loblolly pine genotypes. Furthermore, no studies have measured $k_s$, loss of $k_s$, and stomatal sensitivity to $D$ in loblolly pine clonal varieties. Hence, the objective of this study was to investigate genetic effects on hydraulic conductivity, cavitation resistance, transpiration, canopy conductance, and stomatal sensitivity to $D$, while comparing uniformity of physiological process rates within genotypes representing a range of genetic diversity. Specifically, we hypothesized (1) hydraulic traits, transpiration, and stomatal responses may not differ among genotypes, but physiological uniformity will be greater within genotypes with lower inherent genetic diversity (i.e. clones) than within more genetically diverse (i.e. more genetically heterogeneous) individuals. Hence, we predict that more genetically homogeneous clonally propagated genotypes will show more uniformity in $G_s$ and $E$ than full-sib or half-sib families and (2) genotypes with greater specific hydraulic conductivity and higher rates of transpiration or conductance will be more sensitive to increases in atmospheric evaporative demand. Therefore, unless the tree is anisohydric or not coupled well with the atmosphere, genotypes with higher specific hydraulic conductivity and canopy conductance will be more sensitive to changes in $D$, and will be more susceptible to loss of conductivity. To examine differences in uniformity and genetic effects on physiological process rates and hydraulic function in loblolly pine, we grew nine distinct genotypes in a plantation setting for 3·5 years and quantified productivity and physiological process rates with both field and laboratory measurements.

## MATERIALS AND METHODS

### *Study site and experimental design*

The study site was located at the Hofmann Forest in Onslow County, North Carolina (34°49 · 4′N, 77°18 · 2′W) (Aspinwall *et al*., 2011). The field site was topographically uniform with very little relief. Elevation at the site was ∼19 m above sea level. Mean annual (1971–2000) precipitation was 1435 mm, mean temperature 26·7 °C in July and 7·6 °C in January (National Climate Data Center, NOAA, available at:



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http://cdo.ncdc.noaa.gov/climatenormals/clim20/nc/314 144.pdf, accessed 24 March 2010). The soils consist of a Pantego mucky loam (fine loamy, siliceous, semiactive, thermic Umbric Paleaquults) (USDA, NRCS available at: http://websoilsurvey.nrcs.usda.gov/, accessed 24 March 2010). This soil series consists of very poorly drained, thick loamy deposits with moderate permeability. A naturally regenerated pine plantation had been established on the site prior to the establishment of this experiment. Prior to plantation establishment, drainage ditches were installed to remove excess water and seedlings were planted in rows along elevated beds to improve soil water and temperature conditions (Allen and Campbell, 1988; Allen *et al*., 1990).

In January 2006, the study was established as a single-tree plot design consisting of nine genotypes replicated 20 times. Therefore, the experimental unit was a single tree of each genotype randomly assigned within each replication (Aspinwall *et al*., 2011). Of the nine genotypes in this study, three were half-sib families (HS1, HS2, and HS3), three were full-sib families (FS1, FS2, and FS3), and three were clones (C1, C2, and C3). Half-sib (open-pollinated) families were created by collecting seed from a well-tested mother-tree that had been wind pollinated in a second generation seed orchard. Full-sib (control pollinated) families were produced by selecting and crossing two well-tested parents (McKeand *et al*., 2003). Clonally propagated material originated from somatic tissue culture (somatic embryogenesis) of the best individuals produced from full-sib families. Therefore, from half-sibs to full-sibs to clones, there was a trend of decreasing inherent genetic variation or increasing genetic homogeneity.

All half-sib and full-sib families were second-generation selections from the South Carolina–Georgia coastal plain. The half-sib families in this study were families known to have excellent productivity, stem form, and disease resistance. The full-sib families were elite families selected for high productivity and disease resistance. The clones used in this study were selected based on assessments of stem form, productivity, and rust resistance. In our study, some genotypes were related. One full-sib (FS3) family was a cross of two half-sib families (HS1 and HS3). HS1 was also one of the parents of FS1.

Given that the limited number of clones, full-sibs and half-sibs in this study were specifically selected based on assessments of productivity, disease resistance, and stem form, they are not representative of the range of productivity and physiological performance present in clones, half-sibs and full-sibs of loblolly pine. However, the selected genetic entries in this study do represent distinct levels of inherent genetic diversity within each genetic group. Therefore, the physiological variance within each genotype is a representation of the physiological variance that is expressed at each 'level' of genetic diversity growing under similar operational conditions during the early years of stand development.

Spacing between rows was 6·1 m (20 ft) and within-row spacing was 3·05 m (10 ft). Tree height (m) and ground-line diameter (cm) were measured monthly for 3·5 years after planting and stem volume ($m^3$) index was calculated as the product of height and ground-line diameter squared. On a yearly basis, competing vegetation adjacent to the study trees was manually removed.

### *Sap flow and climate data*

From 28 July 2008 to 7 April 2009, sap flow ($Q$, kg $h^{-1}$) was recorded on sun exposed, lateral branches (8–16 mm diameter, 1–2 m above ground) using 'baby' sensors (Sap flow meter T4·2, EMS, Brno, Czech Republic) and the heat balance method as described by Cermák *et al*. (2004). Sixty-minute averages of sap flow were computed from measurements taken every minute. A total of 12 sensors were installed on four trees of each genetic group. However, due to damaged sensors or environmental factors, data from all 12 sensors were rarely available. Every 2–3 weeks, the sensors were moved to new trees or branches and the data were downloaded from the datalogger. Before and after installation of the sensors, tree height (m), ground-line diameter (cm), branch height (m), branch diameter (mm), and branch length (m) were recorded. Sap flow during the winter months of November, December, January, and February was very low and extremely variable. Therefore, data collected during these months were omitted from the analysis. To account for seasonal effects, monthly data were grouped into seasons; July and August represented the summer, September and October represented the fall, and March and April represented the spring. In total, during the summer, sap flow was measured on 26 trees from within four replications with all three genetic groups and all nine genotypes represented. During the fall, sap flow was measured on nine trees from within four replications with each group of genetic variation represented; however, data were only available on seven of the nine genotypes (no data from C3 or FS1) due to damage to the sensors. Finally, during the spring, sap flow was measured on 16 trees from 8 of the 9 genotypes (no data from FS3) within two replications.

Hourly air temperature ($T_a$, °C), relative humidity (%), dew-point temperature (°C), barometric pressure (mb), and photosynthetically active radiation (PAR, $\mu$mol $m^{-2}$ $s^{-1}$) data were recorded by a climate station (1·2 km from site) located at the Hofmann Forest (NOAA–WRCC, 2009). Air temperature, dew-point temperature, and relative humidity were used to calculate saturated vapour pressure (kPa) and actual vapour pressure (kPa). The pressure difference is equivalent to the vapour pressure deficit ($D$).

After sap flow data collection, branches were removed and needles located distal to the sensors were removed, weighed in the field, and transported to the lab in a cooler. A subsample of fresh needles from each branch was scanned (Epson v700 scanner, Epson Inc., Long Beach, CA, USA) and needle surface area was calculated using ImageJ software (NIH Image software v1·62, http://rsbweb.nih.gov/nih-image). The scanned needles



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were then dried to a constant weight at 70 °C, and the fresh area of the scanned needles was divided by the dry weight to estimate one-sided specific leaf area (SLA, $m^{-2}$ $kg^{-1}$). Total projected (one-sided) branch leaf area ($m^2$) was calculated as the product of the total needle dry weight and SLA. $Q$ was then converted to transpiration per unit projected leaf area ($E$, mmol $m^{-2}$ $s^{-1}$) by dividing $Q$ by the total branch leaf area.

Sap flux-based canopy conductance per unit projected leaf area ($G_s$, mmol $m^{-2}$ $s^{-1}$) was calculated using the simplified Penman–Monteith model:

$$G_s = (RT_a\rho E)/D \quad (1)$$

where $R$ is the universal gas constant (0·46 $m^3$ kPa $K^{-1}$ $kg^{-1}$), $T_a$ is in degrees K, and $\rho$ is the density of water (998 $kg^{-1}$ $m^3$). Due to high boundary-layer conductance in loblolly pine canopies, the simplified calculation is used because $D$ is considered equivalent to the leaf-to-air vapour pressure deficit (Jones, 1992). For $Q$ to be used as a diurnal measure of $E$, the time lag between $E$ and $D$ was adjusted to more closely approximate the relationship between $E$ and $D$ (Phillips *et al*., 1997; Ewers and Oren, 2000). To make this adjustment, sap flow was shifted in time (~30 min) to increase the correlation with $D$. Daily mean and maximum mid-day values for transpiration ($E$ and max-$E$, respectively) and canopy conductance ($G_s$ and $G_{smax}$) were estimated based on measurements taken between the hours of 10 : 00 and 14 : 00. The coefficient of variation (CV) of daily mean mid-day $E$ and $G_s$ was also calculated to examine variability in $E$ and $G_s$ within genotypes over time.

The response of $E$ to changes in $D$ was modelled using the exponential function:

$$E = a\ (1 - e^{-bD}) \quad (2)$$

where a represents the maximum rate of transpiration ($E_{max}$) and $b$ represents the initial increase in $E$ with each unit $D$ (Ford *et al*., 2010). Additionally, Oren *et al*. (1999) showed that under light-saturating conditions, decreasing $G_s$ with increasing $D$ was proportional to $G_s$ at low $D$. Therefore, when PAR is greater than 800 µmol $m^{-2}$ $s^{-1}$, the stomatal sensitivity to $D$ can be calculated using the equation (Oren *et al*., 1999; Domec *et al*., 2009a,b):

$$G_s = b - m \times \ln(D) \quad (3)$$

where $b$ is $G_s$ at $D = 1$ kPa (or $G_{s-ref}$) and $m$ is the rate of stomatal closure with each change in $D$ which indicates the sensitivity of $G_s$ to $D$ ($-dG_s/d\ln D$, in mmol $m^{-2}$ $s^{-1}$ $\ln(kPa)^{-1}$). Stomatal sensitivity to $D$ is constant across a range of $D$, which allows for genotype comparisons to be made, independent of the range of $D$ (Oren *et al*., 1999).

*Specific conductivity*

Maximum specific hydraulic conductivity ($k_{s(max)}$, kg $m^{-1}$ $s^{-1}$ $MPa^{-1}$) and cavitation resistance (% loss of $k_{s(max)}$) were measured on xylem from terminal leader sections (15–20 cm in length) from the previous year of growth, collected in February of 2008. Five replications of xylem segments from all nine genotypes were collected for analysis; however, one sample from clone C2 was damaged, and therefore, a total of 44 samples were measured. All attached foliage or shoots were removed and both ends of each sample were recut under water using razor blades. Samples were soaked under a vacuum for 48 h to refill potentially embolized tracheids. Total xylem segment length (cm) and xylem diameter at each end (cm) of the stem segment were recorded. Average stem volume ($cm^3$) was calculated as the volume of a cylinder using the radius of each end of the stem segment for each volume calculation. $k_s$ was calculated as the mass flow rate of the perfusion of water divided by the pressure gradient across the stem segment, normalized by the xylem cross-sectional area (Domec and Gartner, 2001). Vulnerability curves (VCs) were constructed using the method described by Domec and Gartner (2001). This method uses a double-ended pressure chamber for causing embolism using air injection (Sperry and Saliendra, 1994). The method allows for the calculation of the percentage loss of $k_{s(max)}$ (PLC) as the xylem pressure increases. Before measuring $k_{s(max)}$, deionized-filtered (0·22 µm) water was placed under a vacuum for 24 h to remove air bubbles (gas). The segments were then inserted into the air injection chamber with both ends protruding and attached to a tubing system. Before attachment to the tubing system, razor blades were used to remove bark from both ends of the segments to ensure that the flow of water through the stem was maintained without any leakage. A VC was generated by pressurizing the air chamber to 0·025 MPa to avoid lateral water extrusion from the xylem, and allowing the system to equilibrate for 3 min. Water flow through the xylem segment was initiated and $k_{s(max)}$ was measured using a hydraulic pressure head of 3·5 kPa, which was low enough to avoid refilling of embolized tracheids. Efflux was collected in a 1-ml-graduated micropipette (0·01 ml graduation) and the time required for the meniscus to cross five consecutive graduation marks was recorded. A pressure ($P$) of 0·5 MPa was then applied and held constant for 2 min. After equilibration, the air chamber pressure was reduced to 0·025 MPa, and $k_s$ at $P = 0{\cdot}5$ MPa was measured. This process was repeated for pressures ranging from 0·5 to 4·0 MPa, or until the conductivity of the segment was negligible. The temperature of the water (to correct flow rate for the change in viscosity associated with change in temperature), and the length of each sample was recorded before and after the hydraulic conductivity measurements. VCs were fitted using the least squares approach and the sigmoidal function:

$$PLC = 100/(1 + e^{a(P-P50)}) \quad (4)$$

where PLC is the percentage loss of conductivity $[(k_{s(max)} - k_s(P))/k_{s(max)}]$, the parameter a is an indicator of the slope of the linear part of the VC, and $P_{50}$ is



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the pressure (MPa) at which 50% loss of conductivity occurred. The actual slope (s = a 25) of the linear part of the VC and the pressures at 12% loss of $k_{s(max)}$ ($P_{12} = 2/a + P50$) and 88% loss of $k_{s(max)}$ ($P_{88} = -2/a + P50$) were determined from the fitted curves (Domec and Gartner, 2001). The slope, a, was used to calculate the rate of loss of conductivity ($LC_{rate} = a{\cdot}25$ in % loss $k_{s(max)}$ $MPa^{-1}$). The value $P_{12}$, termed the air entry point (Sparks and Black, 1999), is an estimate of the xylem tension at which runaway cavitation and embolism begin when the resistance to air entry of pit membranes within the conducting xylem is overcome (Sperry and Tyree, 1988). $P_{12}$ is a linear approximation of the true air entry point, which from the VCs, starts very close to $P = 0$, but it provides a useful value for comparing curves. Similarly, $P_{88}$ is the full embolism point, interpreted as an approximation of the actual tension of the xylem before it becomes non-conductive (Domec and Gartner, 2001). After specific conductivity measurements were conducted, each stem segment was dried at 70 °C to a constant weight and wood density (g $cm^{-3}$) was calculated as the dry mass divided by wet volume. While this method does not estimate ring-by-ring density, percent earlywood or latewood, or other anatomical traits, it provides a means for determining the effect of overall wood density on cavitation resistance.

### *Statistical analysis*

The CV was used to quantitatively describe the spread of $E$, max-$E$, $G_s$, and $G_{smax}$ data among genetic groups and within genotypes. To account for the correlation among observations measured on the same tree over time (repeated measures), an ANOVA with a correlated residual structure (Fortin *et al.*, 2007) was used to determine the significance of the main effects of season, replication, genetic group, and genotype and their interactions on mid-day $E$, max-$E$, $G_s$, $G_{smax}$, CV for $E$, and CV for $G_s$ (MIXED procedure, SAS/STAT software v9·2, SAS Institute, Inc.). Because the genotypes included in this study were selected based on previous assessments of productivity and disease resistance, and do not represent the productivity and physiological performance of a loblolly pine population, the genotype effect was considered a fixed effect. Based on AIC and BIC model selection statistics, an autoregressive covariance error covariance structure (AR1) was most effective at minimizing the sum of squared error and was therefore used in the final analysis. Tree ground-line diameter, total height, branch height, branch diameter, and branch length were tested as covariates to account for possible size-related differences in $E$ and $G_s$ among genetic groups or genotypes. For the factors that were significant at $P \leq 0{\cdot}05$, Tukey's adjusted multiple range test was used for comparing group means.

Equation (3) was used to model the response of $G_s$ to changes in $D$. All data collected between the hours of 8 : 00 and 20 : 00 were included in the analysis. To reduce the occurrence of spurious relationships between $G_s$ and $D$ caused by low $D$ or low PAR, $G_s$ data associated with values of $D < 0{\cdot}6$ or PAR $<800$ μmol $m^{-2}$ $s^{-1}$ were removed (Oren *et al.*, 1999; Ewers and Oren, 2000). Season (spring, summer, fall), genetic group (half-sib, full-sib, clone), and genotype effects on $G_{s-ref}$ and stomatal sensitivity were tested using the model:

$$Y = \beta_{0i} + \beta_{1i} \ln D \quad (5)$$

where $Y$ represents estimated $G_s$, $\beta_{0i}$ represents the intercept or $G_{s-ref}$ for effect $i$ (season, genetic group, genotype) and $\beta_{1i}$ represents the slope parameter for effect $i$, or the change in $G_s$ $-dG_s/d \ln D$, i.e. sensitivity) as a function of $D$, where ln is the natural logarithm. Contrast statements were used to test for differences ($P \leq 0{\cdot}05$) in $\beta_{0i}$ and $\beta_{1i}$ among seasons, genetic groups, and genotypes using the GLM procedure of SAS/STAT software v9·2 (SAS Institute, Inc.). Equation (5) was also used to estimate genotype $G_{s-ref}$ and stomatal sensitivity to $D$ within each season so that changes in genotype $G_{s-ref}$ and stomatal sensitivity to $D$ could be examined over time.

Equations (2) and (4) were fit using a mixed-effects nonlinear model (NLMIXED procedure, SAS/STAT software v9·2, SAS Institute, Inc.) for comparison of response curve parameters among genetic groups and genotypes (Peek *et al.*, 2002; Ford *et al.*, 2010). Equation (2) was also fit for each season using the same approach. This approach accounts for repeated measures on the same subject and provides unbiased standard error estimates for season, genetic group, and genotype comparisons. Season, genetic group, and genotype parameters with upper and lower 95% confidence intervals that did not overlap were interpreted as being significantly different (Ford *et al.*, 2010). In Equation (4), parameters a and $P_{50}$, estimated for each genetic group and genotype, were used to estimate $P_{12}$, $P_{88}$, and $LC_{rate}$. While the mixed effects nonlinear approach for parameterization of Equation (4) does not produce standard error and confidence interval estimates for $P_{12}$, $P_{88}$, and $LC_{rate}$, it does provide useful estimates of these parameters for each genetic group and genotype. ANOVA $F$-tests were used to test for differences in $k_{s(max)}$ and wood density across all samples.

To investigate the relationship between canopy conductance, stomatal sensitivity to $D$, and cavitation resistance, linear regression was used to test for a relationship between genotype mean $G_{s-ref}$ or stomatal sensitivity within seasons and over time, and genotype $P_{12}$, $P_{50}$, $P_{88}$, $LC_{rate}$, and $k_{s(max)}$ (REG procedure, SAS/STAT software v9·2, SAS Institute, Inc.). With only nine genotypes, relationships with $P$-values $<0{\cdot}10$ were considered marginally significant.

## RESULTS

### *Growth, transpiration, and canopy conductance*

Ground-line diameter growth differed significantly ($P < 0{\cdot}0001$) among all genetic groups. Half-sib families had significantly higher mean diameter (cm) ($8{\cdot}51 \pm 0{\cdot}05$)



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Table I. Repeated measures ANOVA $F$-values, numerator, and denominator degrees of freedom, and mean-squared error (MSE) for mean and maximum mid-day transpiration ($E$, max-$E$) and canopy conductance ($G_s$, $G_{smax}$), as well as the coefficient of variation (CV) of mid-day $E$ and $G_s$.

| | Num df | Den df | $E$ | max-$E$ | $G_s$ | $G_{smax}$ | $E$ (CV) | $G_s$ (CV) |
|---|---|---|---|---|---|---|---|---|
| Tree height | 1 | 538 | 38·13*** | 30·27*** | 24·80*** | 18·53*** | ns | ns |
| Season (S) | 2 | 538 | 4·54* | 4·06* | 1·83 | 0·91 | 2·82 | 0·73 |
| Replication (R) | 4 | 538 | 3·59* | 3·28* | 3·23* | 2·74* | 0·65 | 0·18 |
| Genetic group | 2 | 538 | 7·87** | 6·62** | 8·75** | 6·87** | 1·16 | 1·38 |
| Genotype (genetic group) | 6 | 538 | 5·69*** | 4·79*** | 4·61** | 3·64** | 0·84 | 0·80 |
| S × genetic group | 2 | 538 | 0·28 | 0·23 | 3·07* | 1·97 | 0·56 | 1·74 |
| S × genotype (genetic group) | 4 | 538 | 1·64 | 1·87 | 10·78*** | 9·17*** | 1·04 | 5·71** |
| R × genetic group | 8 | 538 | 3·53** | 3·04** | 3·13** | 2·52* | 0·62 | 0·59 |
| R × genotype (genetic group) | 13 | 538 | 3·28*** | 2·19** | 2·47** | 2·11* | 0·86 | 0·73 |
| MSE | — | — | 0·06 | 0·07 | 1120·9 | 1937·4 | 344·5 | 329·9 |

When tree height was not a significant covariate (ns), it was omitted from the analysis.
* $P < 0·05$.
** $P < 0·001$.
*** $P < 0·001$.

than full-sibs ($8·12 \pm 0·06$) and clones ($7·29 \pm 0·05$). Mean height (m) was also significantly different ($P < 0·0001$) among groups (half-sibs, $\mu = 4·02 \pm 0·02$; full-sibs, $\mu = 3·74 \pm 0·03$; clones, $\mu = 3·57 \pm 0·02$).

Tree height was a highly significant covariate in the repeated measures ANOVA for $E$ and max-$E$ (Table I). $E$ and max-$E$ were significantly different among seasons, replications, genetic groups, and genotypes (Table I). For both $E$ and max-$E$, there were significant replication × genetic group, and replication × genotype interactions (Table I). Among all genotypes, FS3 and C3 had the highest and lowest mean $E$ and max-$E$, respectively (Figure 1a). Overall, C2, FS1, FS3, and HS1 all had significantly higher mean $E$ than C3 (Figure 1a). C2, FS1, FS3, HS1, HS2, and HS3 also had significantly higher mean rates of max-$E$ than C3 (Figure 1a).

As with $E$ and max-$E$, tree height was a significant covariate in the ANOVA for $G_s$ and $G_{smax}$ (Table I). $G_s$ and $G_{smax}$ were significantly different among replications, genetic groups, and genotypes (Table I). $G_s$ also showed a significant season × genetic group interaction. For both $G_s$ and $G_{smax}$, the season × genotype interaction was highly significant (Table I). Similar to $E$ and max-$E$, there were significant replication × genetic group and replication × genotype interactions for $G_s$ and $G_{smax}$ (Table I). Overall, all genotypes showed considerable variation in $G_s$ from season to season (Figure 1b). C1 showed the most drastic change in $G_s$ across seasons with much higher $G_s$ during the summer than in the spring and fall (Figure 1b). Some genotypes (C2, FS2, and HS3) showed high $G_s$ during the spring and summer and low $G_s$ in the fall. In contrast, other genotypes (HS1 and HS2) showed a linear increase in $G_s$ from spring to fall (Figure 1b). Genotypes also showed similar patterns of variation in mean $G_{smax}$ across seasons (Figure 1c). C1 showed much higher $G_{smax}$ during the summer than in the spring and fall, C2, FS2, and HS3 showed peak $G_{smax}$ during the spring and summer, and HS1 and HS2 showed higher $G_{smax}$ during the fall relative to the spring and summer (Figure 1c).

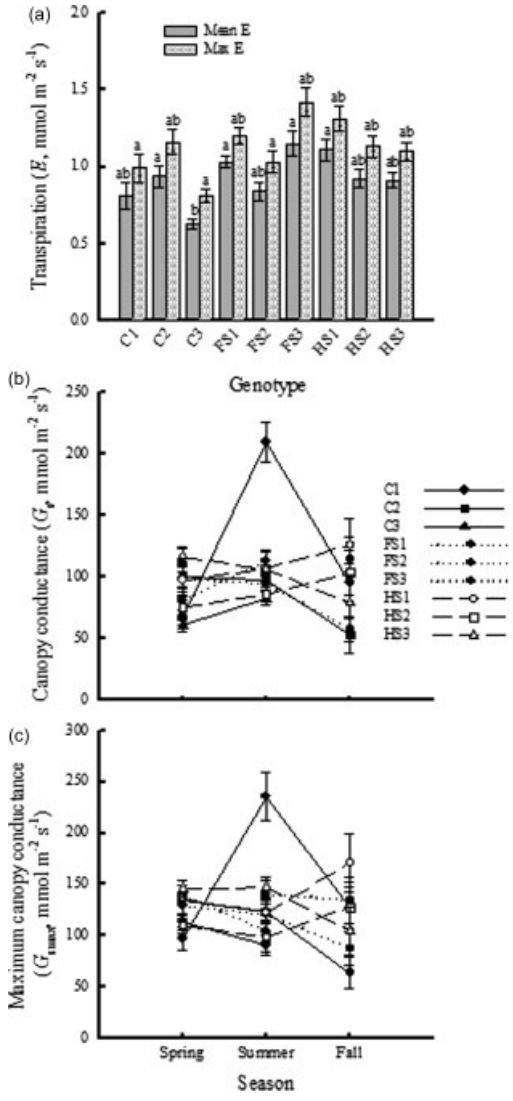


Figure 1. (a) Mean and maximum (± standard error) mid-day transpiration ($E$, mmol $m^{-2}$ $s^{-1}$) across all time periods among different loblolly pine full-sib (FS1, FS2, and FS3), half-sib (HS1, HS2, and HS3), and clonal genotypes (C1, C2, and C3). Means with the same letter are not significantly different at the $P \leq 0·05$ level. (b) Season × genotype interaction for mid-day canopy conductance ($G_s$, mmol $m^{-2}$ $s^{-1}$). (c) Season × genotype interaction for maximum mid-day canopy conductance ($G_{smax}$, mmol $m^{-2}$ $s^{-1}$).



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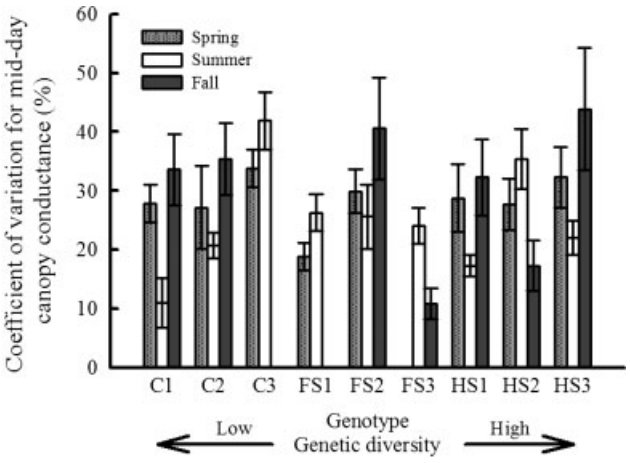


Figure 2. Season × genotype interaction for the coefficient of variation (CV) for mid-day canopy conductance among genotypes representing a range of inherent genetic diversity.

The CV for mid-day $E$ and $G_s$ was not significantly different among seasons, replications, genetic groups, and genotypes (Table I). However, there was a significant season × genotype interaction for the CV of mid-day $G_s$ (Table I). Within C1, C2, HS1, and HS3, CVs for $G_s$ were lowest during the summer (Figure 2). The same genotypes showed much higher CVs for $G_s$ during the fall (Figure 2). In contrast, CVs for $G_s$ within C3, FS1, FS3, and HS2 were highest during the summer (Figure 2). Overall, the physiological uniformity with clonal genotypes was no higher than the physiological uniformity within full-sib and half-sib genotypes.

### *Maximum transpiration, reference canopy conductance, and stomatal sensitivity*

The maximum rate of $E$ in response to increasing $D$ was significantly higher during the summer and fall than during the spring (Figure 3a). The initial increase in $E$ per unit $D$ was significantly higher during the spring and fall than during the summer (Figure 3a). Averaged over the entire growing season, the maximum rate of $E$ and the initial increase in $E$ per unit $D$ was not significantly different among genetic groups. However, over the entire growing season there were significant differences in the response of $E$ to changes in $D$ among genotypes (Table II). C2, FS3, HS1, and HS3 showed the highest maximum $E$ in response to $D$ while C3 showed the overall lowest maximum $E$ in response to changes in $D$ (Table II). In contrast, the initial increase in $E$ per unit $D$ was not significantly different among genotypes (Table II).

$G_{s-ref}$ was significantly different ($P < 0{\cdot}0001$) among all seasons with the highest values in the fall ($134{\cdot}8 \pm 2{\cdot}8$ mmol $m^{-2}$ $s^{-1}$), followed by the summer ($123{\cdot}1 \pm 2{\cdot}2$ mmol $m^{-2}$ $s^{-1}$), and spring ($104{\cdot}9 \pm 1{\cdot}6$ mmol $m^{-2}$ $s^{-1}$). Averaged over the entire growing season, $G_{s-ref}$ was not significantly different ($P = 0{\cdot}54$) among full-sib and half-sib families, but was significantly higher ($P < 0{\cdot}0001$) for both full-sibs and half-sibs than for clones (Figure 3b). Stomatal sensitivity to $D$ did not

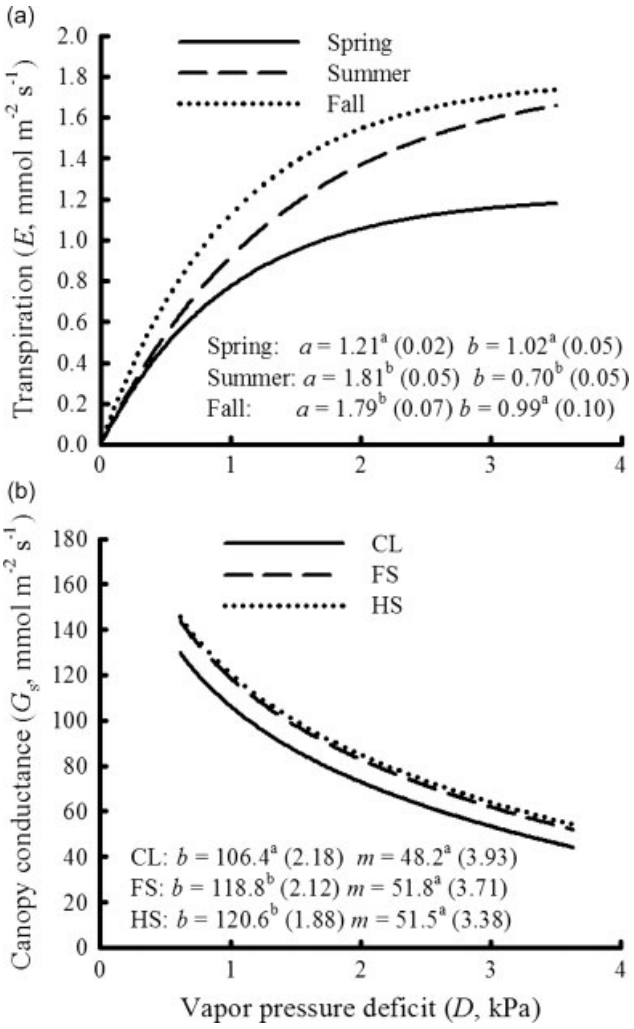


Figure 3. (a) Parameter estimates (± standard error) for the relationship between $E$ and $D$ for each season (SP = spring, SU = summer, and FA = fall) fit using the model: $E = a\ (1 - e^{-bD})$ where a equals the maximum rate of transpiration ($E_{max}$) and $b$ equals the initial slope. (b) Parameter estimates for the relationship between $G_s$ and $D$ by genetic group (CL = clone, FS = full-sib, HS = half-sib) averaged over all time periods. Values for $b$ and $m$ represent $G_{s-ref}$ and $-dG_s/d\ln D$, or reference canopy conductance and stomatal sensitivity to $D$, respectively. Parameter estimates with the same letter are not significantly different at the $P \leq 0{\cdot}05$ significance level.

differ among seasons (all $P > 0{\cdot}14$) or genetic groups (Figure 3b).

When averaged over the entire growing season, the response of $G_s$ to changes in $D$ was significantly different among genotypes (Table II). FS3's $G_{s-ref}$ was significantly higher than all other genotypes, and C1, C2, FS1, HS1, and HS3 had significantly higher $G_{s-ref}$ than C3, FS2, and HS2 (Table II). Genotype mean $G_{s-ref}$ and genotype mean $E_{max}$ showed a significant ($P = 0{\cdot}01$) positive linear relationship (intercept = $-0{\cdot}06$, slope = $0{\cdot}01$), with genotype mean $G_{s-ref}$ explaining 61% of the variation in genotype mean $E_{max}$. As with $G_{s-ref}$, there were significant differences in stomatal sensitivity to $D$ among genotypes (Table II). The stomatal sensitivities of C1, FS1, and FS3, were not significantly different, but all had significantly higher stomatal sensitivities than C2, C3, FS2, HS1, HS2, and HS3 (Table II).



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Table II. Comparison of model parameter estimates for the response of canopy conductance ($G_s$) and transpiration ($E$) to changes in vapour pressure deficit ($D$) among full-sib (FS1, FS2, and FS3), half-sib (HS1, HS2, and HS3), and clonal (C1, C2, and C3) genotypes.

| | Model 1: $E = \mathrm{a}\ (1 - \mathrm{e}^{-bD})$ | | | Model 2: $G_s = b - m \times \ln D$ | | |
|---|---|---|---|---|---|---|
| Genotype | $n$ | a | $b$ | $n$ | $G_{sref}$ | $-\mathrm{d}G_s/\mathrm{d}\ln D$ |
| C1 | 586 | $1{\cdot}29^{ac} \pm 0{\cdot}08$ | $1{\cdot}20^{a} \pm 0{\cdot}17$ | 363 | $127{\cdot}3^{a} \pm 3{\cdot}6$ | $81{\cdot}8^{a} \pm 6{\cdot}7$ |
| C2 | 633 | $2{\cdot}00^{b} \pm 0{\cdot}11$ | $0{\cdot}72^{a} \pm 0{\cdot}10$ | 440 | $123{\cdot}3^{a} \pm 3{\cdot}8$ | $48{\cdot}9^{b} \pm 6{\cdot}2$ |
| C3 | 831 | $1{\cdot}00^{a} \pm 0{\cdot}05$ | $0{\cdot}87^{a} \pm 0{\cdot}13$ | 514 | $83{\cdot}3^{b} \pm 2{\cdot}9$ | $39{\cdot}5^{b} \pm 5{\cdot}5$ |
| FS1 | 567 | $1{\cdot}50^{ac} \pm 0{\cdot}05$ | $1{\cdot}04^{a} \pm 0{\cdot}09$ | 376 | $124{\cdot}6^{a} \pm 3{\cdot}9$ | $64{\cdot}0^{a} \pm 6{\cdot}4$ |
| FS2 | 635 | $1{\cdot}28^{ac} \pm 0{\cdot}07$ | $0{\cdot}77^{a} \pm 0{\cdot}11$ | 410 | $99{\cdot}2^{c} \pm 3{\cdot}3$ | $33{\cdot}7^{b} \pm 6{\cdot}0$ |
| FS3 | 417 | $1{\cdot}90^{b} \pm 0{\cdot}10$ | $1{\cdot}07^{a} \pm 0{\cdot}13$ | 277 | $147{\cdot}1^{d} \pm 4{\cdot}5$ | $73{\cdot}8^{a} \pm 7{\cdot}9$ |
| HS1 | 743 | $1{\cdot}78^{abc} \pm 0{\cdot}13$ | $0{\cdot}59^{a} \pm 0{\cdot}10$ | 459 | $128{\cdot}0^{a} \pm 3{\cdot}4$ | $51{\cdot}4^{b} \pm 6{\cdot}1$ |
| HS2 | 734 | $1{\cdot}47^{ac} \pm 0{\cdot}05$ | $0{\cdot}87^{a} \pm 0{\cdot}09$ | 505 | $105{\cdot}3^{c} \pm 3{\cdot}3$ | $46{\cdot}6^{b} \pm 5{\cdot}4$ |
| HS3 | 692 | $1{\cdot}81^{abc} \pm 0{\cdot}13$ | $0{\cdot}66^{a} \pm 0{\cdot}10$ | 396 | $127{\cdot}8^{a} \pm 3{\cdot}2$ | $48{\cdot}4^{b} \pm 6{\cdot}3$ |

In Model 1, a equals the maximum transpiration ($E_{max}$) and $b$ equals the initial slope. In Model 2, $b$ represents $G_{s-ref}$ ($G_s$ at $D = 1$ kPa) and $m$ represents stomatal sensitivity to $D$ ($-\mathrm{d}G_s/\mathrm{d}\ln D$). All parameters were significant at $P \leq 0{\cdot}05$ level. For Model 1, parameters with lower and upper 95% confidence intervals that did not overlap were considered significantly different. Parameter estimates with the same letter are not significantly different at the $P \leq 0{\cdot}05$ significance level.

Over time, some genotypes showed significant changes in $G_{s-ref}$ and stomatal sensitivity to $D$ (Figure 4a and b). For example, clone C1 had the lowest $G_{s-ref}$ in the spring but the highest $G_{s-ref}$ in the summer before decreasing in the fall. Clone C2's $G_{s-ref}$ was consistent across spring and summer before increasing in the fall while FS2's $G_{s-ref}$ increased from the spring to summer before reaching its lowest value in the fall (Figure 4a). Across genotypes, stomatal sensitivity to $D$ showed no consistent pattern over time (Figure 4b). C1 showed a linear increase in stomatal sensitivity to $D$ from spring to fall while C3, FS2, HS1, and HS2 showed peak stomatal sensitivity in the spring and low sensitivity in the summer and fall (Figure 4b). Nonetheless, over time, genotypes exhibiting higher $G_{s-ref}$ tended to have higher stomatal sensitivity to $D$. Indeed, the overall genotype mean $G_{s-ref}$ showed a significant positive linear relationship with genotype mean stomatal sensitivity (Figure 4c).

*Specific conductivity and percent loss of conductivity*

VCs describing the relationship between xylem pressure and PLC were all highly significant ($P < 0{\cdot}0001$) for each genetic group and genotype (Figure 5). The slope parameter, a, was not significantly different among genetic groups or genotypes (Table III). Estimates of $P_{50}$ were not significantly different among genetic groups; however, among genotypes, C3 and HS2 had significantly higher (more negative) estimates of $P_{50}$ than genotypes C1, C2, FS1, FS2, and HS3 (Table III). As a result, calculated values for $P_{12}$ and $P_{88}$ were also higher (more negative) for C3 and HS2 and lower for C1, C2, FS1, FS2, and HS3 (Table III). Calculated values for $LC_{rate}$ were highest for FS1, HS1, and HS2, and lowest for C1 and FS2 (Table III).

Tree height, diameter, and sample wood density were not significantly correlated with $P_{12}$, $P_{50}$, $P_{88}$, $LC_{rate}$ or $k_s$(max). There were no significant differences in $k_s$(max) or sample wood density among genetic groups ($P = 0{\cdot}21$, $P = 0{\cdot}93$) or genotypes ($P = 0{\cdot}36$, $P = 0{\cdot}86$). Overall, mean $k_{s(max)}$ and wood density was $0{\cdot}92 \pm 0{\cdot}13$ kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$ and $0{\cdot}345 \pm 0{\cdot}003$ g cm$^{-3}$, respectively.

Genotype mean $k_{s(max)}$, $P_{12}$, $P_{50}$, $P_{88}$, and $LC_{rate}$ showed no significant relationship with genotype mean $G_{s-ref}$ or stomatal sensitivity to $D$ over time. However, when analysed by season, there was a marginally significant negative relationship between genotype mean $k_{s(max)}$ and genotype stomatal sensitivity to $D$ during the spring (Figure 6a) which means that genotypes with greater hydraulic conductivity were less sensitive to increases in $D$ during the spring. There were also significant positive relationships between both genotype $P_{88}$ and $LC_{rate}$, and spring $G_{s-ref}$ (Figure 6b and c), indicating that genotypes that were more susceptible to cavitation tended to have higher rates of canopy conductance during the spring.

## DISCUSSION

*Variation in gas-phase water flux as a function of genetic homogeneity*

Contrary to our hypothesis that physiological uniformity would increase with genetic homogeneity, we found no significant differences in the CV for mid-day $E$ among genetic groups or genotypes. Furthermore, the CV for mid-day $G_s$ showed a significant season × genotype interaction. Hence, the CVs for canopy-level gas-phase water flux within more genetically homogeneous individuals were not consistently lower implying that clones may not show greater physiological uniformity than more genetically heterogeneous individuals in operational plantation settings. High physiological variability within clones may be associated with enhanced phenotypic plasticity in response to changes in environmental conditions. Since clones possess low genotypic variation, their phenotypic variability is primarily an expression of environmental variation (Zobel and Talbert, 1984). As



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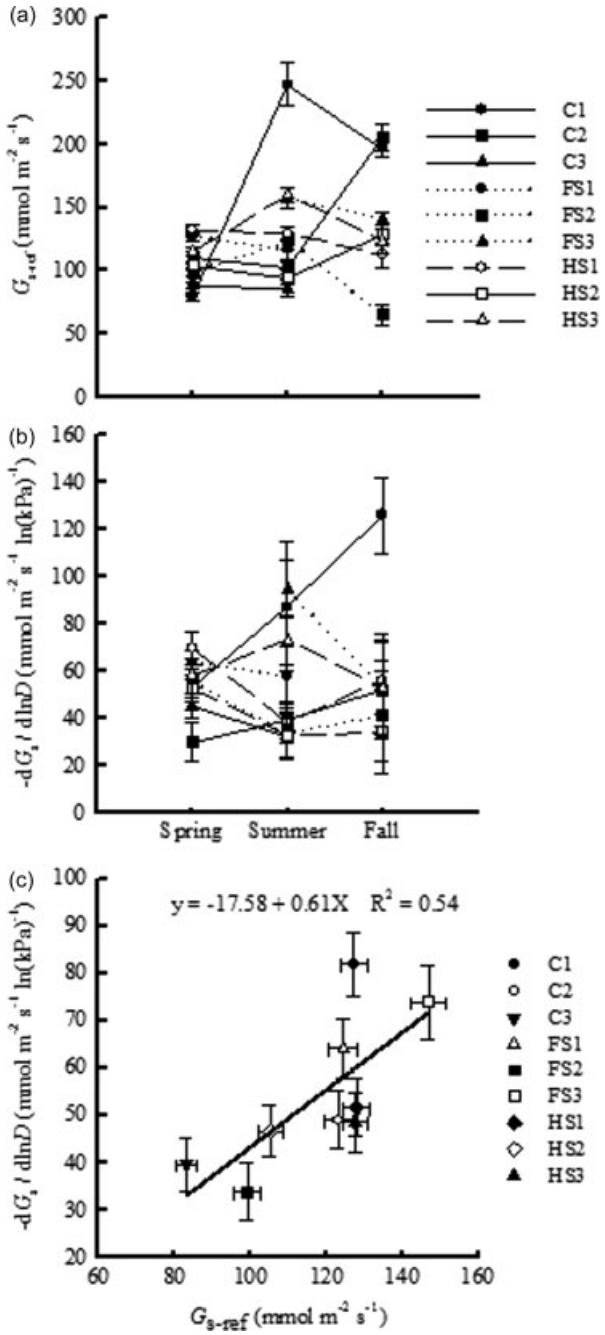


Figure 4. (a) Changes in reference canopy conductance ($G_{s-ref}$, $G_s$ where $D = 1$ kPa) by season for each genotype, (b) changes in stomatal sensitivity to $D$ ($-dG_s/d\ln D$) by season for each genotype. For (a) and (b), each point represents the mean parameter estimate for $G_{s-ref}$ and $-dG_s/d\ln D$ for the given genotype during the given season. Both parameters were estimated using equation 2, where $b$ and $m$ represent $G_{s-ref}$ and $-dG_s/d\ln D$, or reference canopy conductance and stomatal sensitivity to $D$, respectively, and (c) relationship between $G_{s-ref}$ and $-dG_s/d\ln D$ (model $p$-value = 0·025). Each point represents the mean parameter estimates for each genotype over the entire measurement period.

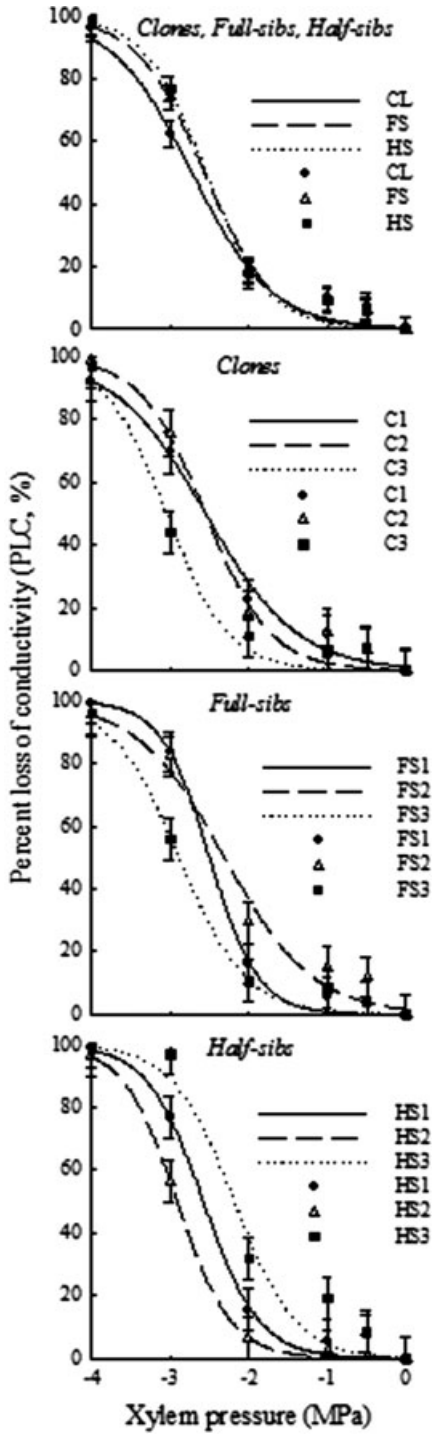


Figure 5. Vulnerability curves (VCs) showing the mean % loss of conductivity as a function of xylem pressure (MPa) for each genetic group (CL = clone, FS = full-sib, and HS = half-sib) and each genotype. For clones each data point represents the mean of $n = 14$ trees. For both full-sib and half-sib genetic groups, each data point represents the mean of $n = 15$ trees. For each genotype (C1, C2, C3, FS1, FS2, FS3, HS1, HS2, and HS3) each point is the mean of $n = 5$ trees, with exception to C2, where $n = 4$ trees.

an example, Orlović *et al*. (1998) found that in *Populus*, significant clonal variability in leaf physiological and anatomical properties was due to changes in site conditions. Conversely, full-sibs and half-sibs have higher amounts of inherent genetic variation allowing a wider range of physiological responses to environmental variation. Paradoxically, higher inherent genetic variation appears to buffer environmental heterogeneity, resulting in greater uniformity of physiological process rates and possibly stand-level resource assimilation.

While there have been no explicit investigations of physiological uniformity across different loblolly pine genotypes, there have been studies which have examined



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Table III. Vulnerability curve parameter estimates (± standard error) determined by the percent loss of conductivity (PLC) response to increases in xylem pressure (MPa) among different loblolly pine genetic groups and genotypes.

| | a | $P_{50}$ (MPa) | $P_{12}$ (MPa) | $P_{88}$ (MPa) | $LC_{rate}$ (% loss $k_{s(max)}$ $MPa^{-1}$) |
|---|---|---|---|---|---|
| Genetic group | | | | | |
| Clones | $1{\cdot}97^{a} \pm 0{\cdot}28$ | $-2{\cdot}73^{a} \pm 0{\cdot}08$ | $-1{\cdot}71$ | $-3{\cdot}75$ | 49·3 |
| Full-sibs | $2{\cdot}29^{a} \pm 0{\cdot}25$ | $-2{\cdot}57^{a} \pm 0{\cdot}06$ | $-1{\cdot}70$ | $-3{\cdot}44$ | 57·3 |
| Half-sibs | $2{\cdot}53^{a} \pm 0{\cdot}29$ | $-2{\cdot}55^{a} \pm 0{\cdot}06$ | $-1{\cdot}76$ | $-3{\cdot}34$ | 63·3 |
| Genotype | | | | | |
| C1 | $1{\cdot}72^{a} \pm 0{\cdot}46$ | $-2{\cdot}55^{a} \pm 0{\cdot}15$ | $-1{\cdot}39$ | $-3{\cdot}12$ | 43·0 |
| C2 | $2{\cdot}41^{a} \pm 0{\cdot}61$ | $-2{\cdot}55^{a} \pm 0{\cdot}14$ | $-1{\cdot}72$ | $-3{\cdot}43$ | 60·3 |
| C3 | $2{\cdot}54^{a} \pm 0{\cdot}73$ | $-3{\cdot}05^{b} \pm 0{\cdot}10$ | $-2{\cdot}26$ | $-3{\cdot}74$ | 63·5 |
| FS1 | $3{\cdot}22^{a} \pm 0{\cdot}45$ | $-2{\cdot}50^{a} \pm 0{\cdot}07$ | $-1{\cdot}87$ | $-3{\cdot}29$ | 80·5 |
| FS2 | $1{\cdot}78^{a} \pm 0{\cdot}37$ | $-2{\cdot}31^{a} \pm 0{\cdot}14$ | $-1{\cdot}19$ | $-3{\cdot}59$ | 44·5 |
| FS3 | $2{\cdot}34^{a} \pm 0{\cdot}55$ | $-2{\cdot}89^{ab} \pm 0{\cdot}08$ | $-2{\cdot}04$ | $-2{\cdot}97$ | 58·5 |
| HS1 | $2{\cdot}80^{a} \pm 0{\cdot}52$ | $-2{\cdot}58^{ab} \pm 0{\cdot}09$ | 1·87 | $-3{\cdot}71$ | 70·0 |
| HS2 | $2{\cdot}92^{a} \pm 0{\cdot}57$ | $-2{\cdot}91^{b} \pm 0{\cdot}06$ | $-2{\cdot}23$ | $-3{\cdot}38$ | 73·0 |
| HS3 | $2{\cdot}57^{a} \pm 0{\cdot}61$ | $-2{\cdot}19^{a} \pm 0{\cdot}10$ | $-1{\cdot}41$ | $-3{\cdot}84$ | 64·3 |

Parameter a is an indicator of the slope of the linear part of the vulnerability curve and $P_{50}$ is the pressure (MPa) at which 50% loss of $k_{s(max)}$ occurred. Parameters $P_{12}$ and $P_{88}$ are estimates of the xylem pressure at which 12 and 88% loss of $k_{s(max)}$ occurs, respectively. $P_{88}$ and $P_{88}$ are termed the air entry point and full embolism point, respectively. $LC_{rate}$ represents the rate at which loss of $k_{s(max)}$ occurs. Since $P_{12}$, $P_{88}$, and $LC_{rate}$ for each genetic group and genotype were calculated using parameter estimates of a and $P_{50}$, standard errors, confidence intervals, and significance tests could not be conducted. Parameters with the same letter had overlapping lower and upper 95% confidence intervals and were therefore not considered to be significantly different.

growth uniformity among genotypes growing across different environments. For instance, several studies have documented the generally high performance stability of open-pollinated loblolly pine families across a wide range of environmental conditions (McKeand *et al*., 1990, 2008; Lopez-Upton *et al*., 1999; Svensson *et al*., 1999). Others have suggested that clones will show more uniform growth and development (Martin *et al*., 2001; Bettinger *et al*., 2009) under intensive management. However, many studies involving clones have been conducted in progeny test conditions (Isik *et al*., 2003) and not under operational plantation conditions where resource availability can vary considerably. Consistent with our results, clones exposed to differences in resource availability have shown considerable physiological and morphological variability. For example, Gebremedhin (2003) found large differences in biomass partitioning within loblolly pine clones growing under different water availabilities. King *et al*. (2008) and Tyree *et al*. (2009) also found considerable variability in physiological process rates within clones growing under different nutrient availabilities. Despite sample size limitations, our results coupled with those of the previous studies suggest that clones may show equal or greater phenotypic plasticity in response to environmental variation, relative to more genetically diverse individuals. In terms of hydrology, greater variability in $E$ and $G_s$ within clones could potentially result in more heterogeneous stand-level water uptake, soil moisture, and productivity. On the other hand, at more uniform sites, low environmental heterogeneity might allow clones to show more uniformity in $E$ and $G_s$, and thus, more uniform ET and draw-down of soil water. Interestingly, the CVs for $G_s$ during the summer were lower for several genotypes and the maximum rate of $E$ in response to $D$ was generally higher during the summer (Figure 3). Together, these results suggest that the impacts of uniformity in gas-phase water fluxes on ET, water yield, and streamflow will be most apparent during the summer months.

### *Genetic effects on $G_s$ and E, $G_{s-ref}$, and stomatal sensitivity to D*

Overall, larger trees had higher rates of $E$ and $G_s$. Similarly, Ewers *et al*. (2001) found that in *P. taeda*, optimal water availability and nutrition resulted in larger trees with higher $G_s$. Pataki *et al*. (1998c) also found that higher leaf-level stomatal conductance was associated with larger increases in basal area increment. The genetic group and genotype differences in $E$ and $G_s$ (Figure 1) are consistent with other studies in loblolly pine where differences in gas-exchange rates among genotypes of varying genetic diversity were significant (Bilan *et al*., 1977; Bongarten and Teskey, 1986; Seiler and Johnson, 1988; King *et al*., 2008; Tyree *et al*., 2009). $G_s$ and $G_{smax}$ both showed significant variation across seasons with one clone in particular showing drastic increases in $G_s$ and $G_{smax}$ during the summer. Several factors including genotype phenology (Dougherty *et al*., 1994), microsite uniformity in herbaceous competition and soil water availability (Ewers *et al*., 2001; Domec *et al*., 2009b), or genetic variation in stomatal traits (stomatal density, stomatal length) (Dillen *et al*., 2008), may have contributed to the variation in our data. Genotypes also could have shown differences in plasticity in response to changes in season, as reflected in the seasonal variability in $G_{s-ref}$ and $G_s$ to $D$. Genetic differences in gas-exchange rates could impact stand-level biomass production as well as carbon assimilation, allocation, and sequestration. Stands established with genotypes that maintain more rapid rates of $G_s$ and $E$ will likely have greater water uptake and ET (Bond *et al*., 2007), reduced soil drainage and lower water yield (Amatya



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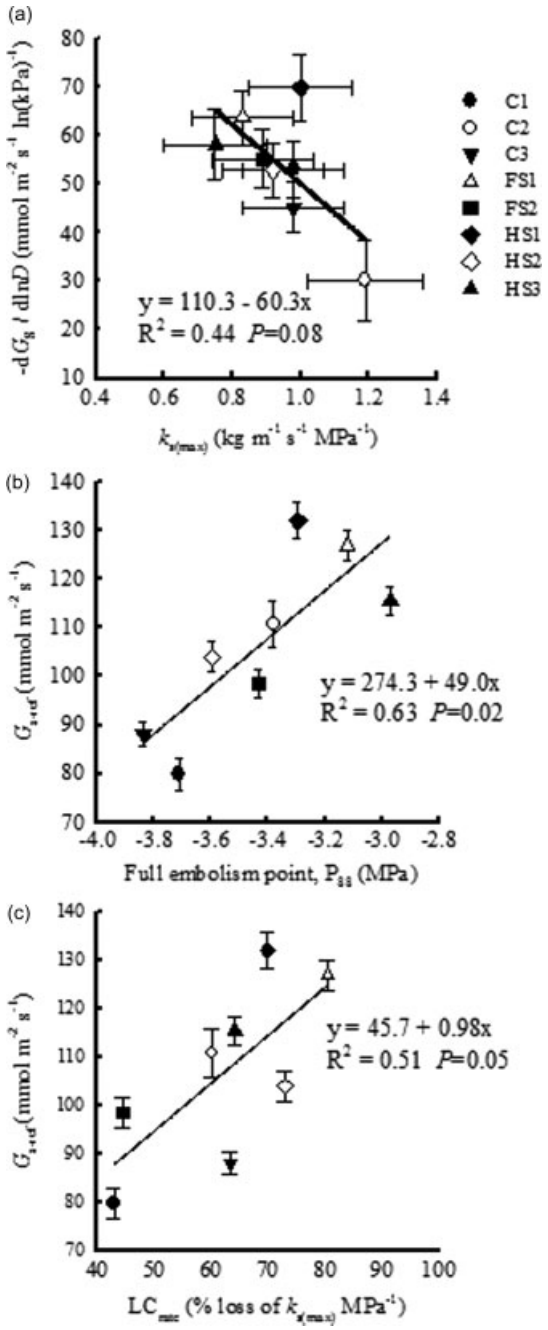


Figure 6. (a) Relationship between mean stomatal sensitivity to $D$ ($-dG_s/d\ln D$) during the spring and mean maximum specific hydraulic conductivity ($k_{s(max)}$), (b) relationship between mean canopy conductance at $D = 1$ kPa ($G_{s-ref}$) during the spring and the mean full embolism point ($P_{88}$), and (c) relationship between mean $G_{s-ref}$ during the spring and mean rate of loss of conductivity ($LC_{rate}$).

*et al*., 1996). Increases in $E$ and $G_s$ during the growing season could also result in reduced stream flows and soil moisture (Huntington, 2006; McLaughlin *et al*., 2007). On the other hand, genotype driven changes in $E$, and thus stand ET, could contribute to a feedback between available soil water and convective precipitation (Juang *et al*., 2007), thereby intensifying the water cycle (Huntington, 2006). Our findings suggest genetic effects on physiological processes will be most pronounced during the summer and fall when the response of $E$ to increasing $D$ is greatest (Figure 3), and therefore overall effects on site water balance will depend on how physiological drivers interact with other aspects of site hydrology, such as the seasonal distribution of precipitation.

Relatedness of some genotypes may explain some associations in mean physiological process rates among genotypes in the current study; however, diverse areas of the genome might respond differently to selective forces which may ultimately lead to phenotypic differences in morphology and physiology (Day *et al*., 2002; Masle *et al*., 2005). Interestingly, in *Populus trichocarpa*, Secchi and Zwieniecki (2010) found an upregulation of genes induced by embolism, followed by a downregulation after embolism, which suggested that there are underlying genetic responses to embolism presence. Furthermore, Watkinson *et al*. (2003) found that drought stress caused contrasting patterns of gene expression for genes encoding expression of photosynthetic traits, heat shock proteins, and enzymes associated with metabolic pathways for carbon allocation in loblolly pine. Therefore, although some genotypes were related, differences in gene transcript abundance or gene expression could have affected the physiological responses of genotypes.

Although we found no differences in $G_s$ sensitivity to $D$ among genetic groups or seasons, there were significant differences in $G_s$ sensitivity to $D$ among genotypes. Pallardy and Kozlowski (1979) also found marked differences in stomatal sensitivity to $D$ within particular *Populus* clones which were related to parental drought resistance. Furthermore, Pinheiro *et al*. (2005) found clonal differences in hydraulic conductivity and stomatal sensitivity to $D$ among different *Coffea canephora*. Clones with higher conductivity and less sensitivity to $D$ were quicker to reach more negative predawn water potentials, while more sensitive clones with lower conductivity exhibited heightened drought tolerance. In contrast, Bongarten and Teskey (1986) found no differences in the response of conductance to changing absolute humidity deficit among different loblolly pine half-sib families. While genotype differences in $G_s$ sensitivity to $D$ were significant, these differences were not consistent across all seasons, which indicated that other variables may have affected genotype stomatal responses. Some genotypes may have been more sensitive to seasonal fluctuations in localized soil water availability which influence $G_s$ (Ekanayake *et al*., 1994). With increases in temperature and decreases in humidity projected to continue (Trenberth *et al*., 2007), stands with more sensitive genotypes may be able to maximize carbon assimilation when atmospheric conditions are favourable, and minimize stress and water loss when conditions are less favourable. Therefore, genetic differences in stomatal sensitivity may be important for maintaining loblolly pine plantation productivity, sustainability and hydrologic balance. Understanding the interaction of genotype effects and soil water availability on $G_s$ sensitivity to $D$ will become increasingly important for selecting the best loblolly pine genotypes as climate change continues to increase the severity and variability of extreme weather events (e.g. hot and dry years).



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In comparison to stomatal sensitivity to $D$, mean genotype $G_{s-ref}$ was less variable over time, with exception to clones. Despite the seasonal variation in both $G_{s-ref}$ and stomatal sensitivity to $D$ among genotypes, stomata responded to $D$ in a manner consistent with protecting xylem integrity and thus capacity for water transport (Oren *et al*., 1999; Domec *et al*., 2009b). Based on the stated hydraulic consideration formulated by Oren *et al*. (1999), stomatal sensitivity to $D$ is expected to be proportional to $G_{s-ref}$ with the proportionality averaging ~0·60, as found in this study, and varying predictably depending on the range of $D$ experienced.

*Genetic effects on hydraulic properties: trade-offs between liquid- and gas-phase water transport*

While we found no differences in cavitation resistance among genetic groups, we did find significant differences in $P_{50}$ among individual genotypes. Similarly, Vander Willigen and Pammenter (1998) and Costa e Silva *et al*. (2004) found that vulnerability to cavitation was strongly related to clone in *Eucalyptus*. Dalla-Salda *et al*. (2009) also found significant variability in embolism resistance among different Douglas-fir (*Pseudotsuga menziesii*) clones. In contrast, Martinez-Vilalta *et al*. (2009) found little variation in embolism resistance across a wide range of *Pinus sylvestris* provenances. We found no significant relationship between $P_{50}$ and wood density, possibly due to the lack of variability in mean wood density among genotypes. Some studies have found a positive association between wood density and $P_{50}$ within and across species (Hacke *et al*., 2001); however, the relationship between wood anatomical traits and embolism resistance among genotypes of a species has been inconsistent. For example, Rosner *et al*. (2008) found a strong relationship between hydraulic properties and basic wood density in 24-year-old *Picea abies* clones, while Rosner *et al*. (2007) found no relationship between wood density and hydraulic vulnerability in 5-year-old *P. abies* clones. However, Rosner *et al*. (2007) found that longer tracheids, high latewood percentage, and thicker cell walls in earlywood were associated with hydraulic vulnerability. Since the stem samples in our study were taken from the previous years' growth on juvenile trees, the wood (juvenile wood) was composed of nearly all earlywood cells. Juvenile wood may show different mechanisms of cavitation resistance than mature wood. The shorter tracheids in juvenile wood require water to pass through more cell walls for a given distance, which increases the resistance to water flow, decreases water flux (Ewers *et al*., 1999), and may influence cavitation resistance (Domec and Gartner, 2002). Meinzer *et al*. (2009) also noted that the temporary release of stored water may govern changes in xylem tension in low wood density trees, whereas embolism avoidance in high wood density trees may be more constrained by xylem anatomical features. In future studies, measurements on samples from older trees with both earlywood and latewood cells may provide additional information on genetic variation in cavitation resistance. This may be especially important given the documented genetic effects on xylem development in loblolly pine (Talbert *et al*., 1983; Egertsdotter *et al*., 2004; Yang and Loopstra, 2005). Furthermore, investigation of variation in hydraulic traits and water transport efficiency throughout an intact plant may provide a better understanding of the dynamic nature of cavitation resistance among different genotypes (Meinzer *et al*., 2010).

Our results suggest that differences in $P_{50}$ among different loblolly pine genotypes may influence individual tree and stand level responses to drought. Stands established with more cavitation resistant genotypes may be able to maintain hydraulic function and $E$ under less favourable conditions, thereby sustaining carbon fixation and productivity. However, during cavitation, water is freed to the transpiration stream which temporarily increases xylem water potential, and thereby allows trees to maintain higher gas-exchange rates (Hölttä *et al*., 2009). If cavitation resistance allows for higher gas exchange over time, this could result in higher carbon sequestration and higher ET (Sun *et al*., 2010). Sustained gas-exchange rates due to greater cavitation resistance could further reduce soil moisture and stream flow (McLaughlin *et al*., 2007) during periods of drought. On the other hand, sustained drought could stimulate hydraulic dysfunction and reduce $G_s$ which may ultimately lead to carbon starvation (Breshears *et al*., 2009), desiccation and forest dieback even within cavitation-resistant genotypes. Drought-induced reductions in stand water uptake could lead to increases in water yield (Potts, 1984), streamflow (Bethlahmy, 1974), and runoff when precipitation does occur (Breshears *et al*., 2005; Huntington, 2006). From an ecohydrological perspective, genetic differences in cavitation resistance could impact stand level water and carbon cycling, alter loblolly pine population genetic composition, and generate land-surface atmospheric feedbacks (Breshears *et al*., 2005).

Lastly, our results provide support for the hypothesis that genotypes with higher physiological process rates will be more susceptible to loss of $k_s$. For example, we found a significant positive association between spring-time $G_{s-ref}$ and $P_{88}$, which indicated that genotypes that are more resistant to catastrophic loss of $k_s$ have limited rates of canopy conductance at low $D$. We also found that as genotype mean $LC_{rate}$ increases, so does spring $G_{s-ref}$, which indicates that genotypes with higher spring-time canopy conductance rates also have a more rapid loss of $k_s$ as xylem pressure increases. We expected that greater stomatal sensitivity to $D$ would be coupled with higher $G_{s-ref}$ and increased hydraulic conductivity. Interestingly, as genotype mean $k_{s(max)}$ increased, spring stomatal sensitivity to $D$ decreased, suggesting that genotypes with more rapid hydraulic conductivity are less sensitive to increases in evaporative demand. Although our results do not elucidate which factors control these relationships, genotype differences in phenology (Dougherty *et al*., 1994), stomatal traits (Dillen *et al*., 2008), or the combination of genetic and environmental effects on xylem formation (Zimmerman and Brown,



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1971; Yang and Loopstra, 2005) could all be potential factors. Additionally, xylem structural features that insure hydraulic safety may not be fully developed in juvenile stems during the spring (Cochard *et al*., 2009; Meinzer *et al*., 2010). Or, as in *Populus*, there may be differences in gene regulation associated with embolism (Secchi and Zwieniecki, 2010). Interestingly, the maximum rate of $E$ in response to $D$ was lowest during the spring which may indicate that $E$ is constrained by xylem hydraulic safety margins (Meinzer *et al*., 2010) during the spring. Overall, our results indicate that some genotypes are at greater risk of cavitation and those genotypes with higher $G_s$ may be most susceptible to catastrophic loss of $k_s$. Given that larger trees tended to have higher rates of $E$ and $G_s$, selection and deployment of highly productive genotypes of varying degrees of genetic diversity should be done carefully to maintain productivity, and to the extent possible, minimize stand vulnerability to drought induced dieback.

## CONCLUSIONS

Contrary to our expectations, our results did not support the hypothesis that physiological uniformity would increase with genetic homogeneity. We conclude that the lack of genetic variation within clones does not necessarily increase physiological uniformity, but to the contrary, may actually increase variation in resource uptake due to a lack of 'genetic buffering' of environmental variation. As a result, under heterogeneous environmental conditions, clonal stands might show more phenotypic variation in productivity, water uptake and ET. Genotype effects on mean and maximum mid-day $E$ were significant and the maximum rate of $E$ in response to increasing $D$ was greatest during the summer and fall. Together, these results suggest that stands on the lower coastal plain (USA) established with more productive genotypes that maintain more rapid rates of canopy-level gas-phase water flux will likely have greater water uptake and ET, lower stream flow, and lower water yield, with the most pronounced effects occurring during the summer and fall. Lastly, our results suggest that genotypes and stands with higher spring-time $G_s$ may be more susceptible to catastrophic loss of $k_s$, carbon starvation, loss of productivity and tree die-off during periods of high temperature, low humidity, and low soil water availability. Loss of hydraulic function coupled with decreases in gas-phase water flux would likely decrease stand ET and increase water yield. At the stand and regional level, genetic differences in drought tolerance could ultimately induce changes in stand dynamics, direct changes in population genetic structure, and create various meteorological feedbacks between the land surface and atmosphere. Further genetic testing and examination of physiological variation among and within different loblolly pine genotypes will help to ensure genetic material is properly matched to suitable sites, especially when incorporating climate change projections.

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