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## Research paper

# Effects of potassium supply on limitations of photosynthesis by mesophyll diffusion conductance in *Carya cathayensis*

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Potassium (K) influences the photosynthesis process in a number of ways; however, the mechanisms underlying the photosynthetic response to differences in K supply are not well understood. Concurrent measurements of gas exchange and chlorophyll fluorescence were made to investigate the effect of K nutrition on photosynthetic efficiency and mesophyll conductance ( $g_m$ ) in hickory seedlings (*Carya cathayensis* Sarg.) in a greenhouse. The results show that leaf K concentrations <0.7–0.8% appeared to limit the leaf net CO<sub>2</sub> assimilation rate ( $A$ ), and that the relative limitation of photosynthesis due to  $g_m$  and stomatal conductance ( $g_s$ ) decreased with increasing supplies of K. However, a sensitivity analysis indicated that  $A$  was most sensitive to the maximum carboxylation rate of Rubisco ( $V_{c,max}$ ) and the maximum rate of electron transport ( $J_{max}$ ). These results indicate that the photosynthetic rate is primarily limited by the biochemical processes of photosynthesis ( $V_{c,max}$  and  $J_{max}$ ), rather than by  $g_m$  and  $g_s$  in K-deficient plants. Additionally,  $g_m$  was closely correlated with  $g_s$  and the leaf dry mass per unit area ( $M_A$ ) in hickory seedlings, which indicates that decreased  $g_m$  and  $g_s$  may be a consequence of leaf anatomical adaptation.

**Keywords:** *Carya cathayensis* Sarg., chloroplast CO<sub>2</sub> concentration, mesophyll conductance, potassium nutrition, photosynthesis.

## Introduction

Chinese hickory (*Carya cathayensis* Sarg.) is distributed throughout the Zhejiang and Anhui provinces, making it the most eastern of Asian hickories. In this region, the soils contain low amounts of total potassium (K) and respond to the addition of fertilizers containing K, which increases K availability (Lu 1998). In fact, only a fraction of the soil K content is available for uptake by plants. Potassium is known to influence fruit yield, and in particular fruit quality (Besford and Maw 1975). Moreover, the rate of K uptake by hickory plants can be limited by high levels of calcium in some soils (Chen et al. 2010), and cation competitive effects frequently lead to K deficiency in the field. The relationship between leaf K concentration and leaf

photosynthesis in deciduous fruit tree species has received little attention, and, in addition, the effect of K deficiency on photosynthesis in hickory plants has not been investigated. Therefore, the major objective of the present study was to determine the effect of K nutrition on photosynthesis capacity in hickory.

Plants absorb K in larger amounts than any other mineral element (except for nitrogen), and K is the nutrient that most frequently limits plant growth and crop yields. Potassium supply affects a wide range of physiological processes in higher plants. The K cation has no structural purpose, but is the most common cation in plant biochemical processes, acting as an activator or cofactor in several enzyme systems. Essential roles

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for K are found in energy transfer and utilization, protein synthesis, carbohydrate metabolism, transport of sugars from leaves to fruits, and production and accumulation of oils (Römheld and Kirkby 2010). Potassium is also the major osmotic solute in plants; the osmotic potential ( $\Psi_s$ ) is considerably lowered, while the pressure potential ( $\Psi_p$ ) significantly increases when K supply is increased (Pervez et al. 2001). Some studies have shown that K fertilization results in a marked improvement in water-use efficiency (Ashraf et al. 2001). Potassium deficiency is generally associated with decreased transpiration rates, and most studies attributing stomatal closure to K deficiency have tended to focus on plants at a very advanced stage of nutritional deficiency (Thiel and Wolf 1997, Römheld and Kirkby 2010). Therefore, plants that have adequate K nutrition will be able to withstand longer periods of low soil moisture. A typical symptom of K deficiency is the wilting of plants in prolonged dry weather.

The role of K in agricultural production is intimately connected with photosynthesis. Potassium influences the process of photosynthesis at many levels, namely synthesis of ATP, activation of the enzymes involved in photosynthesis, CO<sub>2</sub> uptake, balance of the electric charges required for photophosphorylation in chloroplasts, and acting as the counterion to light-induced H<sup>+</sup> flux across the thylakoid membranes (Marschner 1995). There are numerous studies suggesting that the photosynthetic capacities of higher plants change dramatically in response to different supplies of K (Bednarz et al. 1998, Zhao et al. 2001, Basile et al. 2003, Weng et al. 2007, Gerardeaux et al. 2009); however, the underlying mechanisms of photosynthetic acclimation are still not well understood. As the light-dependent uptake of K into guard cells is a critical step in stomatal opening (Shavala 2003), it is likely that stomatal limitations may arise under K deficiency. Stomatal closure in response to K deficiency is well documented and is often considered a major factor that contributes to decreased net photosynthesis (Thiel and Wolf 1997). Conversely, Arquero et al. (2006) reported that K starvation enhanced stomatal conductance in both well-irrigated and water-stressed olive trees, while Basile et al. (2003) showed that leaf K concentration did not significantly influence stomatal conductance, implying that it is unlikely that K deficiency in almond affects photosynthetic rates via stomatal limitations. Numerous studies have also shown that K starvation promotes transpiration (Bednarz et al. 1998, Sudama et al. 1998, Cabañero and Carvajal 2007). However, Bednarz et al. (1998) concluded that stomatal limitations are the major factor that affects leaf photosynthesis in cotton plants during mild K deficiency, whereas biochemical limitations become more important as the severity of K deficiency increases. These contradictions suggest that the effects of K deficiency on plant photosynthesis remain elusive; therefore, it is necessary to re-examine the internal mechanism of plant photosynthesis under the influence of stress induced by K deficiency.

The exact mechanisms of the effects of leaf K status on photosynthesis are still unclear; however, it has been suggested that the activity of Rubisco is an important limiting factor of photosynthesis in rice leaves (Yang et al. 2004, Weng et al. 2007). Recent studies have shown that mesophyll conductance ( $g_m$ ) is finite, leading to a significant draw-down in CO<sub>2</sub> concentrations from the substomatal internal cavities ( $C_i$ ) to the site of carboxylation inside the chloroplast stroma ( $C_c$ ), which may limit photosynthesis as significantly as stomata closure (Warren et al. 2003, 2007, Niinemets et al. 2009a). The chloroplastic CO<sub>2</sub> partial pressure can impact photosynthetic efficiency, as Rubisco activity is induced by  $C_c$  (Galmés et al. 2011). It is known that  $g_m$  can respond to environmental factors such as salt, temperature, nutrients, light and water (Niinemets et al. 2009a), and internal factors including tree size and height, as well as leaf structure and ageing (Niinemets et al. 2009a, Han 2011, Whitehead et al. 2011, Zhu et al. 2011). Additionally, it has been suggested that  $g_m$  is associated with aquaporin (Hanba et al. 2004, Terashima et al. 2006) and carbonic anhydrase activities (Makino et al. 1992). Longstreth and Nobel (1980) showed that the reduction in photosynthesis caused by K deficiency was primarily related to decreased mesophyll conductance; however, this work was conducted under the assumption that mesophyll conductance is only dependent on the photosynthetic rate. Bednarz et al. (1998) reported that K deficiency stress reduced the photosynthetic rate and increased  $C_i$  in cotton, which they interpreted as an indication of a greater limitation in photosynthesis due to mesophyll conductance; however, there is no experimental evidence to indicate that a relative limitation due to mesophyll conductance is affected by K supply in plants. For these reasons, it is desirable to develop a better understanding of the mechanisms by which K supply affects gas exchange in plants. Such knowledge will enable better predictions of photosynthesis, plant productivity and plant responses to nutrition supply.

## Materials and methods

### Plants materials and growth conditions

One-year-old hickory seedlings (*C. cathayensis* Sarg.) were obtained from the Tianze Hickory Company (Zhejiang, China). The seedlings were transplanted into 30.5-cm tall plastic pots with a top diameter of 25 cm, containing full-strength nutrient solution. The composition of the standard nutrient solution was as follows: 2.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.5 mM Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>, 1.0 mM K<sub>2</sub>SO<sub>4</sub>, 0.5 mM MgSO<sub>4</sub>, 12.5 μM H<sub>3</sub>BO<sub>3</sub>, 1.0 μM MnSO<sub>4</sub>, 1.0 μM ZnSO<sub>4</sub>, 0.25 μM CuSO<sub>4</sub>, 0.1 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> and 10 μM EDTA-Fe. The seedlings were grown in a greenhouse with natural sunlight during the day. The mean daytime maximum and minimum temperatures in the greenhouse were

28 and 20 °C, with a constant relative humidity of ~60%. After ~2 months, the composition of the nutrient solution was altered to one of five K concentrations: K0, K1, K2, K3 and K4 containing 0, 0.4, 1.0, 2.0 and 5.0 mM K, respectively. In all cases, Ca(OH)<sub>2</sub> and HCl were used to adjust the pH of the nutrient solution to 5.7. The nutrient solution was changed every 7 days. All the treatments had 10 replicates with a completely random design.

### Measurements of gas exchange and chlorophyll fluorescence

Measurements were made on the youngest fully expanded leaf from 6–8 randomly selected seedlings on the 60th day of the treatment, using leaves developed after the initiation of the K nutrition treatment. Leaf gas exchange and chlorophyll fluorescence were measured simultaneously using a portable infrared gas analyser system (Li-6400, Li-Cor, Lincoln, NE, USA) equipped with an integrated leaf chamber fluorometer (Li-6400-40) at a concentration of 380 μmol mol<sup>-1</sup> CO<sub>2</sub>, 21% O<sub>2</sub> and 50% relative humidity. Leaf chamber temperature was maintained at 28 °C. All measurements were carried out at 1200 μmol m<sup>-2</sup> s<sup>-1</sup>, with 90% red light and 10% blue light, which we previously determined to be just above light saturation for hickory seedlings. Once a steady state was reached (~20 min at a photosynthetic photon flux density (PPFD) of 1200 μmol m<sup>-2</sup> s<sup>-1</sup>), a CO<sub>2</sub> response curve (A–C<sub>i</sub> curve) was performed. The ambient CO<sub>2</sub> concentration (C<sub>a</sub>) was lowered stepwise from 380 to 50 μmol mol<sup>-1</sup>, and then returned to 380 μmol mol<sup>-1</sup> to re-establish the initial steady-state value of photosynthesis. C<sub>a</sub> was then increased stepwise from 380 to 1800 μmol mol<sup>-1</sup>. At each C<sub>a</sub>, photosynthesis was allowed to stabilize for 3–4 min until gas exchange was steady, so that each curve was completed in 35–50 min. Corrections for the leakage of CO<sub>2</sub> in and out of the Li-6400 leaf chamber, as described by Perez-Martin et al. (2009), were applied to all gas-exchange data.

The actual photochemical efficiency of photosystem II (Φ<sub>PSII</sub>) was determined by measuring steady-state fluorescence (F<sub>s</sub>) and maximum fluorescence during a light-saturating pulse (F<sub>m</sub>') following the procedure of Genty et al. (1989):

$$\Phi_{\text{PSII}} = (F'_m - F_s) / F'_m \quad (1)$$

The rate of electron transport estimated from chlorophyll fluorescence is given by the equation (Bilger and Björkman 1990)

$$J = \Phi_{\text{PSII}} \text{PPFD} \alpha \beta \quad (2)$$

where PPFD is the photosynthetic photon flux density, α is leaf absorptance and β is the proportion of quanta absorbed by photosystem II. αβ was determined for each treatment from

the slope of the relationship between Φ<sub>PSII</sub> and Φ<sub>CO<sub>2</sub></sub> (i.e., the quantum efficiency of gross CO<sub>2</sub> fixation), which was obtained by varying light intensity under non-photorespiratory conditions in an atmosphere containing <1% O<sub>2</sub> (Valentini et al. 1995).

### Measurement of mitochondrial respiration rate in the light (R<sub>d</sub>) and intercellular CO<sub>2</sub> compensation point (C<sub>i</sub><sup>\*</sup>)

R<sub>d</sub> and C<sub>i</sub><sup>\*</sup> were determined according to the method of Laisk (1977). A–C<sub>i</sub> curves were measured using an open gas-exchange system (Li-6400, Li-Cor Inc.) equipped with an integrated light source (Li-6400-02) at three different photosynthetically active PPFDs (50, 200 and 500 μmol m<sup>-2</sup> s<sup>-1</sup>) at six different CO<sub>2</sub> levels ranging from 300 to 50 μmol CO<sub>2</sub> mol<sup>-1</sup> air. The curves intersected at the point where A is the same at different PPFDs; therefore, A at that point represents R<sub>d</sub>, and C<sub>i</sub> represents C<sub>i</sub><sup>\*</sup>.

### Calculation of the chloroplastic CO<sub>2</sub> compensation point (Γ<sup>\*</sup>), C<sub>c</sub> and g<sub>m</sub>

From combined gas-exchange and chlorophyll fluorescence measurements, the mesophyll conductance for CO<sub>2</sub> (g<sub>m</sub>) was estimated according to Harley et al. (1992) as

$$g_m = A / (C_i - \Gamma^* (J + 8(A + R_d)) / (J - 4(A + R_d))) \quad (3)$$

where A, C<sub>i</sub>, R<sub>d</sub> and J were determined as previously described for each treatment. Γ<sup>\*</sup> is the chloroplastic CO<sub>2</sub> photocompensation point calculated from the C<sub>i</sub><sup>\*</sup> and R<sub>d</sub> measurements according to the method of Warren et al. (2007) using a simultaneous equation with g<sub>m</sub>:

$$\Gamma^* = C_i^* + R_d / g_m \quad (4)$$

Equation (4) was then substituted into (3) and the value of g<sub>m</sub> was found; then Γ<sup>\*</sup> was calculated. The value of Γ<sup>\*</sup> was found to be slightly higher for the K0-treated plants (53.9 ± 9.6 μmol mol<sup>-1</sup>), compared with the four other treatments (47.3 ± 7.5, 44.9 ± 7.8, 44.7 ± 5.1 and 44.6 ± 8.6 μmol mol<sup>-1</sup> for K1, K2, K3 and K4 treatments, respectively). Changes in Γ<sup>\*</sup> derived using the method of Laisk (1977) have been frequently observed under stress conditions such as drought (Galmés et al. 2006); therefore, we re-calculated g<sub>m</sub> using the non-stressed Γ<sup>\*</sup> values (44.6 μmol mol<sup>-1</sup>), which is a reasonable assumption as Γ<sup>\*</sup> is an intrinsic property of Rubisco and thus varies only by a small amount within a species under different growing conditions. The CO<sub>2</sub> concentration in the chloroplast stroma (C<sub>c</sub>) was calculated using the equation

$$C_c = C_i - A / g_m \quad (5)$$

*Calculation of maximum Rubisco carboxylation rate ( $V_{c,max}$ ), maximum rate of electron transport ( $J_{max}$ ) and the relative limitations in the rate of photosynthesis by stomatal conductance ( $g_s$ ) and mesophyll conductance ( $g_m$ )*

A– $C_i$  curves were transformed to A– $C_c$  curves using the calculated value of  $C_c$ . The maximum carboxylation rate of Rubisco ( $V_{c,max}$ ) and the maximum rate of electron transport ( $J_{max}$ ) were determined from the measured A and  $C_c$  values fitted to the photosynthesis model of Farquhar et al. (1980).

The limitations in the photosynthetic rate imposed by  $g_s$  and  $g_m$  were calculated using the predicted photosynthetic rates, assuming that these conductance values were either infinite or finite, according to Farquhar and Sharkey (1982). From the response of A to  $C_i$ , stomatal limitation ( $L_s$ ) was calculated using

$$L_s = (A_{ca} - A_{ci}) / A_{ca} \quad (6)$$

where  $A_{ca}$  is estimated using the infinite  $g_s$ , and  $A_{ci}$  is estimated using the actual  $g_s$  and  $g_m$ . Similar to  $L_s$ , from the response of A to  $C_c$ , mesophyll limitation ( $L_m$ ) was calculated using

$$L_m = (A_{cc} - A_{ci}) / A_{cc} \quad (7)$$

where  $A_{cc}$  is estimated using the infinite  $g_m$  and actual  $g_s$  values.

*Sensitivity analysis of the key photosynthetic parameters contributing to variation in A*

Photosynthetic rates are influenced by several parameters ( $C_i$ ,  $g_s$ ,  $R_d$ ,  $C_c$ ,  $g_m$ ,  $V_{c,max}$  and  $J_{max}$ ). To quantify the importance of the key photosynthetic parameters in the response of A to reduced K, a sensitivity analysis of the A– $C_c$  curves was carried out using the biochemical model of photosynthesis previously described by Farquhar et al. (1980) according to the methods of Warren and Adams (2004) and Turnbull et al. (2007). Independently, the  $C_i$ ,  $g_s$ ,  $R_d$ ,  $C_c$ ,  $g_m$ ,  $V_{c,max}$  or  $J_{max}$  values for each K treatment (2.0, 1.0, 0.4 and 0 mM K) were substituted into the model, while all the other photosynthetic parameters were kept constant, using the parameters from the 5.0 mM K treatment as control values. The model was re-run for each combination of treatment and photosynthetic parameter, and deviations from the control values were recorded as a proportion. To calculate the sensitivity of A to  $g_s$ ,  $C_c$  was calculated using

$$C_c = C_a - A / (g_s / 1.6) - A / g_m \quad (8)$$

where  $g_s/1.6$  is the stomatal conductance to carbon dioxide, calculated from the stomatal conductance to water.

Altering the biochemical capacity for photosynthesis via  $R_d$ ,  $V_{c,max}$  and  $J_{max}$  will affect  $C_c$  and  $C_i$  unless  $g_m$  and  $g_s$  also respond. Cases with constant  $C_c$  were calculated by a direct substitution of new values into the model and re-calculation of A. If  $g_m$  was assumed constant (i.e., we allowed  $C_c$  to vary and assumed  $C_i$  was invariant), Eq(5) was substituted into the model, and the equations were solved for A. Where we assumed  $g_m$  was constant (i.e., we allowed  $C_c$  and  $C_i$  to vary), Eq(8) was substituted into the model to solve the equations for A.

*Determination of pigment and soluble protein content*

After measuring photosynthesis, a portion of leaf from the same sampling position in each seedling was freeze-clamped into liquid nitrogen and subsequently used for biochemical measurements. Chlorophyll concentration was determined according to the methods of Huang et al. (2004). The total soluble protein (TSP) content was determined with the dye-binding method introduced by Bradford (1976) using bovine serum albumin as a standard.

*Determination of potassium content and leaf dry mass per unit area*

The remaining leaves were collected from the same sampling position of the seedlings, and the area of these leaves was determined using a Li-3000 area meter (Li-Cor). The leaves were subsequently dried for 48 h at 65 °C and used to calculate leaf dry mass per unit area ( $M_A$ , g m<sup>-2</sup>). For the K determination, samples of dried leaves were ground, digested with an acid mixture (HNO<sub>3</sub>:HClO<sub>4</sub>, 3:1, v/v) and the K concentration in the digest was determined using a flame photometer (PFP-7, Barloworld Scientific T/As Jenway, Gransmore Green, UK).

*Statistical methods*

Statistical analysis was performed with SPSS 10.0 software (SPSS, Chicago, IL, USA), using Student's t-test to compare means with the significance level at  $P < 0.05$ .

**Results**

The leaf K content varied significantly among plants from the different nutrient treatments, increasing from 0.52 to 1.42% of dry weight with an increase in K supply from 0 to 5.0 mM (Table 1). The leaf dry mass per unit area ( $M_A$ ) was increased in the KO and K1 treatments compared with the K2, K3 and K4 treatments. The total chlorophyll content was significantly lower in KO-treated plants (241 mg m<sup>-2</sup>), compared with the other four treatments, where the mean total chlorophyll content ranged from 347 to 354 mg m<sup>-2</sup>. A similar trend was noted in the soluble protein content, which was significantly lower in the leaves of KO-treated plants, compared with the other four K treatment concentrations. In contrast, in the KO-treated plants,



Table 1. Effect of K supply on leaf K content, leaf dry mass per unit area ( $M_A$ ), total chlorophyll (Chl  $a + b$ ), ratio of chlorophyll  $a$  to  $b$  (Chl  $a/b$ ) and total soluble protein (TSP) content in the youngest fully expanded leaves of *C. cathayensis* seedlings.

Treatment	K (% dry weight)	$M_A$ (g m <sup>-2</sup> )	Chl $a + b$ (mg m <sup>-2</sup> )	Chl $a/b$	TSP (g m <sup>-2</sup> )
K0 (0 mM)	0.52 ± 0.05d	207 ± 10.6a	241 ± 23b	3.8 ± 0.5a	3.0 ± 0.1b
K1 (0.4 mM)	0.64 ± 0.07c	186 ± 15.2ab	347 ± 46a	2.8 ± 0.3b	3.3 ± 0.2a
K2 (1.0 mM)	0.89 ± 0.06b	161 ± 11.9b	353 ± 21a	2.8 ± 0.2b	3.5 ± 0.2a
K3 (2.0 mM)	0.97 ± 0.11b	151 ± 9.1b	353 ± 31a	2.9 ± 0.4b	3.4 ± 0.3a
K4 (5.0 mM)	1.42 ± 0.15a	156 ± 11.4b	354 ± 28a	2.8 ± 0.2b	3.7 ± 0.5a

Hickory seedlings were grown for 60 days with five different K concentrations in hydroponic solutions. Data are means and SE of 6–8 replicate plants per treatment. Values indicated with different letters are significantly different,  $P < 0.05$ .

the ratio of chlorophyll  $a$  to  $b$  (Chl  $a/b$ ) was significantly higher compared with the other four K treatments.

The K status did not appear to significantly influence leaf CO<sub>2</sub> exchange rates on the first two measurement dates (days 20 and 40, data not shown); however, after ~60 days treatment, K status significantly reduced the leaf photosynthetic performance of hickory seedlings. The net CO<sub>2</sub> assimilation rate ( $A$ ) was significantly reduced in the KO- and K1-treated leaves while K2, K3 and K4 had stable assimilation rates (Table 2; Figure 1). KO plants had significantly lower assimilation rates (7.2 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), compared with the other four treatment groups. Stomatal conductance

( $g_s$ ) and mesophyll conductance ( $g_m$ ) were significantly lower in K-deficient plants (KO and K1 treatments), whereas chloroplastic CO<sub>2</sub> concentrations ( $C_c$ ) increased in the KO-treated plants. There was a small significant increase in  $g_s$ ,  $g_m$  and a decrease in  $C_c$  in K2-, K3- and K4-treated seedlings, and a general trend for intercellular CO<sub>2</sub> concentration ( $C_i$ ) to increase with decreasing K supply was observed (Table 2).

Photosynthesis and  $g_m$  responded curvilinearly to leaf K content (Figure 1a and b). When the leaf K concentrations in hickory seedlings remained >0.7–0.8%, the leaf photosynthetic capacity and  $g_m$  declined by only 5–12%, and the slope of the fitted curves for leaves with a K concentration <0.7% increased.

The  $g_m$  values negatively correlated with  $M_A$  in the hickory seedlings (Figure 2a). Studies have shown that  $g_m$  usually decreases in parallel with  $g_s$  (Galmés et al. 2006, Warren 2008), and we observed that a low K supply decreased  $g_s$  and  $g_m$ , with a strong positive relationship observed between  $g_m$  and  $g_s$  (Figure 2b).

The relative photosynthetic limitation due to mesophyll conductance ( $L_m$ ) was slightly smaller than the relative limitation due to stomatal conductance ( $L_s$ , Figure 3). The mesophyll and stomatal limitations to photosynthesis were both negatively correlated with leaf K concentrations, and the slope of this relationship was similar for both  $L_m$  and  $L_s$ .

The rate of mitochondrial respiration in the light ( $R_d$ ) was not significantly affected by differential K treatments, despite a trend for  $R_d$  to increase with a decreasing K supply (Table 3). The intercellular CO<sub>2</sub> compensation point ( $C_i^*$ ) was also unaffected by K supply. The maximum rate of carboxylation ( $V_{c,max}$ ) was significantly lower in the KO- and K1-treated plants, while the mean values in the three other treatments ranged from 74.5 to 76.5 μmol m<sup>-2</sup> s<sup>-1</sup>. Similarly, the maximum rate of electron transport ( $J_{max}$ ) was significantly lower in KO- and K1-treated plants, with a minor change in  $J_{max}$  observed at the other three K treatments.

The larger  $A$  observed in hickory seedlings treated with 5.0 mM K compared with seedlings receiving lower concentrations of K (0 and 0.4 mM K) was primarily a result of altered  $V_{c,max}$  and  $J_{max}$  (Table 4). When the  $V_{c,max}$  of 2.0 mM K was substituted for that of 5.0 mM K,  $A$  decreased by 2.1–3.3%,

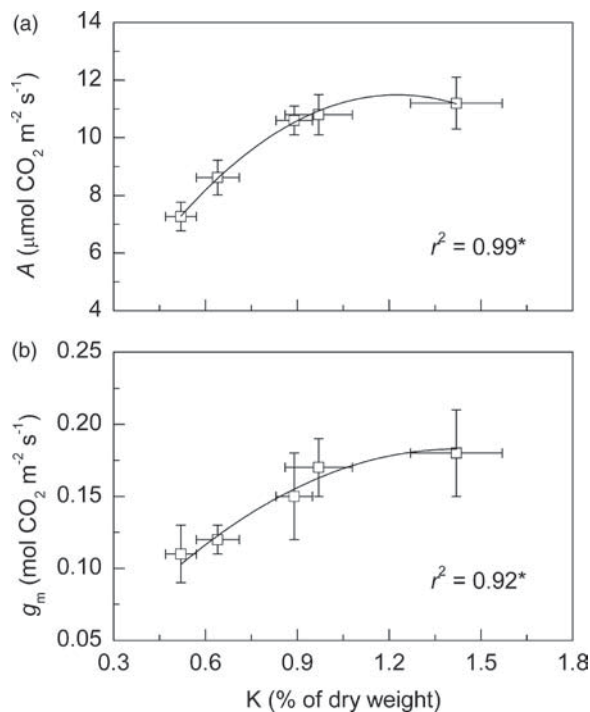


Figure 1. Effects of leaf K concentration on (a) net assimilation rate ( $A$ ) and (b) mesophyll conductance ( $g_m$ ) in the youngest fully expanded leaves of hickory seedlings.  $A$  and  $g_m$  were determined at an ambient CO<sub>2</sub> concentration of 380 μmol mol<sup>-1</sup>, a leaf temperature of 28 °C and a PPFD of 1200 μmol m<sup>-2</sup> s<sup>-1</sup>. Data are means of 6–8 replicate plants; error bars are 1 SE. The significance of the correlation coefficient is indicated by an asterisk beside the regression coefficient ( $r^2$ ),  $P < 0.05$ .

Table 2. Effects of K supply on net CO<sub>2</sub> assimilation rate (*A*), stomatal conductance (*g<sub>s</sub>*), intercellular CO<sub>2</sub> concentrations (*C<sub>i</sub>*), mesophyll conductance (*g<sub>m</sub>*) and chloroplast CO<sub>2</sub> concentrations (*C<sub>c</sub>*) in the youngest fully expanded leaves of hickory seedlings.

Treatment	<i>A</i> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	<i>g<sub>s</sub></i> (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	<i>C<sub>i</sub></i> (μmol mol <sup>-1</sup> CO <sub>2</sub> )	<i>g<sub>m</sub></i> (mol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	<i>C<sub>c</sub></i> (μmol mol <sup>-1</sup> CO <sub>2</sub> )
K0 (0 mM)	7.2 ± 0.5c	0.14 ± 0.02b	247 ± 12a	0.11 ± 0.02b	172 ± 24a
K1 (0.4 mM)	8.6 ± 0.6b	0.17 ± 0.01ab	225 ± 17ab	0.12 ± 0.01b	149 ± 21b
K2 (1.0 mM)	10.6 ± 0.5a	0.19 ± 0.01a	231 ± 19ab	0.15 ± 0.02a	159 ± 12b
K3 (2.0 mM)	10.8 ± 0.7a	0.20 ± 0.00a	228 ± 21ab	0.17 ± 0.02a	163 ± 19b
K4 (5.0 mM)	11.2 ± 0.9a	0.20 ± 0.02a	213 ± 9b	0.18 ± 0.03a	151 ± 10b

Hickory seedlings were grown for 60 days with five different K concentrations in hydroponic solutions. Data are means and SE of 6–8 replicate plants per treatment. Values carrying different letters are significantly different,  $P < 0.05$ .

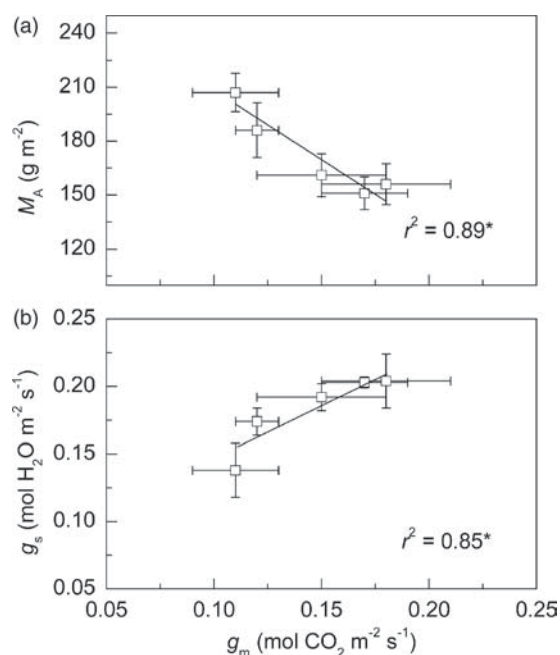


Figure 2. Effects of K supply on relationships between (a) leaf dry mass per unit area ( $M_A$ ) and (b) stomatal conductance ( $g_s$ ) and mesophyll conductance ( $g_m$ ) in the youngest fully expanded leaves of hickory seedlings. The  $g_s$  and  $g_m$  values were determined at an ambient CO<sub>2</sub> concentration of 380 μmol mol<sup>-1</sup>, a leaf temperature of 28 °C and a PPFD of 1200 μmol m<sup>-2</sup> s<sup>-1</sup>. Data are means of 6–8 replicate plants; error bars are 1 SE. The significance of the correlation coefficient is indicated by an asterisk beside the regression coefficient ( $r^2$ ),  $P < 0.05$ .

whereas *A* decreased by 37.4–59.6% when the  $V_{c,max}$  of 0 mM K was substituted for that of 5 mM K. The re-computed *A* values were less affected by substitution of  $J_{max}$  values, compared with substitution of  $V_{c,max}$ . Compared with  $V_{c,max}$  and  $J_{max}$ ,  $R_d$  had very small effects on *A*, and the re-computed *A* values were rather insensitive to  $g_s$  and  $g_m$  in K-deficient hickory seedlings. When the  $g_m$  values of KO-treated plants were substituted for the control plants (5.0 mM K), *A* was 14.6–21.4% smaller. However, when the  $C_i$  and  $C_c$  values obtained at 0 mM K were substituted for the 5.0 mM K  $C_i$  and  $C_c$  values, *A* increased by 13.4 and 24.3%, respectively.

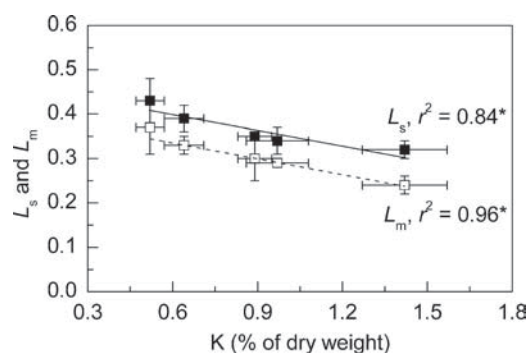


Figure 3. Effects of leaf K concentration on the relative limitations posed by stomatal conductance ( $L_s$ , filled squares, solid line) and mesophyll conductance ( $L_m$ , open squares, dashed line) in the youngest fully expanded leaves of hickory seedlings. Relative limitations were calculated for  $C_a = 380\ \mu mol\ mol^{-1}$  as described in the Materials and methods. Data are means of 6–8 replicate plants; error bars are 1 SE. The significance of the correlation coefficient is indicated by an asterisk beside the regression coefficient ( $r^2$ ),  $P < 0.05$ .

## Discussion

Our results show that a curvilinear relationship exists between leaf photosynthesis and leaf K concentration in hickory seedlings. This result is similar to the K-related differences in *A* previously reported in cotton (Gerardeaux et al. 2009) and almond trees (Basile et al. 2003). The shape of the curve representing the relationship between the photosynthetic rate and leaf K concentration suggests that photosynthesis is minimally impacted at leaf K concentrations >0.7–0.8% of dry weight. The photosynthesis-based critical leaf K concentration for hickory observed is higher than the values of 0.5–0.6% reported for almond (Basile et al. 2003), and 0.4% in olive trees (Arquero et al. 2006).

In hickory seedlings, as K supply reduced,  $g_s$  decreased while  $C_i$  was generally steady or increased, which suggests that the major influence of K on leaf photosynthesis in hickory seedlings may be attributed to a larger mesophyll resistance and/or a lower capacity of the CO<sub>2</sub>-fixation cycle, rather than to stomatal limitations. Similar conclusions have been previously reported for cotton (Zhao et al. 2001, Gerardeaux et al. 2009)

Table 3. Effects of K supply on rate of mitochondrial respiration in the light ( $R_d$ ), intercellular CO<sub>2</sub> compensation point ( $C_i^*$ ), chloroplastic CO<sub>2</sub> compensation point ( $\Gamma^*$ ), maximum rate of carboxylation ( $V_{c,max}$ ) and maximum rate of electron transport ( $J_{max}$ ) calculated from  $A/C_c$  curves.

Treatment	$R_d$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$C_i^*$ ( $\mu\text{mol mol}^{-1} \text{ CO}_2$ )	$V_{c,max}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$J_{max}$ ( $\mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$ )
K0 (0 mM)	0.87 ± 0.10a	38.5 ± 5.8a	35.8 ± 4.7c	114 ± 11.4c
K1 (0.4 mM)	0.84 ± 0.12a	38.9 ± 6.4a	59.7 ± 5.4b	148 ± 12.1ab
K2 (1.0 mM)	0.82 ± 0.09a	39.0 ± 3.9a	74.5 ± 8.6a	166 ± 20.8a
K3 (2.0 mM)	0.82 ± 0.06a	39.8 ± 6.2a	74.2 ± 7.4a	176 ± 19.1a
K4 (5.0 mM)	0.79 ± 0.11a	40.1 ± 5.6a	76.5 ± 7.3a	169 ± 17.5a

Hickory seedlings were grown for 60 days with five different K concentrations in hydroponic solutions. Data are means and SE of 6–8 replicate plants per treatment. Values carrying different letters are significantly different,  $P < 0.05$ .

Table 4. Deviations in the calculated light-saturated CO<sub>2</sub> assimilation rate ( $A$ ) of hickory seedlings subjected to different potassium (K) supply when the biochemical model of photosynthesis of Farquhar et al. (1980) was re-run, substituting each K-induced photosynthetic parameter for the control 5.0 mM K values.

	$C_i$ ( $\mu\text{mol mol}^{-1} \text{ CO}_2$ )	$g_s$ ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )	$R_d$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$C_c$ ( $\mu\text{mol mol}^{-1} \text{ CO}_2$ )	$g_m$ ( $\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$V_{c,max}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$J_{max}$ ( $\mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$ )
% $\Delta A$ (when 5.0 mM K replaced with 2.0 mM K)							
$C_c$ fixed	–	–	–0.3	+10.7	–	–3.3	+4.3
$C_c$ varies ( $C_i$ fixed)	+5.8	–	–0.2	–	–1.8	–2.4	+3.8
$C_c$ varies ( $C_i$ varies)	–	0	–0.5	–	–1.2	–2.1	+3.4
% $\Delta A$ (when 5.0 mM K replaced with 1.0 mM K)							
$C_c$ fixed	–	–	–0.3	+8.1	–	–2.9	–2.5
$C_c$ varies ( $C_i$ fixed)	+7.0	–	–0.2	–	–5.9	–1.9	–2.3
$C_c$ varies ( $C_i$ varies)	–	–1.9	–0.5	–	–4.2	–1.7	–2.2
% $\Delta A$ (when 5.0 mM K replaced with 0.4 mM K)							
$C_c$ fixed	–	–	–0.6	+7.2	–	–27.5	–15.2
$C_c$ varies ( $C_i$ fixed)	+4.6	–	–0.3	–	–19.2	–20.1	–17.5
$C_c$ varies ( $C_i$ varies)	–	–8.6	–0.6	–	–12.7	–17.4	–22.4
% $\Delta A$ (when 5.0 mM K replaced with 0.0 mM K)							
$C_c$ fixed	–	–	–1.0	+24.3	–	–59.6	–24.8
$C_c$ varies ( $C_i$ fixed)	+13.4	–	–0.5	–	–21.4	–42.4	–29.5
$C_c$ varies ( $C_i$ varies)	–	–17.4	–0.7	–	–14.6	–37.4	–36.1

$C_i$ , intercellular CO<sub>2</sub> concentration;  $g_s$ , stomatal conductance;  $R_d$ , mitochondrial respiration rate in the light;  $C_c$ , chloroplast CO<sub>2</sub> concentration;  $g_m$ , mesophyll conductance;  $V_{c,max}$ , maximum carboxylation rate of Rubisco;  $J_{max}$ , maximum rates of electron transport.

and almond (Basile et al. 2003). However, many studies have shown that K-deficient plants have lower  $g_s$  values compared with K-sufficient plants (Peaslee and Moss 1968, Xi et al. 1989). This discrepancy may be related to the experimental system, environmental factors within the experimental field or interspecific differences. Sale and Campbell (1987) concluded that the effect of K deficiency on plant growth was highly dependent on plant species.

Recent reports have shown that  $g_m$  increases with nitrogen and phosphorus supply in *Pinus radiata*; however, due to a concomitant increase in the rate of photosynthesis, the degree of limitation attributable to mesophyll conductance did not change in the different treatments (Bown et al. 2009). Warren (2004) reported that the relative limitation in photosynthesis due to mesophyll conductance increased with increasing nitrogen supply in glasshouse-grown seedlings of the evergreen perennial *Eucalyptus globulus* Labill. Our work measured the effect of K supply on mesophyll conductance, and the data

show that  $g_m$  responds curvilinearly to leaf K concentration. Under normal K status (K3 and K4 treatments) in hickory seedlings, the mean value of  $g_m$  was 0.17 mol m<sup>–2</sup> s<sup>–1</sup>, similar to the values obtained in other broadleaved plants (Manter and Kerrigan 2004). Under such conditions, the mean draw-down from  $C_i$  to  $C_c$  of 64  $\mu\text{mol mol}^{-1}$  was slightly lower than the mean value of 81  $\mu\text{mol mol}^{-1}$  obtained in evergreen perennial *E. globulus* (Warren 2004). In this study, under K-deficient conditions (K0 and K1 treatments),  $g_m$  decreased with decreasing leaf K concentration, in a similar trend to the photosynthetic rate. However, estimates of  $g_m$  are inherently variable irrespective of the method used (Warren et al. 2003). To increase our confidence in the results obtained, we also used the curve-fitting method to estimate  $g_m$  and found that the trends in  $g_m$  were very similar using this method (data not shown).

The parallel decrease observed in both  $g_m$  and photosynthetic rate is consistent with previous findings in a wide range of species (Singsaas et al. 2003, Warren et al. 2003,

Bown et al. 2009, Han 2011, Whitehead et al. 2011). A general trend for increased mesophyll limitation with increasing nitrogen supply has been reported (Warren 2004, Li et al. 2009). Unlike nitrogen treatment, the decreased  $A$  in K-deficient hickory plant leaves was accompanied by a proportional decrease in  $g_s$  and  $g_m$  (Table 2), and the relative limitation in photosynthesis due to  $g_s$  and  $g_m$  decreased with an increasing supply of K (Figure 3). Furthermore, it would appear that there is an approximate scaling of  $g_s$  and  $g_m$  with  $A$  in hickory seedlings; therefore, it seems likely that the large changes in  $A$  observed in different K treatments were due to altered  $g_s$  and  $g_m$ . However, our results show that  $A$  is most sensitive to  $V_{c,max}$  and  $J_{max}$ , with 34.7–59.6% of the variation in  $A$  attributed to  $V_{c,max}$ . The re-calculated value of  $A$  was rather insensitive to  $g_s$  or  $g_m$  compared with  $V_{c,max}$  in K-deficient hickory seedlings (Table 4).  $V_{c,max}$  and  $J_{max}$  are widely accepted as synonymous with Rubisco activity and RuBP regeneration, respectively. In earlier work, K deficiency depressed Rubisco activity in rice plants (Yang et al. 2004, Weng et al. 2007). Although the decreased  $g_m$  observed in K-deprived plants resulted in a decrease in  $CO_2$  concentration from  $C_i$  to  $C_c$  of  $\sim 75 \mu\text{mol mol}^{-1}$  (Table 2), both  $C_i$  and  $C_c$  were slightly increased with a decreasing supply of K, suggesting that stomatal and mesophyll limitations are low in hickory seedlings. Previously, *in vivo* Rubisco deactivation was observed at  $C_c < 100 \mu\text{mol mol}^{-1}$  in the soybean (Flexas et al. 2006), and recently Galmés et al. (2011) suggested that Rubisco activation started to decline at  $C_c < 120 \mu\text{mol mol}^{-1}$  in Mediterranean species. Therefore, it is unlikely that the high  $C_c$  in K-deficient hickory plants leads to deactivation of Rubisco, and we conclude that the photosynthetic response to K supply is the result of a complex interaction between K and a number of biochemical processes, especially Rubisco activation.

It is not known what leads to K-related changes in  $g_m$ . Aquaporin and carbonic anhydrase are involved in  $CO_2$  diffusion (Makino et al. 1992, Hanba et al. 2004, Terashima et al. 2006, Uehlein et al. 2008). Potassium starvation can reduce aquaporin activity and can induce downregulation of aquaporin expression (Liu et al. 2006, Maurel et al. 2008, Kanai et al. 2011), and carbonic anhydrase activities are enhanced by application of K (Mohammad and Naseem 2006). Therefore, further study is required to determine whether the decrease in  $g_m$  observed in K-deficient hickory plants is related to changes in aquaporin and/or carbonic anhydrase.

Several authors have argued that  $g_m$  is largely constitutive, and is determined by leaf structural traits, including the surface area of chloroplasts exposed to intercellular air spaces ( $S_c$ ) and cell wall thickness (Terashima et al. 2006, 2011). In the present study,  $M_A$  increased from 156 to 207  $\text{g m}^{-2}$  with a decrease in the supply of K from 5.0 to 0 mM. This alteration in  $M_A$  confirms the findings of previous research documenting increased  $M_A$  in K-deficient cotton leaves (Pettigrew 1999).

Variations in  $M_A$  are often inversely correlated with  $g_m$  (Niinemets et al. 2005, Hassiotou et al. 2009, Niinemets et al. 2009b), and we observed that this relationship was mediated via variation in K supply in hickory seedlings. Potassium deficiency leads to an increase in non-structural carbohydrates (data not shown, Pettigrew 1999), which may lead to an increase in  $M_A$  (Pettigrew 1999, Poorter et al. 2009). Increased  $M_A$  may mainly reflect an increase in density due to increased cell wall thickness in nutrient-deficient plants (Niinemets et al. 2005, 2009b, Poorter et al. 2009), especially in K-deficient plants (Pettigrew 1999), and one would expect  $S_c$  to increase with increasing  $M_A$ .  $S_c$  has been shown to positively correlate with  $g_m$  (Evans et al. 1994, Terashima et al. 2006). However, Zhao et al. (2001) observed that the K-deficient leaf may have fewer chloroplasts in the mesophyll cells, compared with control plants. O'Toole et al. (1980) reported that leaf K status significantly reduced the cell size and compaction of cells per unit area, resulting in degradation of chloroplasts. In this study, the observed decrease in total chlorophyll content implies that chloroplast degradation may occur. It is known that  $S_c$  is proportional to the number of chloroplasts; therefore, it is possible that the leaves of K-deficient hickory plants have a lower  $S_c$  than K-sufficient leaves. Additionally, increased leaf density may result in an increase in intercellular transfer resistance to  $CO_2$  (Niinemets 1999). In fact, leaves with a higher  $M_A$  have a greater fraction of cell wall components, which leads to less efficient  $CO_2$  diffusion from  $C_i$  to  $C_c$  (Niinemets et al. 2006, Poorter et al. 2009, Hassiotou et al. 2010). Recently, Han (2011) suggested that increased  $CO_2$  diffusive resistance may be a consequence of morphological adaptation when  $g_m$  is closely correlated with  $M_A$  and  $g_s$ . These results suggest that decreased mesophyll conductance may be an inevitable consequence of morphological adaptation to K deficiency in hickory seedlings.

In summary, the photosynthetic capacity of hickory plants was not limited when the leaves had K concentrations  $>0.7$ – $0.8\%$  of dry weight. Mesophyll and stomatal conductance were decreased in K-deficient seedlings; however, the lower photosynthetic rate observed in K-deficient seedlings, compared with K-sufficient seedlings, is more attributable to increased photosynthetic biochemical limitations ( $V_{c,max}$  and  $J_{max}$ ) than changes in  $g_m$  and  $g_s$ .

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