Research paper

Leaf-level gas-exchange uniformity and photosynthetic capacity among loblolly pine (*Pinus taeda* L.) genotypes of contrasting inherent genetic variation

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Variation in leaf-level gas exchange among widely planted genetically improved loblolly pine (*Pinus taeda* L.) genotypes could impact stand-level water use, carbon assimilation, biomass production, C allocation, ecosystem sustainability and biogeochemical cycling under changing environmental conditions. We examined uniformity in leaf-level light-saturated photosynthesis (A_{sat}), stomatal conductance (g_s), and intrinsic water-use efficiency (A_{sat}/g_s or δ) among nine loblolly pine genotypes (selected individuals): three clones, three full-sib families and three half-sib families, during the early years of stand development (first 3 years), with each genetic group possessing varying amounts of inherent genetic variation. We also compared light- and CO_2-response parameters between genotypes and examined the relationship between genotype productivity, gas exchange and photosynthetic capacity. Within full-sib, half-sib and clonal genotypes, the coefficient of variation (CV) for gas exchange showed no consistent pattern; the CV for g_s and δ was similar within clonal (44.3–46.9 and 35.5–38.6%) and half-sib (41.0–49.3 and 36.8–40.9%) genotypes, while full-sibs showed somewhat higher CVs (46.9–56.0 and 40.1–45.4%). In contrast, the CVs for A_{sat} were generally higher within clones. With the exception of δ, differences in gas exchange among genotypes were generally insignificant. Tree volume showed a significant positive correlation with A_{sat} and δ, but the relationship varied by season. Individual-tree volume and genotype volume were positively correlated with needle dark respiration (R_d). Our results suggest that uniformity in leaf-level physiological rates is not consistently related to the amount of genetic variation within a given genotype, and δ, A_{sat} and R_d were the leaf-level physiological parameters that were most consistently related to individual-tree and genotype productivity. An enhanced understanding of molecular and environmental factors that influence physiological variation within and between loblolly pine genotypes may improve assessments of genotype growth potential and sensitivity to global climate change.

Introduction

Genetic improvement of loblolly pine (*Pinus taeda* L.) has contributed to large increases in plantation productivity and, today, tree improvement operations provide forest managers and landowners with a variety of highly productive open-pollinated (half-sib) families and full-sib families, and more recently, varieties (clones) (McKeand et al. 2003, 2006). Each of these groups possesses varying amounts of inherent genetic variation (Namkoong et al. 1966, Zobel and Talbert 1984, White et al. 2007), and since growth of a given tree is a function of both genetics and environment, some increases in productivity may be attributed to greater control of genetic effects on growth. However, the physiological basis of increased productivity in intensively selected genotypes is still poorly understood (Martin et al. 2001, 2005) and little is known about how uniformity of physiological rates varies as a

function of genetic variation. Genetic differences in uniformity of important physiological traits such as photosynthesis, stomatal conductance and water-use efficiency may ultimately impact stand-level carbon assimilation and water use. Greater uniformity in physiology may lead to greater resource capture, resulting in greater stand-level productivity (Bettinger et al. 2009) during the early stages of stand development. Likewise, greater physiological variation could lead to more thorough exploitation of ecosystem niches and greater stand-level productivity. In addition, more or less physiological variation among taxa (i.e., genotypes or genetic categories) could influence stand resistance to climate change (Roth et al. 2007).

Differences in morphological and physiological traits in loblolly pine and other plant species have been reported between and within genotypes with varying amounts of inherent genetic variation (Boltz et al. 1986, Bongarten and Teskey 1986, Seiler and Johnson 1988, Barigah et al. 1994, Gerber and Dawson 1997, Lamhamedi et al. 2000, Emhart et al. 2007, King et al. 2008, Tyree et al. 2009). Within tree species, there is also evidence of significant genetic variation in light- and CO_2-response parameters (i.e., α, quantum yield; R_d, dark respiration; V_{cmax}, carboxylation efficiency; J_{max}, maximum rate of electron transport; Bauerle et al. 2003, Weston and Bauerle 2007). Conversely, some studies have found very little genetic variation in leaf-level physiology (Seiler and Johnson 1988, Cregg 1994). Furthermore, there are many environmental factors (Teskey et al. 1986, Liu and Teskey 1995, Murthy et al. 1996) working interactively at different levels (Yang et al. 2002, Gough et al. 2004, McGarvey et al. 2004) to influence photosynthesis and gas exchange. For this reason, instantaneous measurements of leaf-level gas-exchange and C fixation have been inconsistently related to genotype productivity (Yang et al. 2002, McGarvey et al. 2004, Martin et al. 2005) and, in many of these studies, the relationship between C assimilation and individual-tree and plot-level volume production has been linked to overall leaf area or light interception rather than instantaneous leaf-level photosynthetic rates (Vose and Allen 1988, McCrady and Jokela 1998, Chmura and Tjoelker 2008). To complicate the issue, environmental variables may also affect gene expression of morphological and physiological traits (Day et al. 2002). Despite the strong sensitivity of leaf-level physiological rates to changes in environment and the poor correlation between growth and leaf-level C fixation, there is evidence of a genetic correlation between variation in photosynthesis and growth when measurements account for seasonal variability and changes in tree size (Ledig and Perry 1969, Boltz et al. 1986, Isebrands et al. 1988). Therefore, measurements of instantaneous process rates taken over time would provide a more robust examination of genetic differences in physiological rates and the relationship between leaf-level physiology and genotype productivity.

Although previous studies have investigated genetic effects on gas exchange in loblolly pine, no studies have explicitly investigated genetic differences in uniformity of net CO_2 assimilation under saturating light (A_{sat}), stomatal conductance to water vapor (g_s) and intrinsic water-use efficiency (A_{sat}/g_s or δ) over time and within genotypes representing a range of genetic variation. Therefore, the objectives of this study were to compare uniformity of leaf-level physiological rates between and within half-sib families, full-sib families and clones, to determine how leaf-level physiology varies as a function of genetic variation and whether leaf-level physiological rates across multiple seasons are correlated with productivity. Additionally, we aimed to compare photosynthetic light- and CO_2-response parameters (α, R_d, V_{cmax}, J_{max} etc.) among genotypes and determine the relationship between genotype productivity and photosynthetic capacity. Specifically, our hypotheses were: (i) leaf-level physiological rates (A_{sat}, g_s, δ) will not necessarily differ between half-sib, full-sib and clonal genotypes, but within genotypes, uniformity in these processes will increase as genetic variation decreases; and (ii) physiological rates may not differ between genotypes at any particular measurement date, but when averaged over time, genotypes with higher A_{sat}, g_s and δ will show higher productivity. In other words, those genotypes that maintain consistently higher physiological rates will be more productive.

Materials and methods

Study site and experimental design

The study site was located at the North Carolina State University Hofmann Forest in Onslow County, North Carolina (34°49.4′N, 77°18.2′W) (Aspinwall et al. 2011). Elevation varies between 6.1 and 48.8 m, although our field site was topographically uniform with very little relief. Mean annual (1971–2000) precipitation is 1435 mm, mean temperature 26.7 °C in July and 7.6 °C in January (National Climate Data Center, NOAA, available at http://cdo.ncdc.noaa.gov/climatenormals/clim20/nc/314144.pdf, accessed 24 March 2010). Soils consist of a Pantego mucky loam (fine-loamy, siliceous, semiactive, thermic Umbric Paleaquult). This soil series, common to Atlantic Coast flatwoods, consists of very poorly drained, thick loamy soils with moderate permeability (USDA, NRCS, available at http://websoilsurvey.nrcs.usda.gov/, accessed 24 March 2010). A mature (age ~25), naturally regenerated pine stand, which had been established on the site prior to the establishment of this experiment, was harvested in October 2003. Drainage ditches were installed to remove excess water prior to plantation establishment, and seedlings were planted in rows along elevated beds to improve water and temperature conditions (Allen and Campbell 1988, Allen et al. 1990).

In January 2006, a physiological study was established as a randomized complete block design consisting of nine genotypes, where one tree from each genotype was randomly

inter-planted within each complete replication. Therefore, each tree within a replication was considered a single tree plot. Three of the genotypes were half-sib families (HS1, HS2, HS3), three were full-sib families (FS1, FS2, FS3) and three were clones (C1, C2, C3). Half-sib (open pollinated) families were created by collecting seed from a single well-tested mother tree that has been wind pollinated. 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 were varying amounts of inherent genetic variation. In our study, some genotypes were related. One full-sib family (FS3) was a cross of two half-sib families (HS1 and HS3). HS1 was also one of the parents of FS1. The half-sib and full-sib families were second-generation selections from the South Carolina–Georgia Coastal Plain and all nine genotypes were selected based on assessments of stem form, productivity and rust resistance.

All seedlings were bare root, but C1 and C3 were also planted as containerized stock to examine the effects of initial root morphology. However, after 2 years of growth, there were no significant differences in ground-line diameter or height between the bare-root and containerized trees of C1 ($P = 0.48$ and 0.80 for ground-line diameter, respectively) and C3 ($P = 0.19$ and 0.34 for ground-line diameter, respectively). Because there were no planting stock effects and all other genotypes were established as bare-root seedlings, we removed the containerized trees from the analysis. Spacing between rows was 6.1 m (20 ft) and within-row spacing was 3.05 m (10 ft).

Growth and gas-exchange measurements

Tree height (in m) and ground-line diameter (in cm) were measured monthly for 3.5 years after planting and stem volume (in m^3) index was calculated as the product of height and ground-line diameter squared. From January 2008 to May 2009, light-saturated CO_2 assimilation (A_{sat}, µmol m^{-2} s^{-1}) and stomatal conductance (g_s, mmol m^{-2} s^{-1}) were measured monthly or semi-monthly (total of $n = 14$ measurement dates) using an LI-6400 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA). Intrinsic water-use efficiency (A_{sat}/g_s or δ, µmol mmol^{-1}) was estimated following Cregg et al. (2000). Measurements were taken under ambient temperature conditions and cuvette temperatures averaged 16.6 °C in January, 20.1 °C in March, 32.5 °C in June, 37.3 °C in August, 22.2 °C in October and 23.0 °C in December.

Loblolly pine trees carry 2 years of foliage during the growing season (Vose and Allen 1988) and as needles age they become less efficient at photosynthesis due to excess wax buildup and shading from new branch and needle formation

(Warren 2006). In loblolly pine, needle elongation continues exponentially with peak elongation reached by the end of June, early July (Domec et al. 2009). Therefore, needle gas-exchange measurements were carried out on 1-year-old needles until the end of June, and on current-year needles afterwards, with all needles being fully elongated and fully sun exposed. Five to six replications of each genotype were measured per measurement date. Measurements were taken on three detached fascicles of needles within 3–4 min of removal from the tree. Preliminary measurements showed that A_{sat} of detached needles did not change within 20 min of removal, which is consistent with the findings of other studies (see King et al. 2008). Data were logged once the system coefficient of variation (CV) was ≤3%. Irradiance and reference CO_2 concentration were held constant at 1500 µmol m^{-2} s^{-1} and 360 µmol mol^{-1}, respectively. After the reference CO_2 concentration had stabilized (~5 min), the chamber CO_2 was matched to the reference CO_2 so that the chamber and reference CO_2 concentrations were equilibrated prior to a leaf being inserted into the chamber. All other variables remained at ambient conditions. All gas-exchange measurements were recalculated on a needle area basis following Ginn et al. (1991).

Photosynthetic light- and CO_2-response curves

Four replications of photosynthetic light-response curves were measured on fully elongated needles attached to sun-exposed branches on all nine genotypes in both June and July 2009. One light-response curve dataset measured on genotype HS1 (from July 9) was removed as a result of highly variable chamber conditions. Overall, a total of 71 light-response curves were developed. Initial chamber conditions were set at irradiance = 2000 µmol m^{-2} s^{-1} and CO_2 concentration = 360 µmol mol^{-1}. Next, light intensity was reduced stepwise from 2000 to 1500, 1000, 800, 600, 400, 200 and 0 µmol m^{-2} s^{-1} so that a curve relating A to irradiance could be produced. Chamber conditions were held constant for 2–3 min before data were logged. All gas-exchange measurements were adjusted for needle surface area as described above. The irradiance-saturated photosynthetic rate (A_{max}; µmol m^{-2} s^{-1}), dark respiration rate (R_d; µmol m^{-2} s^{-1}), apparent quantum yield (α) and light compensation point (Γ_i; µmol m^{-2} s^{-1}) were estimated by fitting a non-rectangular hyperbola (Marshall and Biscoe 1980) by non-linear least squares. A/C_i curves were also constructed using the same four replications of all nine genotypes in both June and July 2009. Irradiance was held constant at 2000 µmol m^{-2} s^{-1} while external CO_2 concentration, controlled with a CO_2 mixer, changed to 400, 300, 200, 100, 50, 600, 800 and 1000 µmol mol^{-1} CO_2. The intercellular CO_2 concentration (C_i; µmol mol^{-1}) was recorded and data were logged when chamber conditions reached equilibrium (CV ≤3%). One A/C_i curve dataset measured on genotype FS2 (from 21 July) was removed due to highly variable chamber

conditions. In total, 71 A/C_i curves were developed. Each A/C_i curve was parameterized using the Farquhar model of C_3 photosynthesis (Farquhar et al. 1980). The maximum rate of carboxylation (V_{cmax}; $\mu mol\ m^{-2}\ s^{-1}$), the maximum rate of electron transport under saturated light (J_{max}; $\mu mol\ m^{-2}\ s^{-1}$), the rate of non-photorespiratory CO_2 evolution (R_c; $\mu mol\ m^{-2}\ s^{-1}$) and the intercellular CO_2 concentration at which the rate of CO_2 assimilation changes from Rubisco carboxylation limited to ribulose bisphosphate (RuBP) limited ($C_{i\,tr}$; $\mu mol\ mol^{-1}$) were estimated following Dubois et al. (2007). Rather than fitting each function of the Farquhar model individually, the procedures described by Dubois et al. (2007) were used for simultaneous parameterization with the temperature-adjusted (25 °C) coefficients, K_c, K_o [Michaelis–Menten constants of for CO_2 ($\mu mol\ mol^{-1}$) and O_2 ($mmol\ mol^{-1}$), respectively] and Γ_c (the CO_2 photosynthetic compensation point; $\mu mol\ mol^{-1}$) taken from Bernacchi et al. (2001). In cases where the model failed to converge, the mean C_{itr} was used as a partial constraint on the range of C_{itr}, which allowed for estimation of V_{cmax}, J_{max} and R_c for the entire A/C_i curve (Dubois et al. 2007). Following Rodeghiero et al. (2007), all A/C_i curve parameters were corrected to account for potential chamber diffusion leaks.

Statistical analysis

Variation in A_{sat}, g_s and δ between genetic levels and within genotypes was compared using frequency distributions, the CV and the standard deviation. The assumption of homogeneous variance across genetic levels and genotypes was tested using Levene's test (Levene 1960) ($P \leq 0.05$) for homogeneous variance with Welch's test for equal group means. To account for the correlation among observations measured on the same tree over time, an analysis of variance (ANOVA) with a correlated residual structure (Fortin et al. 2007) was used to determine the significance of the main and interactive effects of month, replication, genetic group and genotype within genetic group on A_{sat}, g_s, δ, A_{max}, α, R_d, Γ_i, V_{cmax}, J_{max}, R_c and C_{itr}. Because the genotypes in this study do not represent all clones, half-sib or full-sib families, we considered the genotype effect to be fixed. All other effects were also considered fixed. Akaike information criteria and Bayesian information criteria were used to determine the correlated residual structure that most effectively minimized the sum of squared error. Each light-response and A/C_i curve was fitted using non-linear least-square regression and the Levember–Marquardt algorithm. Model adequacy (goodness-of-fit) was verified using residual analysis, model F-test and the model coefficient of determination (R^2). Parameter estimates estimated from each light-response and A/C_i curve were combined into one dataset and ANOVA was used to determine the significance of the main and interactive effects of month, replication, genetic group and genotype within genetic group on estimated A_{max}, R_d, α, Γ_i, V_{cmax}, J_{max}, R_c and C_{itr}. Pearson correlation coefficients (r) and

scatter plots were used to determine the relationship between growth (volume, ground-line diameter, height) and A_{sat}, g_s and δ over time. Light- and A/C_i-response curves were fitted using SAS PROC NLIN, and repeated-measures ANOVA was conducted using SAS PROC MIXED. When genotype effects were significant in the ANOVA, Tukey's adjustment was used for pairwise comparison of genotype means. All significance tests were conducted at the $P < 0.05$ significance level (SAS Institute, 2002).

Results

Genetic and seasonal variation in A_{sat}, g_s and δ

At the end of the measurement period, the mean volume growth was significantly different ($P = 0.03$) between genotypes with FS1, FS3, HS1, HS2 and HS3 having consistently higher mean volume than C1, C2, C3 and FS2 (Figure 1a). Tree volume (in m^3) was not a significant covariate for A_{sat}, but was a significant covariate for g_s and δ (Table 1). Mean A_{sat}, g_s and δ were significantly different between months of the year and A_{sat} and δ differed between replications, indicating significant
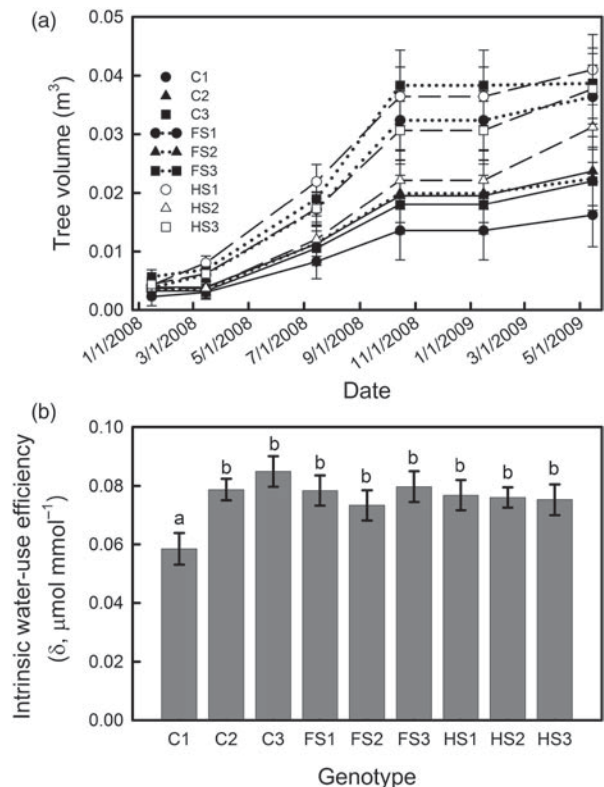

Figure 1. (a) Mean volume growth (±standard error) among different 2- to 3-year-old open-pollinated (half-sib; HS1, HS2, HS3), full-sib (FS1, FS2, FS3) and clonal (C1, C2, C3) genotypes between January 2008 and May 2009. (b) Least-squared means (±standard error) for intrinsic water-use efficiency (δ) over time among different 2- to 3-year-old half-sib, full-sib and clonal genotypes. Based on Tukey's adjustment, means with the same letter are not significantly different at $P \leq 0.05$.

Table 1. *P*-values, degrees of freedom (d.f.) and mean-squared error (MSE) from repeated-measures ANOVA on leaf-level light-saturated photosynthesis (A_{sat}, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}) and intrinsic water-use efficiency (δ; μmol mmol^{-1}) from monthly data collected from January 2008 to May 2009.

	d.f.	A_{sat}	g_s	δ
Tree volume	1	ns	0.0078	0.0002
Month (M)	9	<0.0001	<0.0001	<0.0001
Replication (R)	12	<0.0001	0.6754	<0.0001
Genetic group	2	0.0935	0.2130	0.7922
Genotype (genetic group)	6	0.7460	0.4120	0.0160
M × genetic group	18	0.9046	0.5368	0.2015
M × genotype (genetic group)	54	0.4660	0.1802	0.6165
R × genetic group	24	0.4907	0.3260	0.4711
R × genotype (genetic group)	70	0.0228	0.0468	0.0870
MSE		0.92	528.90	0.00039

Measurements were conducted on 2- to 3-year-old loblolly pine trees growing in a plantation setting on the lower coastal plain of North Carolina (NC). Total tree volume (m^3) was tested as a covariate and when not significant (ns), it was omitted from the analysis.

temporal and microsite variation. On the basis of repeated-measures ANOVA, there were no significant differences in A_{sat}, g_s and δ between genetic groups (clones, full-sibs, half-sibs) and no significant differences in A_{sat} and g_s among genotypes. However, there were significant genotype differences in δ with clone C1 having the lowest overall mean δ (Table 1, Figure 1b). There was a significant replication × genotype interaction for A_{sat} and g_s. Overall, for all gas-exchange parameters, there were no significant interactions between months and genetic groups or genotypes (Table 1).

Mean A_{sat} was generally highest between the months of April and June (5.23–5.78 μmol m^{-2} s^{-1}) and lowest between the months of November and March (2.56–4.70 μmol m^{-2} s^{-1}) (Figure 2a). Mean g_s showed a similar seasonal trend with mean values ranging from 77.25 to 108.37 mmol m^{-2} s^{-1} between April and June, to 42.12–61.15 mmol m^{-2} s^{-1} between November and March (Figure 2b). Peak δ occurred during the winter and spring (0.08–0.12 μmol mmol^{-1}) when g_s and transpiration was low. Hence, the seasonal low mean δ occurred during the summer (0.05–0.06 μmol mmol^{-1}) when the ratio of C fixation to water loss via transpiration was lowest (Figure 2c).

Uniformity of A_{sat}, g_s and δ within full-sib families, half-sib families and clones

Among all nine genotypes, there were no significant differences in the variance of A_{sat} ($P = 0.74$), g_s ($P = 0.06$) and δ ($P = 0.83$). As a group, clones had a significantly lower standard deviation in g_s ($\sigma = 28.8$, $P = 0.005$) than full-sibs or
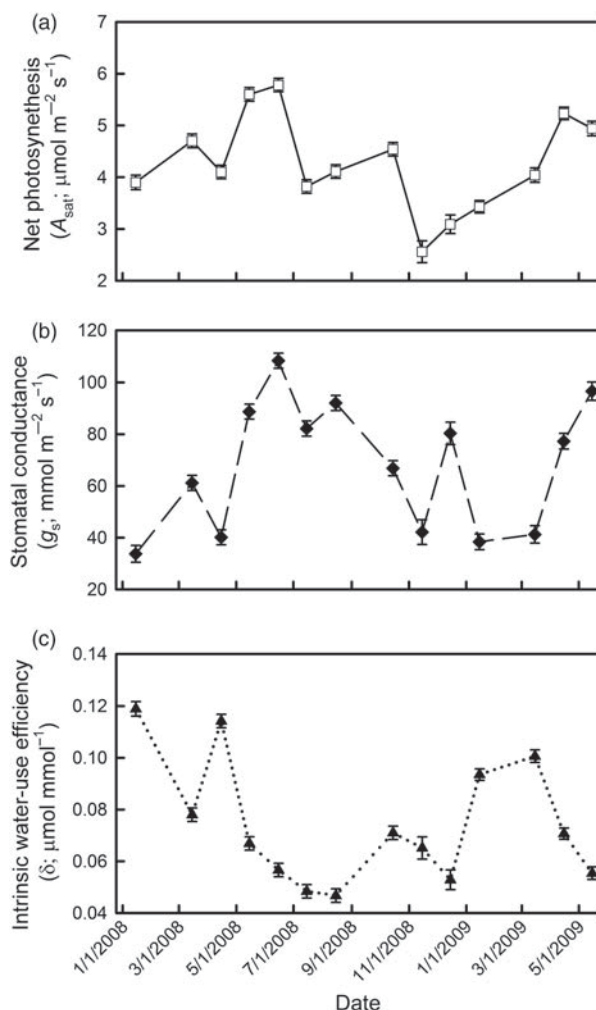

Figure 2. Mean monthly (a) light-saturated photosynthetic rate (A_{sat}), (b) stomatal conductance (g_s) and (c) intrinsic water-use efficiency (δ) across different 2- to 3-year-old loblolly pine clones, full-sib families and half-sib families growing in a plantation on the lower coastal plain of North Carolina.

half-sibs ($\sigma = 37.3$ and $\sigma = 32.7$, respectively). There were no significant differences in the standard deviation of A_{sat} ($P = 0.30$) or δ ($P = 0.45$) among genetic groups. When comparing the CV within genotypes, uniformity in A_{sat} was greatest within full-sib genotypes, slightly lower within half-sibs and lowest within clones (Figure 3a–c). In contrast, uniformity in g_s and δ was generally higher within individual clones and half-sib genotypes than within full-sib genotypes (Figure 3).

Overall mean A_{sat} within individual genotypes ranged from 4.73 (FS3, HS3) to 3.79 (C2) μmol m^{-2} s^{-1} (Figures 3a and 4c). Genotype mean g_s and δ ranged from 55.0 to 79.1 mmol m^{-2} s^{-1} and 0.070 to 0.080 μmol mmol^{-1}, respectively (Figures 3d and 4i). In addition to having the least uniformity in A_{sat}, clone C2 also had the lowest mean g_s while full-sib genotypes FS2 and FS3 had the highest mean g_s (Figure 3d and f). Without accounting for month, replication and interactive
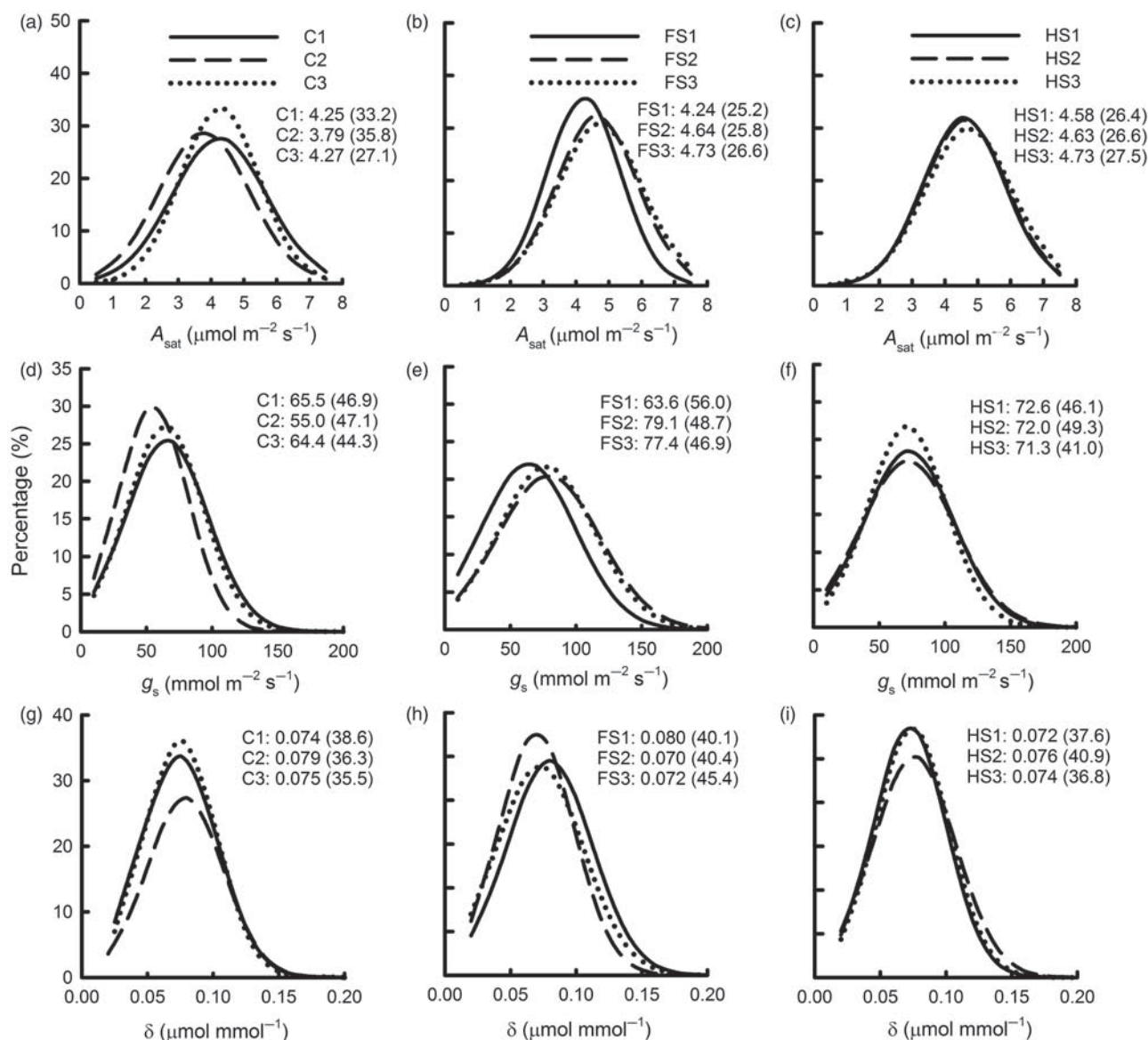
Figure 3. Distribution of leaf-level light-saturated net photosynthetic rate (A_{sat}; a–c), stomatal conductance (g_s; d–f) and intrinsic water-use efficiency (δ; g–i) between and within different 2- to 3-year-old loblolly pine clones (C1, C2, C3), full-sib families (FS1, FS2, FS3) and half-sib families (HS1, HS2, HS3) growing in a plantation on the lower coastal plain of North Carolina. Raw mean gas-exchange values for each genotype are shown with the CV in parentheses.

effects with ANOVA, clones had the widest range of genotype means for A_{sat}, full-sibs had the widest range of genotype means for g_s and δ, and half-sibs had the narrowest range of means for A_{sat}, g_s and δ (Figure 3a and i).

Photosynthetic light- and CO₂-response curves

The non-rectangular hyperbola model provided an excellent fit for each individual photosynthetic light-response curve ($P < 0.0001$). Significance tests of the parameter estimates of all 71 curves indicated that there were no significant differences in A_{max}, α, R_d or Γ_i between genetic groups or genotypes within each group (Table 2). There were no significant differences between replications and there were no significant

interactions between genotypes or genetic groups and replications or measurement months (Table 2). Since the effects in the ANOVA were not significant for any of the light-response curve parameters, one model fit to all the data resulted in an estimated A_{max} and R_d of 8.5 ± 0.5 and 0.98 ± 0.13 μmol m⁻² s⁻¹, respectively. The fitted Γ_i was 64.5 μmol m⁻² s⁻¹ and the overall estimated α was 0.023 ± 0.005.

The method for fitting each A/C_i dataset provided significant ($P < 0.05$) and repeatable estimates of V_{cmax}, J_{max}, R_c and C_{itr}. Analysis of variance on all 71 A/C_i datasets showed no significant differences in V_{cmax}, R_c or C_{itr} between measurement dates, and no significant differences between genetic groups or genotypes (Table 3). Overall mean V_{cmax}, R_c and C_{itr} was 19.1 ± 0.8,

Table 2. ANOVA P-values, d.f. and MSE for genetic effects on photosynthetic light-response curve parameters in a 3-year-old loblolly pine plantation on the coastal plain of North Carolina.

	d.f.	A_{max}	α	R_d	Γ_i
Month (M)	1	0.0241	0.5198	0.0004	<0.0001
Replication (R)	3	0.3246	0.4195	0.2017	0.3809
Genetic group	2	0.2989	0.2434	0.2287	0.8996
Genotype (genetic group)	6	0.1032	0.9203	0.4527	0.6427
M × genetic group	2	0.8531	0.4060	0.3012	0.9848
M × genotype (genetic group)	6	0.1820	0.9539	0.1878	0.5598
R × genetic group	6	0.6423	0.6009	0.5892	0.8902
R × genotype (genetic group)	18	0.8830	0.5134	0.8824	0.9507
MSE		4.93	0.0004	0.24	629.34

A_{max} is the maximum rate of light-saturated photosynthesis (μmol m^{-2} s^{-1}); α is the apparent quantum yield; R_d is the dark respiration rate (μmol m^{-2} s^{-1}), and Γ_i is the light compensation point (μmol m^{-2} s^{-1} PPFD).

Table 3. ANOVA P-values, d.f. and MSE for genetic effects on photosynthetic CO_2-response curve parameters in a 3-year-old loblolly pine plantation on the coastal plain of North Carolina.

	d.f.	V_{cmax}	J_{max}	R_c	C_{itr}
Month (M)	1	0.309	0.101	0.185	0.667
Replication (R)	3	0.015	0.001	0.496	0.005
Genetic group	2	0.097	0.810	0.121	0.090
Genotype (genetic group)	6	0.054	0.001	0.151	0.085
M × genetic group	2	0.827	0.573	0.605	0.449
M × genotype (genetic group)	6	0.746	0.034	0.423	0.985
R × genetic group	6	0.129	0.007	0.177	0.033
R × genotype (genetic group)	18	0.357	0.012	0.737	0.625
MSE		51.1	8.95	0.34	12301

V_{cmax} is the maximum rate of carboxylation (μmol m^{-2} s^{-1}), J_{max} is the maximum rate of electron transport (μmol m^{-2} s^{-1}), R_c is the rate of nonphotorespiratory CO_2 evolution (μmol m^{-2} s^{-1}) and C_{itr} is the intercellular CO_2 concentration (μmol mol^{-1}) at which the rate of CO_2 assimilation transitions from Rubisco carboxylation limited (A_c) to ribulose bisphosphate (RuBP) limited (A_j).

0.85 ± 0.1 and 249 ± 19.8 μmol m^{-2} s^{-1}, respectively. J_{max} showed a significant month × genotype interaction (Table 3). For measurements taken during June 2009, there were no significant differences in mean J_{max} between genotypes after Tukey's adjustment (Figure 4). For measurements taken during July 2009, half-sib HS1 showed significantly lower J_{max} (23.0 ± 1.5 μmol m^{-2} s^{-1}) than all other genotypes (Figure 4). Overall, half-sib HS3 showed the highest mean J_{max} during July (40.2 ± 1.5 μmol m^{-2} s^{-1}) (Figure 4).

Relationship between growth, leaf-level gas exchange and photosynthetic capacity

Over time, A_{sat} showed a significant positive correlation with individual-tree volume ($r = 0.33$, $P = 0.02$), tree height ($r = 0.35$ $P = 0.01$) and tree ground-line diameter ($r = 33$, $P = 0.02$) in July 2008, and a significant negative correlation with ground-line diameter in January 2008 ($r = -0.30$, $P = 0.04$). δ also showed a significant positive correlation with tree volume ($r = 0.46$, $P = 0.001$), tree height ($r = 0.42$ $P = 0.002$) and tree ground-line diameter ($r = 0.48$, $P = 0.0003$) in July 2008. Overall, within a given month, there was no relationship
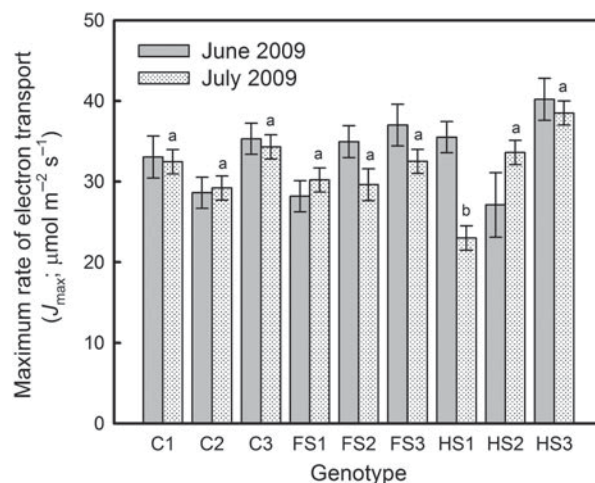

Figure 4. Least-squared means (±standard error) for the maximum rate of electron transport (J_{max}) among different loblolly pine clones (C1, C2, C3), full-sib families (FS1, FS2, FS3) and half-sib families (HS1, HS2, HS3) during June and July 2009. During June 2009, J_{max} was not significantly different ($P \leq 0.05$) between genotypes after Tukey's adjustment. However, there were significant differences in J_{max} between genotypes during July 2009. Means with the same letter are not significantly different at $P \leq 0.05$.

between g_s and any measured growth trait. When relating genotype mean monthly rates of A_{sat}, g_s and δ to genotype mean tree volume, there was a significant positive correlation between mean volume and mean δ in January 2008 ($r = 0.83$, $P = 0.005$) and July 2008 ($r = 0.73$, $P = 0.02$). Genotype mean α was negatively associated with genotype mean ground-line diameter ($r = -0.62$, $P = 0.08$) and volume ($r = -0.60$, $P = 0.09$). In contrast, genotype mean ground-line diameter was positively associated with genotype mean R_d ($r = 0.60$, $P = 0.09$). V_{cmax}, J_{max} and C_{itr} showed no associations with any growth traits. However, there was a significant positive relationship between R_c and both tree height ($r = 0.28$, $P = 0.04$) and ground-line diameter ($r = 0.34$, $P = 0.009$).

Discussion

Genotype variation and uniformity in A_{sat}, g_s and δ

We expected that within individual clones, which have no genetic variation from one tree to the next, uniformity in A_{sat}, g_s and δ would be significantly higher relative to uniformity within full-sib and half-sib genotypes, which have greater tree-to-tree genetic variation. However, there were no consistent patterns in the CV of A_{sat}, g_s and δ among clones, full-sibs and half-sibs. For A_{sat}, the CV within individual clones tended to be higher relative to the CV within full-sib and half-sib genotypes. Conversely, the CV for g_s and δ within individual clones tended to be lower relative to the CV within full-sib and half-sib genotypes. The lack of uniformity differences among genotypes of contrasting inherent genetic variation is similar to the results of another study involving the same genotypes where we found no uniformity differences in sap flow-based canopy-level conductance or transpiration (Aspinwall et al. 2011). Other than our results, we have found no other studies in loblolly pine or any other tree species that have explicitly compared uniformity in physiological rates among genotypes with varying amounts of inherent genetic variation. However, for integrated traits such as growth, wood density and disease resistance, others have generally found less variation among full-sib and half-sib families, relative to clones, when making across-site comparisons (Cumbie 2002, Isik et al. 2005). For example, the performance of half-sib families with high levels of genetic diversity has been relatively stable across sites within climatic zones (McKeand et al. 2006); however, genotypes with less inherent genetic diversity (clones and/or full-sibs) have, in some cases, shown a greater potential for genotype × environment interactions across sites or silvicultural prescriptions (Roth et al. 2007). Gebremedhin (2003) also found large differences in biomass partitioning among clones growing under different water availabilities. These studies suggest that more genetically diverse taxa have greater stability across varying environments. Therefore, our hypothesis was that when growing at one site, under similar environmental conditions, more

genetically diverse full-sib and half-sib genotypes will show a wider range (i.e., higher CV) of physiological rates relative to clonal genotypes. Contrary to our expectation, our results showed that less genetically diverse genotypes did not consistently express greater physiological uniformity. In fact, for some traits, more genetically diverse genotypes showed equal or higher uniformity than clones, which suggests that greater inherent genetic diversity could also enhance the buffering capability against within-site environmental heterogeneity, which may, in turn, result in more uniform physiology.

Given that we found a significant replication × genotype interaction for some physiological parameters, microsite variation probably had an impact on tree growth and physiological performance. Variation in water availability, intergenotypic competition or competition for resources from other woody and herbaceous plants may have all impacted genotype uniformity (Miller et al. 1991). However, there is contradictory evidence of the impact of intergenotypic competition on genotype performance (Adams et al. 1973, Tuskan and van Buijtenen 1986, Foster et al. 1998, Roth et al. 2007), and lower Coastal plain sites such as ours are generally not water limited (Allen and Campbell 1988). Even so, measurements of leaf water potential may have provided insight into whether competition or microsite variation contributed to heterogeneity in leaf-level physiology. Although we have found no evidence of differences in specific leaf area (SLA), whole-tree leaf area or canopy architecture in a separate study involving the same genotypes (M.J. Aspinwall, unpublished data), leaf area or canopy heterogeneity (Chmura et al. 2007, Emhart et al. 2007) could have also contributed to some physiological variation.

Additionally, environmental heterogeneity may have acted as a selective pressure and altered patterns of gene expression, and thus, physiology (Day et al. 2002). For example, Watkinson et al. (2003) found distinct patterns of drought-induced gene expression associated with photosynthetic traits and metabolic activity in loblolly pine, and Secchi and Zwieniecki (2010) found changes in gene expression associated with aquaporin activity and embolism in *Populus*. Tschaplinski et al. (2006) also identified several quantitative trait loci (QTL) associated with osmotic potential in selected *Populus* genotypes growing under contrasting water availabilities. At the population level, there may also be specific genes associated with physiological capacity and metabolic activity. Eckert et al. (2010a) identified 5 single-nucleotide polymorphisms associated with stress response in loblolly pine, and 24 loci were associated with genes that encoded proteins involved in a wide range of physiological processes. In *Pinus pinaster*, Costa et al. (1998) identified several drought-responsive proteins linked with photosynthesis, cell elongation and antioxidant metabolism. Similarly, Dubos et al. (2003) identified several transcript-derived fragments in *P. pinaster* related to water-stress response in *Arabidopsis* and loblolly pine. Despite these advances in our molecular

understanding of physiological responses, physiological variation within such a wide range of high- and low-diversity loblolly pine genotypes remains poorly understood. Nonetheless, our results suggest that, under operational plantation conditions, genotypes with little or no inherent genetic variation may show greater sensitivity to changes in within-site environmental conditions. These results agree with the basic principles of forest genetics and tree breeding where phenotypic variance within more genetically homogeneous genotypes is primarily an expression of environmental variation (Namkoong et al. 1966, Zobel and Talbert 1984). Consequently, if enhanced stand uniformity and productivity are the objective, silvicultural prescriptions that increase site uniformity should be appropriately matched with suitable genotypes so as to maximize growth and physiological potential.

Seasonal and genetic variation in gas exchange

The seasonal patterns of photosynthesis, stomatal conductance and transpiration in this study were similar to those found by Yang et al. (2002), Tang et al. (2003) and Gough et al. (2004), with peak A_{sat} occurring in the spring and minimum A_{sat} occurring during the winter months. There was a strong seasonal pattern for g_s, similar to that of A_{sat}, with peak rates occurring during the spring and summer when evaporative demand and temperature were high (Teskey et al. 1986). The seasonal pattern of C fixation was probably influenced by several factors, including temperature (Ellsworth 2000), humidity, water availability and light intensity and interception (Teskey et al. 1986, Teskey et al. 1994, Gough et al. 2004).

Although no studies have compared uniformity in physiological rates within loblolly pine genotypes representing a range of genetic variation, several studies have investigated differences in physiological rates among loblolly pine genotypes. The lack of genetic differences in A_{sat} among half-sib families in our study was similar to the results of Seiler and Johnson (1988), Samuelson (2000) and McGarvey et al. (2004), who found no significant differences in photosynthesis among different half-sib families of loblolly pine. Gebremedhin (2003) also found no differences in gas exchange between 30 loblolly pine clones. In contrast, under drought conditions, Gonzalez-Benecke and Martin (2010) found differences in stomatal control between two loblolly pine seed sources. King et al. (2008) and Tyree et al. (2009) also found significant variability in leaf-level physiological processes among different loblolly pine clones, and studies of other commercially important plantation species have found similar differences in leaf-level physiology among clones from within families (Barigah et al. 1994, Lamhamedi et al. 2000).

Given that the genotypes in this study were mainly lower Coastal plain selections, we might have found significant differences in physiology if we had compared genotypes adapted to a broader range of edaphic conditions (Boltz et al.

1986, Bongarten and Teskey 1986, Barnes 2002). Decades of selection and breeding of highly productive individuals may have also resulted in more physiologically homogeneous genotypes with similar molecular control over physiology. If this were true, we might expect unimproved loblolly pine to have somewhat lower physiological functioning than improved varieties. However, if we compare net photosynthetic rates from studies involving unimproved loblolly pine versus studies involving improved varieties, there are no clear differences in leaf-level physiology. For example, in studies planted with first-generation half-sib families (Seiler and Johnson 1988, Maier et al. 2002, Yang et al. 2002, Tang et al. 2003, McGarvey et al. 2004), gas-exchange values (A_{sat}, g_s) are similar to those of unimproved loblolly pine (Cregg et al. 1993). King et al. (2008) and Tyree et al. (2009) show somewhat higher net photosynthetic rates within individual clones but the differences are, in most cases, confounded by age and environmental effects. Overall, there remains very little published information on the physiology of loblolly pine clones and relatively little is known regarding the molecular regulation of physiological processes in loblolly pine, let alone specific genotypes. However, at the loblolly pine population level, recent findings have shown a molecular basis for a host of adaptive and physiological traits (Eckert et al. 2010a, 2010b). Moreover, with high levels of genetic diversity, even within selected and improved populations of loblolly pine (Schmidtling et al. 1999), the potential for significant physiological variation among loblolly pine genotypes remains. Nonetheless, among the genotypes in study, variation in leaf-level physiology was negligible over time, which follows many other studies in loblolly pine that have found no significant physiological differences between genotypes (Yang et al. 2002, Nelson and Johnsen 2008).

Overall, δ was the only gas-exchange parameter that showed significant genotype differences and no genotype × month interaction. This difference was primarily due to clone C1 having a very low mean δ relative to all other genotypes. Studies in loblolly pine (Gebremedhin 2003) and other conifer species have also found genetic differences in both δ and carbon isotope (¹³C) discrimination (Δ). Aitken et al. (1995) found significant variation in Δ among populations of Douglas-fir. Cregg et al. (2000) found significant variation in Δ among provenances of ponderosa pine and Olivas-García et al. (2000) found significant differences in Δ among different ponderosa pine families. Moreover, Baltunis et al. (2008) found that across >1000 loblolly pine clones, rankings for Δ were relatively stable across two different sites and the heritability of Δ was similar to that of total tree height (0.23–0.33). Furthermore, there was a significant negative correlation between height and Δ at the parental, full-sib family and family within clone level, which indicated that taller trees had greater water-use efficiency. Other studies have also found similar correlations between

height and Δ (Zhang et al. 1994, Johnsen et al. 1999). Our results indicated a significant positive correlation between δ and growth during the summer; however, this relationship varied in other seasons. Grossnickle et al. (2005) found high δ during the summer that was associated with low Δ. If Δ had been measured rather than δ, the relationship between water-use efficiency and volume may have been stronger, since Δ may provide a more integrated measure of δ (Grossnickle et al. 2005). Even so, δ measured over time does provide information on water-use efficiency that incorporates underlying phenological or morphological properties that may have influenced the relationship between water-use efficiency and Δ (Meinzer et al. 1992). For example, variability in SLA may have influenced δ since thicker leaves (lower SLA) increase resistance to CO_2 diffusion through the stomata and into the chloroplast. The increased resistance may reduce the CO_2 concentration at the fixation site, thereby decreasing Δ and increasing δ (Vitousek et al. 1990). Interestingly, Chmura and Tjoelker (2008) found significant differences in SLA between different loblolly pine families, with SLA increasing with canopy depth. In our study, all needles were selected from sun-exposed branches and needles located on the canopy edge. Therefore, if there were subtle differences in SLA, it was not due to selection of needles from different canopy positions (Chmura and Tjoelker 2008). Furthermore, biomass harvests and foliage sampling have found no differences in SLA or total tree leaf area among the genotypes in this study (M.J. Aspinwall, unpublished data).

The moderate to high calculated heritability for Δ and significant correlations between growth and Δ (Johnsen et al. 1999, Baltunis et al. 2008) suggest that traits such as δ could be important for improving the efficiency of carbon assimilation without sacrificing productivity. Most studies have found significant relationships between Δ and g_s and Δ has been associated with genes controlling stomatal traits and water-use efficiency in *Arabidopsis* (Masle et al. 2005). Tschaplinski et al. (2006)'s identification of QTL associated with osmotic potential in *Populus* suggests that there could be similar sets of genes associated with stomatal traits that regulate plant water status allowing for continued carbon gain during drought stress. The potential isolation of similar genes in loblolly pine could have major implications for selection of high water-use efficiency genotypes (Baltunis et al. 2008). Furthermore, deployment of genotypes with advantageous δ or g_s may confer genotype differences in heat avoidance, i.e., evaporative cooling (Radin et al. 1994), and may have implications for forest survival and productivity under changing climatic conditions. Overall, our results provide further evidence of the relationship between water-use efficiency and growth; however, improvement of these traits may necessitate an understanding of seasonal and genotypic patterns of growth and physiological activity (Cregg et al. 2000).

Light and A/C_i curve parameter estimates among genotypes

We found no significant differences in A_{max}, α, R_d or Γ_i between genetic groups or genotypes within each group. Similarly, Naidu et al. (1993), Zhang et al. (1997) and Samuelson et al. (2001) found no differences in A_{max}, α, R_d or Γ_i in loblolly pine growing under different environmental and cultural treatments. Overall, our estimates of α were higher than those of Zhang et al. (1997) and lower than those of Naidu et al. (1993), but similar to those of Samuelson et al. (2001).

In terms of genetic effects, Grossnickle and Fan (1998) also found no differences in photosynthetic response to light intensity among six clones of interior spruce (*Picea glauca* × *Picea engelmannii*) and Grulke et al. (1993) found no differences in the Γ_i, A_{sat}, α or R_d among two full-sib families of *Pinus ponderosa* growing under ambient and elevated CO_2. In comparison, Bassman and Zwier (1991) found significant differences in the light saturation point of different ecotypes of *Populus*. In general, our results agree with other genetic studies involving conifers in which genotype differences in light-response parameters have been small and insignificant.

In comparison with other studies involving genetic effects on the biochemical limitations to photosynthesis (Gerber and Dawson 1997, Weston et al. 2007), genetic variation in CO_2-response parameters was generally insignificant. Overall, mean estimated V_{cmax} (19.1 μmol m^{-2} s^{-1}) was slightly higher than that reported by Wullschleger (1993) (15–17 μmol m^{-2} s^{-1}) in loblolly pine, and slightly lower than those estimated by Ellsworth (2000). The genotype mean J_{max} (23.0–40.2 μmol m^{-2} s^{-1}) fell well within the range of J_{max} (20–39 μmol m^{-2} s^{-1}) in loblolly pine (Wullschleger 1993). The significant month × genotype interaction for J_{max} indicated that, over time, genotypes differed in their potential capacity for electron transport or the efficiency of photosystem II in capturing light energy (Koehn et al. 2003). Other studies have also found significant genetic variation in biochemical limitations to photosynthesis (Gerber and Dawson 1997, Weston et al. 2007). In fact, Weston et al. (2007) found differences in RNA transcript abundance between contrasting *Acer rubrum* genotypes, which suggests that there may be a molecular basis for biochemical differences in photosynthetic capacity. However, the genetic effects on J_{max} in our study may be overestimated due to the significant differences in J_{max} between replications. Differences in J_{max} may also be related to subtle differences in the light environment in which the needles had become acclimated to, or differences may have been due to variation in chlorophyll fluorescence (Koehn et al. 2003). Nonetheless, to identify the importance of differences in biochemical CO_2 fixation parameters, it may be valuable to determine the relationship between C fixation and

C allocation to biochemical compounds and different plant parts (Wullschleger 1993).

Relationship between growth, gas exchange and photosynthetic capacity

In our results, A_{sat} and tree size were positively correlated during January and July 2008. Some studies have found similar results with a significant positive relationship between growth and C fixation (Cuelemans and Impens 1983, Boltz et al. 1986, Major and Johnsen 1996), while others have found a negative relationship between leaf-level C fixation and tree size (Samuelson et al. 1992, Marshall et al. 2001). Additionally, genotypes with higher mean leaf-level α tended to have lower mean volume and diameter growth. In contrast, Groninger et al. (1996) found that although shade-grown loblolly pine produced less biomass, quantum efficiency did not differ between larger, sun-exposed seedlings and smaller, shade-grown seedlings. However, Zhang et al. (1997) showed that shade-grown loblolly pine tended to have lower A_{sat} and R_d, and higher α. Although our trees were not shade grown and needles were not shaded, these results may help to explain the relationships among the measured parameters.

Interestingly, we found a negative correlation between genotype mean α and mean genotype volume. In contrast, Brodribb and Feild (2000) found that maximum photosynthetic rate was constrained by stem hydraulic conductivity, and hydraulic conductivity was positively associated with growth rate, whereas leaf-specific hydraulic conductivity and α showed a strong positive association, which suggests that growth rate and α are positively correlated. Differences in results may be due to the limited number of genotypes in our study, in fact, on an individual-tree basis, we found no relationship between growth and most photosynthetic response parameters. At both the individual-tree and genotype level, needle respiration rates were positively associated with ground-line diameter growth. In comparison, Samuelson (2000) determined that families of slash pine and loblolly pine that demonstrated more rapid growth also had lower leaf maintenance respiration rates. Differences in these results may be due to the fact that we estimated R_d from daytime light-response curves and Samuelson (2000) took direct measures of leaf dark respiration.

Finally, there is strong evidence suggesting that canopy light interception is more positively associated with productivity than leaf-level photosynthetic rate (McCrady and Jokela 1996, McGarvey et al. 2004, Chmura and Tjoelker 2008). Phenological differences in shoot elongation (Dougherty et al. 1994, Barnes 2002), leaf area dynamics (Vose et al. 1994), timing and rate of height and diameter growth (Emhart et al. 2006) and the number and length of growth flushes (Bridgwater 1990, Dewald et al. 1992) may also be better indicators of genotype productivity. Additionally, variation in C allocation to secondary defense compounds (Aspinwall 2010), biomass partitioning (Bongarten and Teskey 1987, Retzlaff et al. 2001, Chmura et al. 2007, Tyree et al. 2009) and other morphological parameters such as foliage and branch morphology likely contribute to the complexities of genotype growth potential.

In conclusion, our findings suggest that δ, A_{sat} and R_d are the leaf-level physiological parameters that are most significantly related to individual-tree and genotype productivity in loblolly pine. Furthermore, under operational management conditions, physiological uniformity was not consistently related to genetic homogeneity, which implies that leaf-level physiology, and conceivably stand uniformity, will only be maximized when suitable genotypes are matched with silvicultural prescriptions that reduce within-site heterogeneity. Awareness of the underlying environmental factors that influence physiological variation both within and between loblolly pine genotypes will improve assessments of the potential for greater stand-level productivity and sensitivity to global climate change. Moreover, advancements in our molecular understanding of complex physiological traits will improve our knowledge of physiological variation across a range of genetic improvement.

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