

Quantifying stomatal and non-stomatal limitations to carbon assimilation resulting from leaf aging and drought in mature deciduous tree species

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Summary Gas exchange techniques were used to investigate light-saturated carbon assimilation and its stomatal and non-stomatal limitations over two seasons in mature trees of five species in a closed deciduous forest. Stomatal and non-stomatal contributions to decreases in assimilation resulting from leaf age and drought were quantified relative to the maximum rates obtained early in the season at optimal soil water contents. Although carbon assimilation, stomatal conductance and photosynthetic capacity (V_{cmax}) decreased with leaf age, decreases in V_{cmax} accounted for about 75% of the leaf-age related reduction in light-saturated assimilation rates, with a secondary role for stomatal conductance (around 25%). However, when considered independently from leaf age, the drought response was dominated by stomatal limitations, accounting for about 75% of the total limitation. Some of the analytical difficulties associated with computing limitation partitioning are discussed, including path dependence, patchy stomatal closure and diffusion in the mesophyll. Although these considerations may introduce errors in our estimates, our analysis establishes some reasonable boundaries on relative limitations and shows differences between drought and non-drought years. Estimating seasonal limitations under natural conditions, as shown in this study, provides a useful basis for comparing limitation processes between years and species.

Keywords: A/C_i curve, leaf age, maple, oak, photosynthetic limitations, stomata.

Introduction

Limitations to light-saturated carbon assimilation rates are associated with either the diffusion of carbon dioxide to the intracellular leaf space, controlled by stomatal functioning, or biochemical processes within the leaf. Common nomenclature for this separation is stomatal limitation versus non-stomatal limitation, and quantitative methods have been derived to assess these relative limitations based on gas exchange data (Jones 1985, Assmann 1988, Jones 1998). Quantitative limita-

tion analyses can be obtained directly from the response of assimilation (A) to the intercellular carbon dioxide concentration (C_i) (A/C_i curves).

Two general approaches have been proposed for the quantitative analysis of limitations to light-saturated photosynthesis on the basis of the A/C_i response. Most commonly reported is the sensitivity approach, which is used to estimate the relative sensitivity of carbon assimilation to stomatal conductance (parameter l_g in Jones 1985, Cornic et al. 1983, Hutmacher and Krieg 1983, Comstock and Ehlerlanger 1984, Harley et al. 1986, Teskey et al. 1986, Kubiske and Abrams 1992, 1993, Ni and Pallardy 1992, Stewart et al. 1994, Kubiske et al. 1996, Escalona et al. 1999). As noted in previous studies (Assmann 1988, Jones 1998), the sensitivity approach cannot be used to determine absolute or relative limitations to assimilation between two different states.

Much less frequently applied in practice is the limitation partitioning approach, which calculates the absolute and relative stomatal and non-stomatal limitation partitioning of assimilation between two different states (Jones 1985, Assmann 1988). The limitation partitioning approach is useful for evaluating limitation processes between two different states, such as drought and well-watered or changes in leaf age or light quality (Assmann 1988). In principle, this method can be used to investigate limitation partitioning over any period of time, assuming that a reference standard or maximum assimilation rate can be defined.

We undertook a 2-year study of light-saturated photosynthetic rates for five species in a mature deciduous forest. Both leaf age and drought reduce photosynthetic capacity in this canopy (Wilson et al. 2000). Based primarily on a limitation partitioning approach, we obtained quantitative seasonal estimates of how these reductions limit assimilation rates relative to changes in stomatal conductance. We re-derived the general theory underlying the limitation partitioning approach, discussed in Jones (1985), to evaluate partitioning directly from the parameters of the Farquhar et al. (1980) model. The analytical problems associated with path dependency (Jones 1985),

patchy stomatal closure (Terashima 1992), mesophyll diffusion resistance (Roupsard et al. 1996) and defining maximum or standard values are discussed. The absolute and relative limitation processes were expected to differ between years because in one year (1998) there was especially low rainfall during the summer months, whereas in the other year (1997), rainfall was near normal.

Our specific goals were (1) to outline the methods used to calculate stomatal and non-stomatal limitation partitioning based on the model parameters of Farquhar et al. (1980), and (2) to evaluate the seasonal trends and seasonal integration of stomatal and non-stomatal limitations in a deciduous forest, focusing on the effects of leaf age and the 1998 drought.

Materials and methods

Site

All measurements were performed near the upper crown of 10 mature overstory trees in a 60-year-old closed deciduous forest at Walker Branch watershed within the Department of Energy reservation at Oak Ridge, TN (35°58' N and 84°17' W, 365 m a.s.l.). Measured trees included chestnut oak (*Quercus prinus* L.), white oak (*Q. alba* L.), red maple (*Acer rubrum* L.), sugar maple (*A. saccharum* Marsh.) and blackgum (*Nyssa sylvatica* Marsh). These species comprise more than 75% of the basal area in this overstory (Hanson et al. 1998). The maximum canopy height is about 26 m, and the maximum leaf area index is about 6. Mean annual rainfall and temperature are 1372 mm and 13.9 °C, respectively. Rainfall is distributed fairly equally among seasons, but with occasional summer droughts. Details concerning other biological and environmental aspects of this site have been described by Johnson and Van Hook (1989).

Single-leaf gas exchange measurements were performed from four towers in close proximity. One meteorological tower was not a part of any manipulative experiment, and gave access to a single white oak tree. Three towers were in 80 × 80 m treatment areas (throughfall displacement experiment (TDE), Hanson et al. 1998, Wullschleger et al. 1998). Since July 1993, one treatment has received ambient precipitation, whereas the remaining two treatments have been subjected to a 33% reduction (dry treatment) or a 33% enhancement (wet treatment) of ambient precipitation. Most of the measurements were made above 20 meters. On each tree, an attempt was made to measure leaves on the same branch or branches at similar heights throughout both seasons.

Experimental methods

Gas exchange measurements were performed on about 500 overstory leaves with a steady-state gas exchange system (LI-6400 Li-Cor, Lincoln, NE) over the two years. Photosynthetically active radiation was at least 1100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which was above the 95% threshold for photosynthesis. Each leaf was initially measured at a reference ambient carbon dioxide concentration of 360 $\mu\text{mol mol}^{-1}$. The A/C_i (assimilation versus internal leaf carbon dioxide concentration) response

curves were performed on about one third of the measured leaves by manipulating the reference carbon dioxide concentrations between 50 and 1800 $\mu\text{mol mol}^{-1}$ in about 11 steps, of which four were at or less than 200 $\mu\text{mol mol}^{-1}$. Because full A/C_i curves seriously constrained replication, only measurements at ambient carbon dioxide were performed in about two thirds of the leaves. Most measurements were made in mid-morning when leaf temperature was between 20 and 30 °C and humidity was near ambient.

Periodically, within each of the TDE treatments, soil water contents were measured at 0- to 300-mm depth, the portion of the soil profile containing at least 70% of all roots, with a time-domain reflectometer (Soil Moisture Equipment Corp., Santa Barbara, CA). In 1998, soil water content over the 300-mm profile was also measured hourly at the meteorological tower site with four water content reflectometers (Item CS615, Campbell Scientific Inc., Logan, UT) installed horizontally in the side of a subsequently backfilled soil pit. Soil water contents were converted to matric potential (MPa) based on soil water release curves measured at the site (Hanson et al. 1998).

Calculation of V_{cmax}

In the Farquhar et al. (1980) model, photosynthesis is limited either by the maximum rate of carboxylation (V_{cmax}) or the maximum rate of electron transport (J_{max}), parameters that indicate internal biological limitations on photosynthetic capacity. The procedure we used to calculate V_{cmax} is described in Wilson et al. (2000). Briefly, nonlinear regression methods (Draper and Smith 1966) were used to estimate V_{cmax} and dark respiration (R_d) when C_i was less than or equal to 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and when substrate limitations (Rubisco and CO_2) inhibited photosynthesis (Wullschleger 1993). Michaelis constants and values for the transition state activation energies were from Harley et al. (1992).

The model indicated that virtually all of the light-saturated assimilation rates for external carbon dioxide concentrations at or below ambient were limited by the maximum carboxylation rate (V_{cmax}) (Wilson et al. 2000). Therefore, we used the parameter V_{cmax} to describe light-saturated photosynthetic capacity at ambient carbon dioxide concentrations. We found a good correlation between one-point estimates of V_{cmax} , based on only the ambient carbon dioxide measurement and a mean value of R_d , and V_{cmax} estimated from the full A/C_i curve ($r^2 = 0.97$). Because analysis based on full A/C_i response curves indicated that the results were not sensitive to whether the full response curves or the one-point method was used, all results shown in this study are based on the one-point method. We used the Arrhenius function in Walcroft et al. (1997) to represent the temperature dependence of V_{cmax} , because it provided a good fit to the data (Wilson et al. 2000).

Quantitative limitation analysis

Light-saturated photosynthesis was always substrate-limited at ambient external carbon dioxide concentrations, so assimilation rate (A) can be written as (Farquhar et al. 1980):

$$A = \frac{V_{\text{cmax}} C_i}{C_i + K_c (1 + O/K_o)} \left(1 - \frac{\Gamma_*}{C_i} \right) - R_d, \quad (1)$$

where R_d is the rate of dark respiration, and K_c and K_o are the Michaelis constants for CO_2 and O_2 (concentration given by O), respectively. The CO_2 compensation point (Γ_*) is estimated from $O/2\tau$, where τ is the specificity factor of Rubisco (Harley et al. 1992). If O , leaf temperature, τ and R_d are constant, then by definition the total derivative of Equation 1 is:

$$dA = \frac{\partial A}{\partial C_i} dC_i + \frac{\partial A}{\partial V_{\text{cmax}}} dV_{\text{cmax}}. \quad (2)$$

Neglecting the normally small contribution of evaporation interacting with CO_2 diffusion (Ball 1987), C_i can be written as:

$$C_i = C_a - \frac{A}{g_t}, \quad (3)$$

where C_a is external carbon dioxide concentration (mol mol^{-1}) and g_t is total conductance of CO_2 to the leaf intercellular space ($\mu\text{mol m}^{-2} \text{s}^{-1}$), including both boundary and stomatal components. Assuming there is no change in the CO_2 concentration and that stomatal conductance is much less than the boundary layer conductance, conditions were generally satisfied in our measurements at ambient CO_2 :

$$dC_i \approx \frac{A}{g_{\text{sc}}} d \ln g_{\text{sc}} - \frac{dA}{g_{\text{sc}}}, \quad (4)$$

where g_{sc} is stomatal conductance to CO_2 .

When changes in photosynthetic capacity are much greater than changes in dark respiration (a condition also typically found over the season in our measurements):

$$\frac{\partial A}{\partial V_{\text{cmax}}} \approx \frac{A}{V_{\text{cmax}}}. \quad (5)$$

Substituting Equations 1, 4 and 5 into Equation 2 and rearranging gives:

$$d \ln A = l_g \ln g_{\text{sc}} + (1 - l_g) d \ln V_{\text{cmax}}, \quad (6)$$

with l_g , having a value between zero and one, being identical to that defined using Method V in Jones (1985):

$$l_g = \frac{\partial A / \partial C_i}{g_{\text{sc}} + \partial A / \partial C_i}. \quad (7)$$

The left-hand side of Equation 6 is the relative change in A (i.e., dA/A). The first term on the right-hand side of Equation 6 is the relative change in A resulting from a change in stomatal conductance, and is the product of a sensitivity (l_g) and a relative change in stomatal conductance ($dg_{\text{sc}}/g_{\text{sc}}$ or $d \ln g_{\text{sc}}$). The second term on the right-hand side represents changes in A re-

sulting from changes in photosynthetic capacity (non-stomatal term), and is also the product of a sensitivity ($1 - l_g$) and a relative change in V_{cmax} ($d \ln V_{\text{cmax}}$). The values of l_g and $1 - l_g$ can also be regarded as indicators of stomatal and non-stomatal controls of assimilation, respectively. However, actual limitations in A and the relative stomatal and non-stomatal components depend not only on l_g , but also on $d \ln g_{\text{sc}}$ and $d \ln V_{\text{cmax}}$ (i.e., on the extent to which g_{sc} and V_{cmax} are changed).

Relative stomatal limitation (σ) can be defined from Equation 6, with a value typically between zero and one, as the ratio of the change in assimilation resulting from changes in stomatal conductance to the total measured change in assimilation:

$$\sigma = \frac{l_g d \ln g_{\text{sc}}}{l_g d \ln g_{\text{sc}} + (1 - l_g) d \ln V_{\text{cmax}}}. \quad (8)$$

The value $1 - \sigma$ is then the relative non-stomatal limitation in A resulting from changes in V_{cmax} . The value of σ has the same theoretical definition as in Jones (1985), but Jones uses an alternative mathematical approach and does not incorporate the model parameters of Farquhar et al. (1980). Note that when $d \ln V_{\text{cmax}} = d \ln g_{\text{sc}}$, $\sigma = l_g$.

For Equation 8 to be useful in practice, the right-hand side must be approximated using finite differencing from gas exchange measurements at two different times. For each tree measured, it was assumed that, at some period of the season, assimilation was maximal and that we could estimate the stomatal (σ) and non-stomatal limitations ($1 - \sigma$) for the remainder of the year from a finite difference form of Equation 8, using this maximum rate as a standard. First, for each tree, the day over the two seasons with the mean maximum photosynthesis (A_{max}) was identified, usually corresponding to the mean of three or more leaves. The values of V_{cmax} and g_{sc} normalized to 25°C for that measurement day were denoted as $V_{\text{cmax}+}$ and $g_{\text{sc}+}$. For all other days, $d \ln g_{\text{sc}}$ and $d \ln V_{\text{cmax}}$ were approximated from the difference in V_{cmax} and g_{sc} on that day from these maximum values ($V_{\text{cmax}+}$ and $g_{\text{sc}+}$). The values of g_{sc} and $\partial A / \partial C_i$ used in Equation 8 are approximated by the means between the two measurement times. The value of $\partial A / \partial C_i$ can be estimated directly from Equation 1 at the operational values of C_i at ambient external CO_2 . Absolute stomatal (L_S) and non-stomatal (L_{NS}) limitations were computed by multiplying each side of the finite difference form of Equation 8 by the total limitation (ΔA), the measured reduction in assimilation from the maximum value so that $L_S + L_{NS} = \Delta A$:

$$L_S = \sigma \Delta A, \quad (9a)$$

$$L_{NS} = (1 - \sigma) \Delta A. \quad (9b)$$

We also computed σ from the two state-function equations described in Jones (1985, Equations 25 and 28) to examine whether our calculations of σ differed significantly from the two proposed by Jones (1985).

In this study, measurements were made in the morning

when leaf temperature was typically between 20 and 30 °C, a range in which photosynthesis was relatively insensitive. However, because leaf temperature was not constant, Equation 2 should theoretically include a temperature response term, which greatly confounds the simplicity of the analytical solutions. Simulations with the Farquhar et al. (1980) model were performed with the measured temperature differences, which verified that the effects of changes in morning temperature were nonzero but usually small relative to the stomatal and non-stomatal limitations. Therefore, the analytical solutions presented above were accepted as close approximations.

Path-dependent methods

One implied assumption in Equation 8 is that changes in stomatal conductance and V_{cmax} are independent. If changes in these two quantities are not completely independent, this approach is not rigorously correct. This introduces the concept of path dependence (Jones 1985), illustrated in Figure 1, which shows an example of measured and modeled responses of assimilation to C_i (A/C_i curve) on the same branch at two different times. The two lines progressing from the lower left to upper right, the slopes of which are related to V_{cmax} (see Equation 1), depict the simulated response of assimilation to C_i based on measurements (symbols) at two different times. The point A_A is the measured assimilation rate at ambient external carbon dioxide early in the season and is given by the intersection of the two solid lines, the A/C_i curve (demand function) and another line with a slope of $-g_t$ (the supply function) (Farquhar and Sharkey 1982). At some later time, the photosynthetic capacity (V_{cmax}) and stomatal conductance were reduced and the assimilation rate was reduced to A_D (intersection of the two dashed lines).

The total reduction in assimilation is $A_A - A_D$. In the path-de-

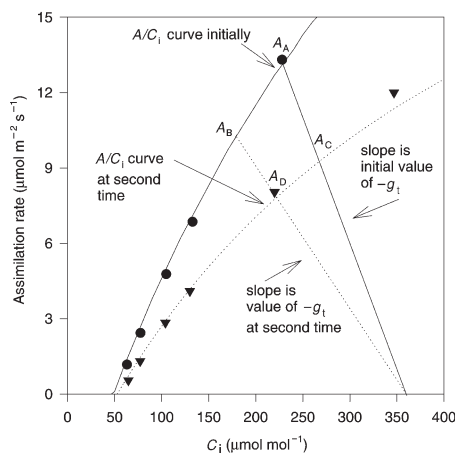


Figure 1. Measured light-saturated response of assimilation (A) to intercellular carbon dioxide concentration (C_i) at 25 °C for a white oak tree on Day 138 (●, rate at ambient CO_2 labeled A_A) and Day 272 (▼, rate at ambient CO_2 labeled A_D), 1998. Simulations based on the measurements are shown as solid (Day 138) or dashed (Day 272) lines. Also shown are the demand functions with slopes of $-g_t$ (the total gas phase conductance to carbon dioxide).

pendent approaches outlined in Jones (1985) and Assmann (1988), stomatal limitation between these two states can be calculated assuming one of two paths ($A_A \rightarrow A_B \rightarrow A_D$ or $A_A \rightarrow A_C \rightarrow A_D$). The first path assumes that changes in stomatal conductance occur first, and that V_{cmax} changes in response to conductance changes. The second path assumes V_{cmax} changes first and stomatal conductance then adjusts to these changes. The two corresponding values of σ (σ_s for stomatal first and σ_v for V_{cmax} first) from this technique are $(A_A - A_B)/(A_A - A_D)$ and $(A_C - A_D)/(A_A - A_D)$, respectively. In our study, the points A_B and A_C were obtained from simulations with the Farquhar et al. (1980) model. In practice, it is especially difficult to determine the extent of independence between changes in the A/C_i response and stomatal conductance (Jones 1985, Assmann 1988). Therefore, the results discussed are based primarily on Equation 8, but the path-dependent methods were also evaluated to indicate the possible range of limitation partitioning assuming extreme cases of path dependence.

Results

Climate and soil water content

During most of the 1997 growing season, precipitation was near or above normal and soil water content was not limiting at any of the sites or treatments (Figure 2). The spring and early summer of 1998 were wet, but total precipitation for July–September 1998 was 150 mm, or only about 36% of normal. Soil water deficits developed in midsummer and by the first of September soil water potentials were below -2.0 MPa on all but the wet TDE treatment (Figure 2). Although the drought was not as severe at the wet treatment area as at the other treatment areas, differences in soil water content were much greater between years than between TDE treatments. Therefore, although some treatment effects were possible, the gen-

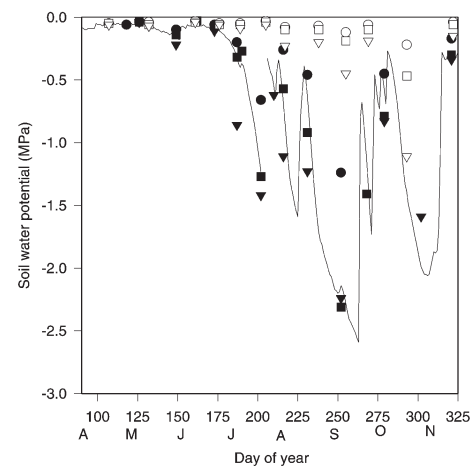


Figure 2. Soil water potential in the 0- to 300-mm horizon in the wet (○), ambient (□) and dry (▼) TDE treatments in 1997 (open symbols) and 1998 (closed symbols). The solid line represents the soil water potential in 1998 at the meteorological tower.

eral effects of drought were evaluated across years and not across TDE treatments.

Light-saturated photosynthetic rates

Figure 3 shows the seasonal time course of light-saturated carbon assimilation for six trees during the two years. During both years, light-saturated assimilation and stomatal conductance were typically greater in the two oak species than in the maple species (Figure 3, Table 1). Maximum assimilation

rates were about 15 to 18 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the oaks and 10 to 12 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the maples (Table 1). Maximum stomatal conductances to water vapor were about 0.300 and 0.180 $\text{mol m}^{-2} \text{s}^{-1}$ in the oaks and maples, respectively (Table 1). Photosynthetic rates were closer to maximum for a longer duration in oaks, but in most trees there was a seasonal decrease from maximum rates that was evident several months before final senescence. As a result, for a considerable portion of the season, most trees, but especially red maple, were operating well below early season maximum rates.

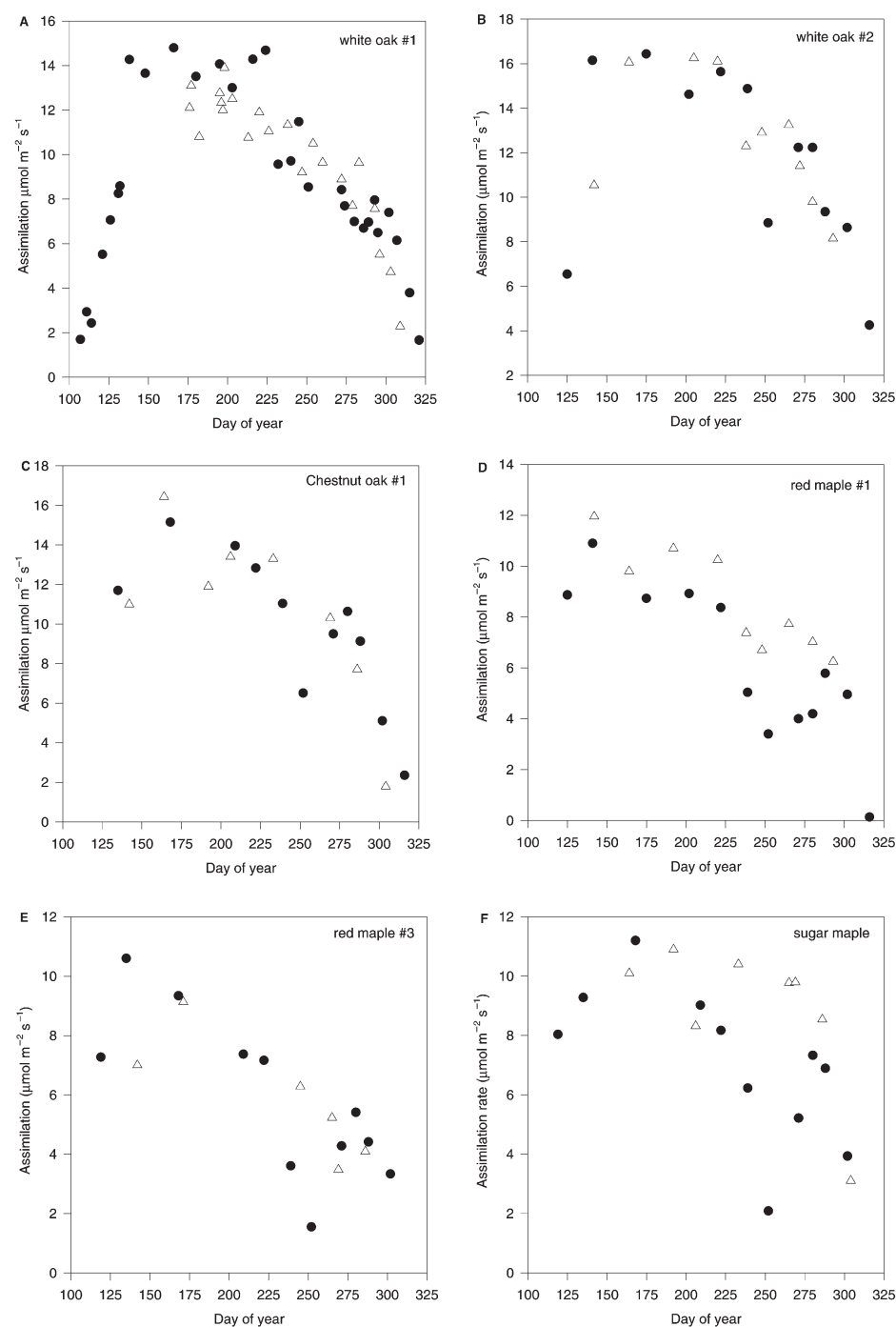


Figure 3. Light-saturated photosynthetic rates in 1997 (Δ) and 1998 (\bullet) for six overstory trees at external carbon dioxide concentrations of 360 $\mu\text{mol mol}^{-1}$. (A) White oak at the meteorological tower, (B) white oak in the ambient TDE treatment, (C) chestnut oak in the wet TDE treatment, (D) red maple in the ambient TDE treatment, (E) red maple in the dry TDE treatment, and (F) sugar maple in the wet TDE treatment.

Table 1. Mean maximum daily photosynthetic rates (A_{\max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) measured for each tree during the two years. Also given are the values of $V_{\text{cmax}+}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$) at 25 °C and stomatal conductance to water vapor ($g_{\text{s}+}$, $\text{mol m}^{-2} \text{s}^{-1}$). Abbreviation: TDE refers to throughfall displacement experiment.

Tree	A_{\max}	$V_{\text{cmax}+}$	$g_{\text{s}+}$	Location
White oak No. 1	14.3	54.5	0.250	Meteorology tower
White oak No. 2	16.4	64.2	0.326	TDE ambient
White oak No. 3	9.8	37.8	0.171	TDE dry
Chestnut oak No. 1	15.2	54.1	0.294	TDE wet
Chestnut oak No. 2	19.1	77.4	0.300	TDE dry
Red maple No. 1	11.9	46.7	0.170	TDE ambient
Red maple No. 2	11.4	55.4	0.127	TDE dry
Red maple No. 3	10.6	41.7	0.183	TDE wet
Sugar maple	11.2	42.3	0.178	TDE wet
Blackgum	10.2	40.8	0.113	TDE dry

In addition to the seasonal decline in assimilation, most trees experienced a period of enhanced reductions during the drought in 1998. The response to drought ranged from small and transient (Figure 3A) to extreme and of extended duration (Figures 3D and 3E). In some trees, drought had large effects on assimilation, but mainly only during the most extreme period of soil drying (Figures 3B and 3C). The effect of drought on assimilation was generally less noticeable in white oak than in the other species. Following a modest rainfall and only partial recovery of soil water deficits (Figure 2), assimilation rates in most trees increased to expected late-season values (Figure 3). Although the seasonal and drought-induced decreases in assimilation were generally correlated with decreases in stomatal conductance during both years, the reductions in assimilation were not exclusively caused by stomatal closure, because leaf age and drought also affected V_{cmax} (Wilson et al. 1999).

Limitation analysis

Figure 4 shows graphical examples of how drought and leaf age affected A/C_i curves, stomatal conductance and V_{cmax} for a chestnut oak and a sugar maple tree. In the chestnut oak (Figure 4A), maximum early season assimilation rate (A_{\max}) for either year was $15.2 \mu\text{mol m}^{-2} \text{s}^{-1}$, with a V_{cmax} of $54.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ and g_{s} of $0.294 \text{ mol m}^{-2} \text{s}^{-1}$ (supply and demand curves indicated by dash-dot lines and intersecting at A_{\max} in Figure 4A). An analysis of the effects of leaf age independent of the effects of drought indicated that in late summer 1997 (Day 269) the soil was still fairly wet (Figure 2), but V_{cmax} and the corresponding demand function (A/C_i response) had decreased ($32.4 \mu\text{mol m}^{-2} \text{s}^{-1}$, dashed line in Figure 4A), probably because of leaf aging. Stomatal conductance was still almost identical to the maximum values common early in both 1997 and 1998 (supply function is nearly identical to that associated with A_{\max} in Figure 4A). As a result, assimilation was $10.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ (square labeled A_{1997} in Figure 4A). The values L_S and L_{NS} were 0.0 and $4.9 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, indicating that the limitations due to leaf age in this tree were

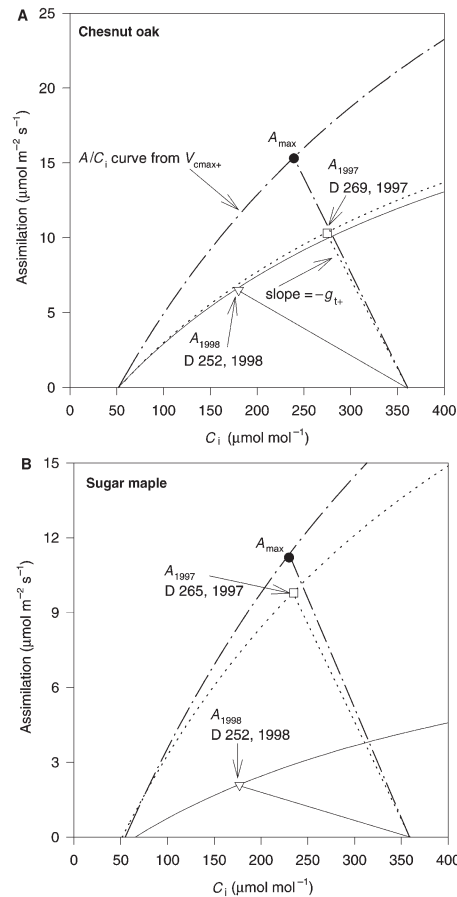


Figure 4. (A) Measured mean photosynthetic rate and C_i for chestnut oak tree No. 1 on Day 168, 1998 (●, A_{\max}), Day 252, 1998 (▽), and Day 269, 1997 (□). Also shown as slightly curved lines are the simulated responses of A to C_i based on the respective daily values of V_{cmax} on Day 168, 1998 (dash-dot lines), Day 252, 1998 (solid line), and Day 269, 1997 (dashed line). The three straight lines are of slope $-g_i$ and approximate the intercepts of the A/C_i curves at the measured photosynthetic rates. (B) Same as (A), except for a sugar maple. The respective days in (B) are Day 168, 1998 (●, dash-dot line), Day 252, 1998 (▽, solid line) and Day 265, 1997 (□, dashed line).

exclusively caused by non-stomatal reductions in V_{cmax} (σ was approximately zero).

Late in the season during the 1998 drought, V_{cmax} and the A/C_i response were similar to what was observed in late 1997 ($31.4 \mu\text{mol m}^{-2} \text{s}^{-1}$, solid line), but stomatal conductance ($0.067 \text{ mol m}^{-2} \text{s}^{-1}$) and the slope of the supply function were reduced by more than a factor of four. As a result, light-saturated assimilation rate was $6.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (triangle labeled A_{1998} in Figure 4A), a total reduction in assimilation of $8.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($A_{\max} - A_{1998}$ in Figure 4A). The values of L_S ($5.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) and L_{NS} ($3.4 \mu\text{mol m}^{-2} \text{s}^{-1}$) in 1998 are the result of both leaf age and drought, and unlike values observed in 1997, suggest a significant stomatal limitation ($\sigma = 0.61$). Figure 4A also shows that the limitation in response to drought, isolated from leaf age ($A_{1998} - A_{1997} = 3.8 \mu\text{mol m}^{-2} \text{s}^{-1}$), was almost exclusively stomatal (i.e., the A/C_i response and V_{cmax} were nearly identical).

The leaf age and drought responses in the sugar maple tree differed from those in the chestnut oak tree (Figure 4B). Late in the 1997 season (Day 265), assimilation was reduced by only 13% from the maximum rate ($A_{\max} - A_{1997}$), indicating a small seasonal effect (A_{1997} in Figure 4b) that was primarily non-stomatal ($\sigma = 0.24$). However, during the drought in 1998 (Day 252), assimilation was only 19% of maximum and 21% of the value during the similar period in 1997 (A_{1998} in Figure 4B). Both sugar maple and chestnut oak had reduced photosynthetic rates during drought, but unlike the chestnut oak (Figure 4A), V_{\max} in sugar maple was substantially reduced during the drought compared with the corresponding time in 1997 (lower solid line in Figure 4B). As a result, both stomatal conductance and V_{\max} contributed to reductions in ambient photosynthetic rates during drought. The relative stomatal limitation (σ) due to both drought and leaf age in 1998 was 0.53.

To provide a summary of the limitation partitioning for all trees, estimates were made of relative limitations (σ) for each tree during both years. Computations were made between July 19 (Day 200) and October 15 (Day 288), a period before most leaves showed pigmentation or other visible signs of senescence, but when assimilation rates were reduced as the leaves aged or experienced drought. Over this period, absolute stomatal (L_S) and non-stomatal (L_{NS}) limitations were summed for each tree, and the relative stomatal limitation (σ) was computed from these sums. Mean values of l_g (Equation 7) were also computed (Table 2).

The mean reduction in assimilation caused by stomatal limitation differed between years ($P < 0.001$), 26% in 1997 and 46% in 1998 (σ in Table 2). Lower mean C_i in 1998 also indicates greater stomatal limitation during that year (Table 2). Figure 5 shows the seasonal progression of the mean total limitation ($L_{NS} + L_S$, Figure 5A), absolute stomatal limitation (L_S , Figure 5B), absolute non-stomatal limitation (L_{NS} , Figure 5C) and relative stomatal limitation (σ , Figure 5D) when tree and

species variability were eliminated by averaging all measured overstorey trees in each year. During both years, the total limitation increased with leaf age and was an important fraction of the maximum assimilation rates (Figure 5A). In 1998, the limitation patterns, both absolute and relative, deviated from those in 1997. First, the total limitation was much greater during the drought in 1998 compared with the similar period in 1997 (Figure 5A). At the peak of the drought, the mean total limitation ($L_S + L_{NS}$) was $9.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in 1998, whereas it was about $4.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ for the same period in 1997. In 1998, the period of drought-enhanced limitation was evident for several months, but by Day 280 there was a recovery and both years indicated similar limitations in assimilation, although the soil was still drier in 1998 (Figure 2).

In addition to the greater total limitation in 1998 than in 1997, Figures 5B–D illustrate temporal differences between years in the absolute and relative contributions from stomatal and non-stomatal sources. In 1997, both the absolute stomatal and non-stomatal components increased monotonically as the season progressed (leaves aged), and L_{NS} was typically about four times greater than L_S (σ was often near 0.25, Figure 5D). Both L_S and L_{NS} were greater during the 1998 drought than during 1997, indicating that both processes resulted in important limitations during drought (Figures 5B and 5C). However, compared with 1997, L_S increased proportionally more during drought than L_{NS} , and the relative stomatal limitation, σ , exceeded 0.50 during the peak drought period.

The average limitation imposed by drought, independent of leaf age, was estimated from the differences in L_S and L_{NS} with (1998) and without (1997) drought (ΔL_S and ΔL_{NS}). The relative limitation imposed by stomata exclusively in response to drought ($\Delta L_S / (\Delta L_S + \Delta L_{NS})$) during the peak period of soil drying is around 0.75, considerably greater than that from leaf age.

Path dependence, l_g and comparison with other techniques

There was a tight correlation between the value of σ used in this study and the values calculated from the two methods described by Jones (1985) ($r^2 = 0.99$ and 0.97 for Equations 25 and 28 in Jones 1985, respectively). A non-zero intercept of 0.05 (both methods) and a slope of 0.89 (both methods) indicate a small bias between the methods at extreme values of σ . The mean standard deviation between our method and the two methods of Jones (1985) were 0.02 and 0.03. All three equations (Equation 8 and Equations 25 and 28 in Jones 1985) indicate similar statistically significant differences in σ between the two years.

When the path-dependent methods described in Jones (1985) and Assmann (1988) were applied to our data, the seasonal mean σ ranged from 0.15 (σ_v , changes in V_{\max} occur first) to 0.37 (σ_s , changes in stomatal conductance occur first) in 1997 and from 0.27 (σ_v) to 0.63 (σ_s) in 1998. Table 3 shows the mean values of σ_v and σ_s for each of the trees over the two years based on the path-dependent methods. For all trees, the annual values of σ_v and σ_s bracket the values obtained with Equation 8 (Table 2).

Table 2. Mean annual values of σ , C_i and l_g for the period July 19 to October 15 (Days 200–288) during 1997 and 1998. Also indicated are pairwise t -tests indicating the probability of similar means for 1997 and 1998 (* = $P < 0.05$ and ** = $P < 0.01$).

Tree	σ^{**}		C_i^*		l_g^*	
	1997	1998	1997	1998	1997	1998
White oak No. 1	0.24	0.35	236	222	0.30	0.35
White oak No. 2	0.23	0.44	241	221	0.27	0.34
White oak No. 3	0.36	0.76	265	224	0.21	0.35
Chestnut oak No. 1	0.03	0.40	271	230	0.17	0.31
Chestnut oak No. 2	–	0.50	–	197	–	0.45
Red maple No. 1	0.14	0.36	222	214	0.35	0.40
Red maple No. 2	0.47	0.41	198	219	0.48	0.40
Red maple No. 3	0.32	0.46	264	215	0.27	0.41
Sugar maple	0.35	0.44	228	216	0.32	0.39
Blackgum	0.16	0.39	268	225	0.26	0.43
Annual mean	0.26	0.46	244	217	0.29	0.38

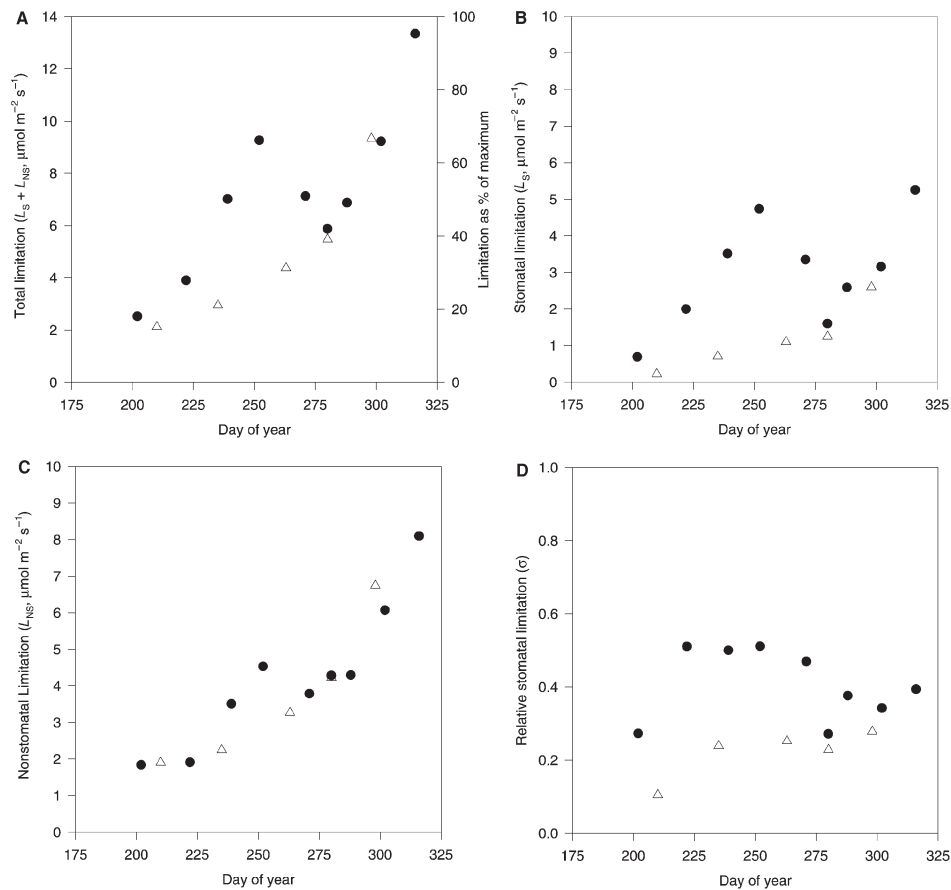


Figure 5. (A) Mean total limitation ($L_S + L_{NS}$), (B) absolute stomatal limitation (L_S), (C) absolute non-stomatal limitation (L_{NS}) and (D) relative stomatal limitation (σ) for all overstory trees versus day of year in 1997 (Δ) and 1998 (\bullet). In 1997, all trees were not measured on identical days, so dates are approximate to within one week.

Figure 6 shows values of σ (Equation 8) plotted against l_g (Equation 7) for each tree and measurement day. The two values are similar when photosynthetic capacity and stomatal conductance change in the same relative proportions (i.e., when $d \ln V_{\text{cmax}} = d \ln g_{\text{sc}}$ in Equation 8), but will differ when rel-

ative changes in photosynthetic capacity (V_{cmax}) and stomatal conductance are not closely coupled. There was a good correlation between σ and l_g in 1997 (intercept = -0.02 , slope = 0.99 , $r^2 = 0.78$), but the correlation was weak during the drought year (1998) (intercept = 0.10 , slope = 0.84 , $r^2 = 0.27$).

Table 3. Mean annual values for both years of the path-dependent values of relative stomatal limitation (σ_v and σ_s) for the period July 19 to October 15 (Days 200–288). Also indicated are pairwise t -tests indicating the probability of similar means for 1997 and 1998 (* = $P < 0.05$, and ** = $P < 0.01$).

	σ_v^*		σ_s^{**}	
	1997	1998	1997	1998
White oak No. 1	0.13	0.20	0.36	0.51
White oak No. 2	0.16	0.32	0.33	0.57
White oak No. 3	0.31	0.70	0.40	0.85
Chestnut oak No. 1	0.01	0.27	0.03	0.51
Chestnut oak No. 2	–	0.30	–	0.70
Red maple No. 1	0.08	0.15	0.21	0.57
Red maple No. 2	0.23	0.13	0.74	0.69
Red maple No. 3	0.09	0.20	0.57	0.72
Sugar maple	0.31	0.24	0.39	0.64
Blackgum	0.04	0.14	0.27	0.64
Mean	0.15	0.27	0.37	0.63

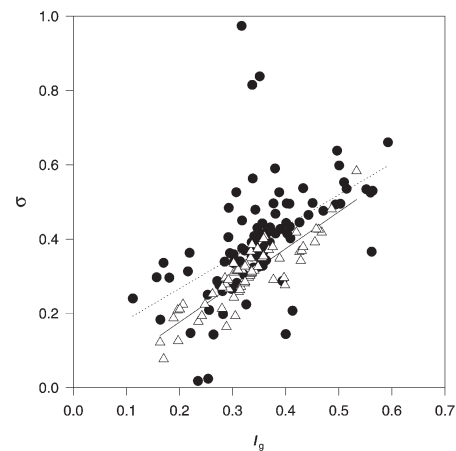


Figure 6. Daily values of σ plotted against l_g for each tree in 1997 (Δ) and 1998 (\bullet). The regression lines are 1997 (solid line) and 1998 (dotted line).

Discussion

Validity of approach

One of the compromising assumptions in our approach is that a meaningful maximum assimilation rate (V_{cmax}) and stomatal conductance can be defined. This maximum value serves as a standard for computing limitations throughout both seasons. Although the maximum rate necessarily depends on the frequency and the precise conditions during our measurements, measurements were performed at least 10 times per year on most trees. Sensitivity tests also showed that the relative, and in many cases the absolute, limitation partitioning was not highly dependent on whether we chose the maximum assimilation rate, the second highest assimilation rate or chose maximum rates independently for each year. Most importantly, because we chose a single maximal value applicable for both years, all limitation calculations were evaluated against a single standard. Therefore, although a precise definition of absolute limitations may be somewhat arbitrary, the difference in limitation partitioning between years, one of the key objectives of this study, can be determined more objectively.

A second assumption is that the relative partitioning can be determined without considering the path dependence (Jones 1985, Assmann 1988), implying that changes in photosynthetic capacity and stomatal conductance occur independently. Because of the correlation between assimilation and stomatal conductance that is often found (Wong et al. 1979), dependence between the two is often suspected. The uncertainty of path dependence puts a considerable range on our seasonal estimates, as shown by the discrepancy between σ_s and σ_v . However, one plausible hypothesis is that leaf age directly affects biochemical properties of leaves, and that stomatal conductance merely adjusts to this change in photosynthetic capacity (Field 1987). In this scenario, we would assume that changes in photosynthetic capacity occur first and that σ should be estimated with σ_v , which would lower our estimates of relative stomatal limitation when leaf age effects are most important (1997). Because of the link between tissue hydraulics or hormonal activity, or both, and stomatal action in deciduous trees (Loewenstein and Pallardy 1998), it would appear less probable that changes in stomatal conductance during drought are driven first because of reduced photosynthetic capacity. Therefore, our estimates of σ during the year when drought limitations were important (1998) would probably not be substantially reduced, whereas they may be when leaf age effects were most important (1997), and the effect of path dependence would not likely alter our conclusion about the differences in relative partitioning between years.

Comparison of σ with methods proposed in Jones (1985) and with l_g

The close relationship between l_g and σ in 1997 suggests that l_g measured at a single time may serve as a reasonable surrogate for σ when relative changes in photosynthetic capacity and stomatal conductance between two different states are

similar, and C_i remains relatively constant. However, it is difficult to determine if this condition is reasonable *a priori*, and as indicated by the weaker correlation between l_g and σ in 1998, stomatal conductance and V_{cmax} did not decrease in the same relative proportions when drought effects were occurring (generally $d\ln g_{\text{sc}} > d\ln V_{\text{cmax}}$). Actual limitations are taken into account by σ . The similar values of σ computed from our methods and from Equations 25 and 28 of Jones (1985) suggest that the three methods are reasonably comparable.

Limitations resulting from leaf aging

When leaf age effects were not confounded by drought, limitations on light-saturated assimilation rates were primarily mediated through diminished photosynthetic capacity (V_{cmax}) and not stomatal closure. This interpretation is subject to the assumption that the extent of patchy stomatal closure (Terashima 1992) and the diffusive resistance within the mesophyll (Parkhurst 1994, Epron et al. 1995) do not substantially increase with leaf age. These processes could create an artificial A/C_i response and overestimate changes in photosynthetic capacity (Laisk 1983). The diffusive mesophyll resistance may increase if lignin concentrations and leaf mass per unit area significantly increase with leaf age (Niinemets and Kull 1998). However, leaf mass per unit area did not change appreciably after early spring (Wilson et al. 2000). Actual decreases in biochemical capacity appear at least equally likely (Field 1987). Because nitrogen translocation accounts for only a small portion of the reduction in photosynthetic capacity for trees in this forest (Wilson et al. 2000), decreases in Rubisco allocation (Rey and Jarvis 1998) or ozone damage (Hanson et al. 1994) are potential agents that may decrease V_{cmax} over time. The seasonal decrease in photosynthesis over the season appears to be greater than in other temperate deciduous forests (Jurik 1986, Reich et al. 1991, Sullivan et al. 1996, Bassow and Bazzaz 1998).

Limitations resulting from drought

Drought in 1998 generally reduced V_{cmax} , g_{sc} , and light-saturated photosynthesis below values for the same period in 1997. Other gas exchange experiments with trees under natural conditions have also implicated a role of non-stomatal limitation during drought (Briggs et al. 1986, Weber and Gates 1990, Kubiske et al. 1996). Patchy stomatal closure is sometimes observed during drought (Terashima 1992), and apparent reductions in photosynthetic capacity deduced from gas exchange measurements are not always detected when analyzed by other methods (Cornic 1994). However, patchy closure is not a universal response and may be more likely when stress is applied rapidly than under more natural conditions (Gunasekera and Berkowitz 1992). Even with the possibility that we overestimated non-stomatal contributions during drought because of changes in patchy stomatal closure or diffusion in the mesophyll, stomatal limitation increased substantially more than non-stomatal limitation during drought. We estimate that the mean relative stomatal limitation in response to drought is around 75%, and possibly approaches

100%, if patchy closure is present. Non-stomatal limitation of assimilation during drought does not exceed 25% and may be less.

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References

- Assmann, S.M. 1988. Stomatal and non-stomatal limitations to carbon assimilation: an evaluation of the path-dependent method. *Plant Cell Environ.* 11:577–582.
- Ball, J.T. 1987. Calculations related to gas exchange. *In* Stomatal Function. Eds. E. Zeiger, G.D. Farquhar and I.R. Cowan. Stanford University Press, Stanford, CA, pp 445–476.
- Bassow, S.L. and F.A. Bazzaz. 1998. How environmental conditions affect canopy leaf-level photosynthesis in four deciduous tree species. *Ecology* 79:2660–2675.
- Briggs, T.W., T.W. Jurik and D.M. Gates. 1986. Non-stomatal limitation of CO₂ assimilation in three tree species during natural drought conditions. *Physiol. Plant.* 66:521–526.
- Comstock, J. and J. Ehleringer. 1984. Photosynthetic responses to slowly decreasing leaf water potentials in *Encelia frutescens*. *Oecologia* 61:241–261.
- Cornic, G. 1994. Drought stress and high light effects on leaf photosynthesis. *In* Photoinhibition of Photosynthesis from Molecule to Field. Eds. N.R. Baker and J.R. Bowyer. Bioscience Publications, pp 297–313.
- Cornic, G., J.-L. Prioul and G. Louason. 1983. Stomatal and non-stomatal contribution in the decline in leaf net CO₂ uptake during rapid water stress. *Physiol. Plant.* 58:295–301.
- Draper, N.R. and H. Smith. 1966. Applied regression analysis. John Wiley and Sons, Inc., New York, 706 p.
- Epron, D., D. Godard, G. Cornic and B. Gentry. 1995. Limitation to net CO₂ assimilation rate by internal resistances to CO₂ transfer in the leaves of two tree species (*Fagus sylvatica* L. and *Castanea sativa* Mill.). *Plant Cell Environ.* 18:43–51.
- Escalona, J.M., J. Flexas and H. Medrano. 1999. Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. *Aust. J. Plant Physiol.* 26:421–433.
- Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149:78–90.
- Farquhar, G.D. and T.S. Sharkey. 1982. Stomatal conductance and photosynthesis. *Annu. Rev. Plant Physiol.* 33:317–345.
- Field, C. 1987. Leaf-age effects on stomatal conductance. *In* Stomatal Function. Eds. E. Zeiger, G.D. Farquhar and I.R. Cowan. Stanford University Press, Stanford, CA, pp 367–384.
- Gunasekera, C. and G.A. Berkowitz. 1992. Heterogeneous stomatal closure in response to leaf water deficits is not a universal phenomenon. *Plant Physiol.* 103:629–635.
- Hanson, P.J., L.J. Samuelson, S.D. Wullschlegler, T.A. Tabberer and G.S. Edwards. 1994. Seasonal patterns of light-saturated photosynthesis and leaf conductance for mature and seedling *Quercus rubra* L. foliage: differential sensitivity to ozone exposure. *Tree Physiol.* 14:1351–1366.
- Hanson, P.J., D.E. Todd, M.A. Huston, J.D. Joslin, J.L. Croker and R.M. Auge. 1998. Description and field performance of the Walker Branch throughfall displacement experiment: 1993–1996. ORNL/TM-13586, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, 36 p.
- Harley, P.C., R.B. Thomas, J.F. Reynolds and B.R. Strain. 1992. Modelling photosynthesis of cotton in elevated CO₂. *Plant Cell Environ.* 15:271–282.
- Harley, P.C., J.D. Tenhunen and O.L. Lange. 1986. Use of an analytical model to study limitations on net photosynthesis in *Arbutus unedo* under field conditions. *Oecologia* 70:393–401.
- Hutmacher, R.B. and D.R. Krieg. 1983. Photosynthetic rate control in cotton. Stomatal and nonstomatal factors. *Plant Physiol.* 73:658–661.
- Johnson, D.W. and R.I. Van Hook, Eds. 1989. Analysis of biogeochemical cycling processes in Walker Branch Watershed. Springer-Verlag, New York, 401 p.
- Jones, H.G. 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant Cell Environ.* 8:95–104.
- Jones, H.G. 1998. Stomatal control of photosynthesis and transpiration. *J. Exp. Bot.* 49:387–398.
- Jurik, T.W. 1986. Seasonal patterns of leaf photosynthetic capacity in successional northern hardwood tree species. *Am. J. Bot.* 73:131–138.
- Kubiske, M.E. and M.D. Abrams. 1992. Photosynthesis, water relations, and leaf morphology of xeric versus mesic *Quercus rubra* ecotypes in central Pennsylvania in relation to moisture stress. *Can. J. For. Res.* 22:1402–1407.
- Kubiske, M.E. and M.D. Abrams. 1993. Stomatal and non-stomatal limitations of photosynthesis in 19 temperate tree species on contrasting sites during wet and dry years. *Plant Cell Environ.* 16:1123–1129.
- Kubiske, M.E., M.D. Abrams and S.A. Mostoller. 1996. Stomatal and non-stomatal limitations of photosynthesis in relation to the drought and shade tolerance of tree species in open and understory environments. *Trees* 11:76–82.
- Laik, A. 1983. Calculation of leaf photosynthetic parameters considering the statistical distribution of stomatal apertures. *J. Exp. Bot.* 34:1627–1635.
- Loewenstein, N.J. and S.G. Pallardy. 1998. Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of young plants of four temperate deciduous angiosperms. *Tree Physiol.* 18:421–430.
- Ni, B.-R. and S.G. Pallardy. 1992. Stomatal and non-stomatal limitations to net photosynthesis in seedlings of woody angiosperms. *Plant Physiol.* 99:1502–1508.
- Niinemets, U. and O. Kull. 1998. Stoichiometry of foliar carbon constituents varies along light gradients in temperate woody canopies: implications for foliage morphological plasticity. *Tree Physiol.* 18:467–479.
- Parkhurst, D.F. 1994. Diffusion of CO₂ and other gases inside leaves. *New Phytol.* 126:449–479.
- Reich, P.B., M.B. Walters and D.S. Ellsworth. 1991. Leaf age and season influence the relationships between leaf nitrogen, leaf mass per area and photosynthesis in maple and oak trees. *Plant Cell Environ.* 14:251–259.

- Rey, A. and P.G. Jarvis. 1998. Long-term photosynthetic acclimation to increased atmospheric CO₂ concentration in young birch (*Betula pendula*) trees. *Tree Physiol.* 18:441–450.
- Roupsard, O., P. Gross and E. Dreyer. 1996. Limitation of photosynthetic activity by CO₂ availability in the chloroplasts of oak leaves from different species during drought. *Ann. Sci. For.* 53: 243–254.
- Stewart, J.D., A. Zine El Abidine and P.Y. Bernier. 1994. Stomatal and mesophyll limitations of photosynthesis in black spruce seedlings during multiple cycles of drought. *Tree Physiol.* 15:57–64.
- Sullivan, N.H., P.V. Bolstad and J.M. Vose. 1996. Estimates of net photosynthetic parameters in mature forests of the Southern Appalachians. *Tree Physiol.* 16:397–406.
- Terashima, I. 1992. Anatomy of non-uniform photosynthesis. *Photosynth. Res.* 31:195–212.
- Teskey, R.O., J.A. Fites, L.J. Samuelson and B.C. Bongarten. 1986. Stomatal and non-stomatal limitations to net photosynthesis in *Pinus taeda* L. under different environmental conditions. *Tree Physiol.* 2:131–142.
- Walcroft, A.S., D. Whitehead, W.B. Silvester and F.M. Kelliher. 1997. The response of photosynthetic model parameters to temperature and nitrogen concentration in *Pinus radiata* D. Don. *Plant Cell Environ.* 20:1338–1348.
- Weber, J.A. and D.M. Gates. 1990. Gas exchange in *Quercus rubra* (northern red oak) during a drought: analysis of relations among photosynthesis, transpiration, and leaf conductance. *Tree Physiol.* 7:215–225.
- Wilson, K.B., P.J. Hanson and D.D. Baldocchi. 2000. Spatial and seasonal variability of photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest. *Tree Physiol.* 20:565–578.
- Wong, S.C., I.R. Cowan and G.D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282: 424–426.
- Wullschlegel, S.D. 1993. Biochemical limitations to carbon assimilation in C₃ plants—a retrospective analysis of A/C_i curves from 109 species. *J. Exp. Bot.* 44:907–920.
- Wullschlegel, S.D., P.J. Hanson and T. Tschaplinski. 1998. Whole-plant water flux in understory red maple exposed to altered precipitation regimes. *Tree Physiol.* 18:71–79.

