RESEARCH PAPER

Loss of photosynthetic efficiency in the shade. An Achilles heel for the dense modern stands of our most productive C₄ crops?

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Received 1 October 2016; Editorial decision 11 November 2016; Accepted 11 November 2016

Editor: Susanne von Caemmerer, Australian National University

Abstract

The wild progenitors of major C₄ crops grew as individuals subjected to little shading. Today they are grown in dense stands where most leaves are shaded. Do they maintain photosynthetic efficiency in these low light conditions produced by modern cultivation? The apparent maximum quantum yield of CO₂ assimilation (ΦCO₂max), a key determinant of light-limited photosynthesis, has not been systematically studied in field stands of C₄ crops. ΦCO₂max was derived from the initial slope of the response of leaf CO₂ uptake (A) to photon flux (Q). Leaf fractional light absorbance (α) was measured to determine the absolute maximum quantum yield of CO₂ assimilation on an absorbed light basis (ΦCO₂max,abs). Light response curves were determined on sun and shade leaves of 49 field plants of Miscanthus × giganteus and Zea mays following canopy closure. ΦCO₂max and ΦCO₂max,abs declined significantly by 15–27% (P<0.05) with canopy depth. Experimentally, leaf age was shown unlikely to cause this loss. Modeling canopy CO₂ assimilation over diurnal courses suggested that the observed decline in ΦCO₂max,app with canopy depth costs 10% of potential carbon gain. Overcoming this limitation could substantially increase the productivity of major C₄ crops.

Key words: C₄ photosynthesis, canopy photosynthesis, corn, crop photosynthesis, crop yield, food security, maize, miscanthus, quantum yield, shade acclimation, planting density.

Introduction

In modern intensive systems, crops form dense canopies where both sun and shade leaves contribute to photosynthetic carbon assimilation and productivity. Shaded leaves are estimated to contribute about 50% of total canopy carbon gain and therefore the efficiency with which shade leaves use light is a critical factor determining crop yield potential.

Abbreviations: α, leaf fractional light absorbance (0 to 1, dimensionless); ΦCO₂max, maximum quantum yield of CO₂ assimilation on an absorbed light basis, i.e. maximum of ΔA/ΔQabs as defined by the initial slope of the response of A to Qabs (mol mol⁻¹); ΦCO₂max,app, maximum quantum yield of CO₂ assimilation on an incident light basis, i.e. maximum of ΔA/ΔQ as defined by the initial slope of the response of A to Q (mol mol⁻¹); ΦCO₂max,app,PSII, maximum quantum yield of CO₂ assimilation corrected for PSII quantum efficiency on an incident light basis (mol mol⁻¹); ΦCO₂max,app,PSII,operating, operating quantum yield of PSII photochemistry (mol mol⁻¹); A, net leaf CO₂ uptake (µmol m⁻² s⁻¹); Aₙ, net canopy CO₂ uptake per unit ground area (µmol m⁻² s⁻¹ or mol m⁻² day⁻¹); Aₚ, light-saturated A (µmol m⁻² s⁻¹); gₛ, stomatal conductance (µmol m⁻² s⁻¹); cₛ, intercellular CO₂ concentration (µmol mol⁻¹); LAI, leaf area index (m² m⁻²); Q, incident photosynthetic photon flux density (µmol m⁻² s⁻¹); Qabs, absorbed photosynthetic photon flux density, i.e. α·Q (µmol m⁻² s⁻¹); Rₑ, leaf dark respiration (µmol m⁻² s⁻¹); Vₚ, rate of PEP regeneration, CO₂ saturated rate of photosynthesis (µmol m⁻² s⁻¹); Vₚₕₚₘ, maximum rate of PEP carboxylation (µmol m⁻² s⁻¹).
(Baker et al., 1988; Long, 1993; Hikosaka et al., 2016). This proportion will increase as planting densities increase. In high light, the rate of leaf CO₂ uptake (A) is limited by capacity for carboxylation and regeneration of the acceptor molecule for CO₂. In low light, however, A is primarily dependent on the ability of the leaf to capture light and convert it with maximum efficiency towards carbon assimilation.

Photosynthetic efficiency under limiting light is defined by the apparent maximum quantum yield of CO₂ assimilation (Φ_{CO₂,max,app}), measured as the initial slope of the response of A to incident photon flux (Q). Φ_{CO₂,max,app} is the product of leaf fractional light absorbance (α) and the intrinsic maximum efficiency with which the leaf can transduce absorbed photons into net CO₂ assimilation, i.e. the absolute maximum quantum yield of CO₂ assimilation (Φ_{CO₂,max,abs}) (Long et al., 1993). Φ_{CO₂,max,app} is the key determinant of efficiency of leaf photosynthesis under light-limiting conditions (Baker et al., 1988; Long, 1993; Long and Hallgren, 1993; Long et al., 1993; Singsaas et al., 2001; Long et al., 2006).

In the classical studies of shade adaptation in C₃ plants, it was found that Φ_{CO₂,max,app} was maintained or increased in shaded adapted leaves, maximizing the use of light in the shade. At the same time capacity for light-saturated photosynthesis (Aₛ) declined, reflecting in particular a decrease in Rubisco content ( Björkman, 1981; Givnish, 1988). As canopies develop, this appears to be a component of a broad acclimation strategy in which various leaf traits are adjusted to optimize resource use with increasing shade (Niinemets et al., 2015).

Φ_{CO₂,max,app} was constant in all green leaves irrespective of leaf position and canopy depth in two independent studies of photosynthesis in field stands of modern cultivars of wheat (Triticum aestivum L.) (Beyschlag et al., 1990; Hoyaux et al., 2008). Similarly, Φ_{CO₂,max,app} did not vary with depth into the canopy in wild oats (Avena fatua L.) growing in a wheat crop (Beyschlag et al., 1990), and did not vary in grapevine (Vitis vinifera L.) leaves throughout the canopy (Cartechini and Palliotti, 1995). Therefore, it appears that Φ_{CO₂,max,app} is maintained as expected in the lower canopy of these field-grown C₃ crops. However, a study of perennial forage grasses showed much greater reductions in photosynthesis and productivity in C₄ species relative to their C₃ counterparts upon shading in the field, suggesting a possible difference between the two photosynthetic types in their ability to acclimate to shaded field conditions ( Kephart et al., 1992). In today’s intensive cultivation, C₄ crops are grown at high population densities leading to leaf area indices (LAI), i.e. layers of leaves per unit ground area, of up to 6 (Dohleman and Long, 2009; Tian et al., 2011; Srinivasan et al., 2016). Continued development of germplasm capable of planting at still higher densities will likely lead to even higher LAI and more shaded layers (Li et al., 2015). It is therefore critical to know whether key C₄ crops are capable of maintaining Φ_{CO₂,max,app} as leaves become progressively shaded in the field with canopy development, as in the classical studies of shade acclimation in C₃ species.

While studies of Φ_{CO₂,max,app} and Φ_{CO₂,max,abs} span a wide variety of species and environments ( Björkman and Demmig, 1987; Long et al., 1993), none have focused on field stands of C₄ crops grown under the high density populations of modern cultivation. In a natural environment, the C₄ understory shrub Euphorbia forbesii Sherff. maintained a high Φ_{CO₂,max,app} in a forest understory (Pearcy and Calkin, 1983). Here, however, the leaves develop in the shade while in canopies of maize (Z. mays L.) and other C₄ crop stands leaves develop in full sunlight and are then shaded by younger leaves. In general, less is known about how light-limited photosynthesis-acclimates in crop canopies in the field, even though other aspects of shade acclimation such as specific leaf area, light-saturated photosynthetic capacity and nitrogen content have been examined extensively in forests and some crop stands (Anten et al., 1995, 1998; Brooks et al., 1996; Drouet and Bonhomme, 1999; Niinemets et al., 2015; Niinemets, 2016a,b).

In prior studies of Φ_{CO₂,max,app} and Φ_{CO₂,max,abs} in C₄ plants, ‘shade’ treatments have typically been obtained by growing plants at low light levels or shading them with neutral density shade cloth (Ludlow and Wilson, 1971; Ehleringer and Pearcy, 1983; Pearcy and Franceschi, 1986; Tazoe et al., 2008). This likely oversimplifies the shade conditions present in field canopies, where reduced light quantity is accompanied by changes in light quality, wind, humidity and temperature (McCree, 1972; Burkey and Wells, 1991; Niinemets and Villadares, 2004; Gutschick, 2016). Most notably, shade cloth fails to mimic the declines in blue and in red to far red ratio, both of which are now known to be critical to several developmental processes (Chen et al., 2004).

With the perceived need to increase crop production, given forecasts of future demand (Long et al., 2015b), it becomes increasingly important to understand leaf photosynthetic shade response of major C₄ crops and in turn whether this could affect canopy photosynthesis and productivity (Miguez et al., 2009; Zhu et al., 2010; Yin and Struik, 2012, 2015). Maize (Z. mays L.) is the largest single primary foodstuff produced globally, with one-third of that production in the US corn belt (FAOSTAT, 2016; USDA-NAASS, 2016). Miscanthus × giganteus (Green et Deu.) is one of the most productive second generation bioenergy crops (Clifton-Brown et al., 2004; Arundale et al., 2014a; Heaton et al., 2008, 2010). These important crops were chosen for this study to represent established or emerging agricultural systems, examined near the center of their US areas of production, where some of the highest yields of both crops have been reported (Dohleman and Long, 2009; Long et al., 2015a). They are members of the grass tribe Andropogoneae and closely related to the two other major C₄ crops based on global production: sorghum (Sorghum bicolor (L.) Moench) and sugarcane (Saccharum officinarum L.). All members of this tribe belong to the same clade of C₄ evolution and are classified as ‘NADP-ME type’ (Sage et al., 2011).

The hypothesis that Φ_{CO₂,max,app} and Φ_{CO₂,max,abs} are maintained or increased in lower canopy leaves of these crops, as anticipated from the shade response observed in C₃ species, was tested. Leaf gas-exchange measurements combined with measurements of absorbance were used to determine Φ_{CO₂,max,app}, α and Φ_{CO₂,max,abs} in upper and lower canopy leaves of field stands of...
M. × giganteus and Z. mays. Measured values of photosynthetic parameters were then integrated into a crop canopy model to determine the effect of shade acclimation on total crop carbon assimilation.

Materials and methods

Plant material

Plants were sourced from mature replicated stands of M. × giganteus and Z. mays on the farm of the University of Illinois Agricultural Research Station near Champaign, IL, USA (40°02′N, 88°14′W, 228 m above sea level) in two consecutive growing seasons. Soils at this site are deep Drummer/Flanagan series (a fine silty, mixed, mesic Typic Endoaquoll) with high organic matter typical of the central Illinois region of the Corn Belt. Established, unfertilized field plots of the ‘Illinois’ clone of M. × giganteus were used, as described previously (Dohleman and Long, 2009; Dohleman et al., 2012; Arundale et al., 2014a,b). On adjacent plots, a high-yielding modern Z. mays hybrid, cv. Dekalb DK61-69, was planted, once soil temperature exceeded 10 °C. Both crops were rainfed and the Z. mays received standard fertilization of 180 kg [N] ha⁻¹, prior to planting, in line with regional production practice. Once the canopy of each crop had closed (ca. LAI>3) measurements began and were spread across the growing season, ceasing with the beginning of senescence of the Z. mays crop. Achieved stand density, also in line with current agronomic practice, was approximately 8 plants m⁻² for Z. mays (Dohleman and Long, 2009). The original stands of M. × giganteus were planted at 1 plant m⁻², but tillering resulted in a stem density of approximately 100 tillers m⁻² in subsequent years (Heaton et al., 2008). This led to an LAI during this period of ~4 in plots for Z. mays and 4–6 for M. × giganteus (Dohleman et al., 2009). To allow transfer to the laboratory for photosynthetic analysis, stems of each plant were cut at the base before dawn, the cut ends immersed in water and immediately recut to avoid any air blockage in the xylem. This avoided possible effects of photoprotection or transient water stress that might develop over the course of the day. Prior use of this technique has shown that detached shoots of both crops maintain photosynthetic rates at least equal to that of field plants for 24 hours after cutting (Leakey et al., 2006; Dohleman et al., 2009).

To isolate the effect of age on M. × giganteus leaves, in a separate experiment, six plants were grown in a soil-free medium (LC1, Sungro Horticulture, Agawam, MA, USA) in 23-liter pots in a controlled environment greenhouse, maintained at 25–30 °C. Pots were kept well-watered and fertilized once per week with a 20:20:20 N:P:K commercial fertilizer (Peter’s Professional; The Scotts Co., Marysville, OH, USA), applied at the manufacturer’s recommended rate. High pressure sodium lamps ensured a minimum Q of 300 μmol m⁻² s⁻¹ and a 14 h day length. Leaves were tagged on emergence of the ligule, and as other leaves formed above, these were artificially held to the side to avoid any shading of the tagged leaves over the next 60 d.

Canopy light profile

The fraction of Q intercepted by the canopy was measured from late June to mid-August by simultaneously measuring Q above the mature crop canopy with a point quantum sensor (Model LI-190; LI-COR, Inc., Lincoln, NE, USA) and with a line quantum sensor (Ceptometer, Model PAR-80, Decagon Devices, Inc., Pullman, WA, USA) within the canopy. The line sensor was lowered from the top of the canopy to the base in 10 cm steps, and the proportion of incident Q remaining was calculated. These measurements were made between 10:00 h and 14:00 h on clear sky days when incident Q was ≥1400 μmol m⁻² s⁻¹.

Photosynthesis measurements

On a single tiller of each plant of M. × giganteus or the sole stem of each Z. mays plant, the lowest fully green leaf and the highest fully developed leaf, as indicated by ligule emergence, were selected for measurement. Leaf CO₂ and water vapor exchange were measured in cuvettes with controlled temperature, humidity and photon flux within a portable open gas-exchange system incorporating infra-red CO₂ and water vapor analysers, and a modulated chlorophyll fluorometer (LI 6400 and LI 6400–40; LI-COR, Inc.). Leaves of both species were placed in the cuvette with incident Q set to 2000 μmol m⁻² s⁻¹, block temperature to 30 °C, [CO₂] to 400 μmol mol⁻¹ and leaf-to-air water vapor pressure deficit to 1.3 kPa. Light was provided by the integrated red (635 nm wavelength) and blue (465 nm wavelength) light-emitting diodes (LED) such that 10% of the light was blue, and the remainder red.

Leaves were allowed to acclimate (60–90 min) until A reached a steady state, then light response curves were determined by decreasing Q to progressively lower levels (2000, 1500, 1000, 500, 200, 180, 160, 140, 120, 100, 80, 60, 40, 20, and 0 μmol m⁻² s⁻¹). Leaves were allowed to acclimate to each step reduction in Q, as assessed by a resumption of a steady-state A, typically requiring 5–10 min. As a check for any hysteresis in the response of A to Q, similar measurements were made on three separate plants in reverse starting from zero and progressively increasing to Q=2000 μmol m⁻² s⁻¹, with acclimation of 15–30 min between changes in photon flux.

Upon acclimation to each photon flux, gas-exchange data were recorded and A, g, and intercellular CO₂ concentration (ci) calculated (von Caemmerer and Farquhar, 1981). On a subset of these, modulated fluorescence measurements were made, as in Yin et al. (2014), to derive operating quantum yield of PSII photochemistry (ΦPSII) using a multiphasic flash protocol (Loriaux et al., 2013). Light response curves were described by a four-parameter non-rectangular hyperbola and fit by a maximum-likelihood routine (Long and Hållgren, 1993). The four parameters are the initial slope of the response, the y-axis intercept, which represents dark respiration (Rd), the upper asymptote (Amax), and a convexity factor (θ) describing the rate of transition between the initial slope and asymptote with respect to Q.

After each light curve was completed, leaf fractional light absorbance of photosynthetically active photon flux (a) was calculated and weighted for 90% red (635 nm wavelength) and 10% blue (465 nm wavelength) to match cuvette illumination. Measurements were made as in Singsaas et al. (2001) by placing the leaf on the entry and then exit ports of a Taylor integrating sphere with attached illuminator and measuring optics (LI-1800-12; LI-COR). The signal was processed through a fiber optic grating spectrometer (USB2000; Ocean Optics, Dunedin, FL, USA) and analysed with the spectrometer operating software (Spectrasuite; Ocean Optics). Absorbed photosynthetic photon flux density (Qabs) for the leaf in the cuvette was then calculated as a Q, assuming that absorbance by non-photosynthetic pigments was negligible, as indicated by the observed spectra (Hikosaka et al., 2016).

Although an estimate of ΦCO₂,max,app is given by fitting the hyperbola to the response of A to Q, this estimate can be affected by values of A above the initial slope of the response curve (Long et al., 1993; Yin et al., 2014). A more accurate estimate of ΦCO₂,max,app was obtained from linear regression of A against Q for six light levels, between Q=40 and 140 μmol m⁻² s⁻¹. ΦCO₂,max,abs was obtained from linear regression of A against Qabs for these same light levels.

It has been suggested that ΦCO₂,max,app can be underestimated due to decline in ΦPSII with increasing Q, even at very low light. An alternative method for calculation of ΦCO₂,max,app to correct for this has been proposed (Yin et al., 2014). While this calculates the theoretical maximum quantum yield for CO₂ assimilation, the observed linear response we have reported as ΦCO₂,max,app is the actual achieved maximum and is the value that contributes directly to canopy carbon assimilation. The response of A to Q may deviate from linearity at very low light due to increased respiration, i.e. the Kok effect, and at high light when A is no longer strictly light-limited. Performing a linear regression with data points deviating from linearity would produce erroneous estimates of ΦCO₂,max,app and ΦCO₂,max,abs (Yin et al., 2014). To avoid this, we ensured that the relationship of A to Q was...
linear over the light range used (Q=40–140 μmol m⁻² s⁻¹) by examining the distribution of residuals and testing their normality for each of the regressions. Details of the statistical analysis of slopes is given below. For comparison of results from this method and that of Yin et al. (2014), maximum quantum yield of CO₂ assimilation corrected for PSII quantum efficiency on an incident light basis (Φ_CO₂,max,app,PSII) was calculated as in Yin et al. (2014) in 13 plants of Z. mays and 15 plants of M. × giganteus for which fluorescence data was recorded, as described above.

To distinguish the effect of leaf age from leaf light history on lower canopy photosynthetic efficiency an additional greenhouse experiment was undertaken with M. × giganteus, as described above. The above gas exchange measurements were repeated on the uppermost leaf in which the ligule had just emerged on six plants, and repeated on the same leaf 30 and 60 days later and after several leaves had formed above on the same stem.

Statistical analysis
Data were analysed using PROC MIXED (SAS Institute Inc., Cary, NC, USA), and graphical displays made with SigmaPlot 11.0 software (Systat Software Inc., San Jose, CA, USA). A randomized complete block mixed model ANOVA was performed on field data to analyse the fixed effect of canopy position (C), species (S) as well as their fixed interaction (C*S), while blocking by the random main effect of year (T). Here, ε is represents a random error term for the model. This analysis was performed on all photosynthetic parameters of interest, with Yijk corresponding to A_sat, Φ_CO₂,max,abs ⋅ Φ_CO₂,max,app ⋅ R_s, or ε.

PROC UNIVARIATE (SAS Institute Inc.) was used to verify normality of the ANOVA residuals using the Shapiro–Wilk test, with a 1% threshold probability of committing a type 1 error. Because measurements from the lower canopy were inherently more variable than from the upper, and variances differed between species, homogeneity of variance could not be assumed. Therefore, the repeated measures option of PROC MIXED was used to allow variance to differ between canopy levels and between species. When analysing Φ_CO₂,max,app and Φ_CO₂,max,abs least squares were weighted by the inverse of the variance of each slope calculation; this was to incorporate variability of each regression into the overall statistical model. An upper and a lower canopy leaf were measured on each of 49 plants, leading to 40 and 53 layers containing equal fractions of LAI. For each layer, sunlit-shaded leaf areas and direct and diffuse light fluxes were calculated hourly throughout the day. Light within each canopy layer was used to calculate the rate of photosynthesis of both sunlit and shaded leaves with a coupled steady-state biochemical and stomatal model (Collatz et al., 1992). Rates were then integrated through the canopy to compute hourly rates of CO₂ assimilation per square meter of ground area, as described previously (Miguez et al., 2009). Solar radiation, temperature, relative humidity, and wind speed were compiled from the nearest Surface Radiation Network (SURFRAD) site (40.05N, −88.37W) for 2012.

In the steady-state biochemical model of C₃ photosynthesis used in BioCro, A_sat is determined by capacity for phosphoenol pyruvate (PEP) carboxylation (V_max) at low c, and by capacity for PEP regeneration (V_pp) at moderate c. Since previous studies of both crops have shown A_sat to be determined entirely by V_max under field conditions, except during severe drought (Dohleman et al., 2009; Leakey et al., 2004, 2006), V_max was assumed equivalent to A_sat+R_s. The exponential decline of photosynthesis parameters (V_max, R_s, and Φ_CO₂,max,app) was simulated after setting values at the top and bottom of the canopy to those measured in this field study, using an extinction coefficient per LAI layer (K=0.1) to vary the parameters between the two measured points. Selection of K was based on the observed decline in leaf N, as a proxy of photosynthetic capacity, measured previously in this M. × giganteus crop (Wang et al., 2012).

Results
Light level declined exponentially with depth into the canopy, most markedly in M. × giganteus (Fig. 1). The lowest fully green leaf was approximately 1.3 m below the canopy surface in the stands of M. × giganteus and 2 m in Z. mays. At those canopy levels, the measured photosynthetic photon flux density (Q) was 5–10% of that at the canopy surface (Fig.1). This corresponds to an overlying leaf area of between 4.4 and 5.8 m⁻².

Leaf fractional light absorptance (α) was significantly and 3% greater in lower compared with upper canopy leaves of M. × giganteus, but not different between canopy levels in Z. mays (Tables 1 and 2). By comparison with upper canopy leaves, values for Φ_CO₂,max,abs and Φ_CO₂,max,app in the lower canopy were significantly decreased by 27–29% in M. × giganteus and by 14–15% in Z. mays (Tables 1 and 2, and Fig. 2B). This reduction was also apparent when the A–Q response was determined by increasing, rather than decreasing, Q, and when determined with adjustment for decline in PSII quantum efficiency (P<0.05) (Tables 1 and 2, and Supplementary Fig. S1 at JXB online). Residuals of each regression used to calculate Φ_CO₂,max,app and Φ_CO₂,max,abs were normally distributed, and therefore they were randomly distributed around Q and Q_abs, respectively (Supplementary Figs S2–S5). This indicates A is linearly related to Q from Q=40 to 140 μmol m⁻²

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s⁻¹ and that the quantum yields measured did represent the true maxima achieved by the measured leaves.

At higher light levels (Q=500 to 2000), the lower canopy of both Z. mays and M. × giganteus had lower photosynthetic rates than the upper canopy (Fig. 2A). This is confirmed by the significant main effect of canopy position on \( A_{\text{sat}} \) (Tables 1 and 2). Relative to the upper canopy leaves, lower canopy values for \( A_{\text{sat}} \) declined by 42% for \( M. \times \) giganteus and by 35% for \( Z. \) mays (Table 2). Lower canopy dark respiration (\( R_d \)) declined by 29% and 69% relative to the upper canopy in \( M. \times \) giganteus and \( Z. \) mays, respectively (Tables 1 and 2). \( M. \times \) giganteus leaves artificially maintained in unshaded conditions in the separate greenhouse experiment showed no significant decline in \( \Phi_{\text{CO}_2,\text{max,abs}} \) (\( F=1.43; P>0.1 \)) or \( \Phi_{\text{CO}_2,\text{max,app}} \) (\( F=0.02; P>0.1 \)) over 60 days (Fig. 3).

Fig. 1. Photon flux (Q), as a proportion of that at the upper surface of the canopy, plotted against depth into the canopies of the field stands of Miscanthus × giganteus Greef et Deu. and Zea mays L. Measurements were made between 10.00 h and 14.00 h on clear sky days from July to August. On the x-axis, 0 indicates the upper surface of the canopy. Each point is the mean ±1 SE of eight independent measurements taken at a given depth from the canopy surface. Arrows indicate approximate canopy depths where lower canopy leaves were selected from both species: 1.3 m for \( M. \times \) giganteus and 2 m for \( Z. \) mays; these corresponded to an overlying LAI of 5.8 and 4.4, respectively. Leaves referred to as upper canopy (full sunlight) were those at the surface (canopy depth=0) and those referred to as lower canopy are indicated by arrows, where photon flux was reduced by about 90%.

Table 1. The significance of differences in light-saturated net leaf CO₂ uptake (\( A_{\text{sat}} \)), maximum quantum yield of CO₂ assimilation on an absorbed light basis (\( \Phi_{\text{CO}_2,\text{max,abs}} \)), maximum quantum yield of CO₂ assimilation on an incident light basis (\( \Phi_{\text{CO}_2,\text{max,app}} \)), maximum quantum yield of CO₂ assimilation corrected for PSII quantum efficiency on an incident light basis (\( \Phi_{\text{CO}_2,\text{max,app,PSII}} \)), leaf dark respiration (\( R_d \)), and leaf fractional light absorptance (\( \alpha \)) between upper and lower canopy leaves of Miscanthus × giganteus Greef et Deu. and Zea mays L.

| Effect                        | \( A_{\text{sat}} \) | \( \Phi_{\text{CO}_2,\text{max,abs}} \) | \( \Phi_{\text{CO}_2,\text{max,app}} \) | \( \Phi_{\text{CO}_2,\text{max,app,PSII}} \) | \( R_d \) | \( \alpha \) |
|-------------------------------|----------------------|------------------------------------------|------------------------------------------|----------------------------------------------|-----------|-----------|
| Canopy position main effect    | 74***                | 9.1*                                     | 7.15*                                    | 16.94***                                     | 67***     | 5.0*      |
| Canopy position × species      | 1.3                  | 9.63*                                    | 8.73*                                    | 2.28                                         | 2.8#      | 8.8*      |

Losses in total crop carbon assimilation due to the measured declines in \( A_{\text{sat}} \) and \( \Phi_{\text{CO}_2,\text{max,app}} \) with canopy depth were simulated in the BioCron mechanistic model of crop canopy photosynthesis. Scenario 1 represented the hypothetical condition of no decline in these parameters. The effect of the actual decline (scenario 1 vs. 4; Fig. 4A) was evident throughout the day and across the whole month (Fig. 4A, B). Integrated across the month the combined decline in \( A_{\text{sat}} \) and \( \Phi_{\text{CO}_2,\text{max,app}} \) cost 15% of potential carbon gain relative to the hypothetical situation of no decline in either parameter (scenario 1 vs. 4; Table 3). Maintaining \( A_{\text{sat}} \) as constant into the canopy, but allowing \( \Phi_{\text{CO}_2,\text{max,app}} \) alone to decline as observed, resulted in a 4% increase in canopy carbon gain (scenario 2 vs. 4). Maintaining \( \Phi_{\text{CO}_2,\text{max,app}} \) at the upper canopy value into the lower canopy, but allowing \( A_{\text{sat}} \) to decline as observed, resulted in a 10% increase in canopy carbon gain (scenario 3 vs. 4; Table 3).

Discussion

In contrast to findings of the classical studies of shade acclimation, the maximum quantum yield of leaves showed a significant decline under the shade conditions of the lower canopy of these two C₄ crops. The observation that the absolute and apparent maximum quantum yield of CO₂ assimilation (\( \Phi_{\text{CO}_2,\text{max,abs}} \) and \( \Phi_{\text{CO}_2,\text{max,app}} \)) both decline in field stands of these highly productive C₄ crops appears new and surprising. Even when quantum yield was adjusted for decline of PSII quantum efficiency at low light (Yin et al., 2014), \( \Phi_{\text{CO}_2,\text{max,app,PSII}} \) was significantly reduced in the lower canopy (Tables 1 and 2, and Fig. 2B). However, for the purposes of this study the decline in \( \Phi_{\text{CO}_2,\text{max,app}} \) is the important measure, since based solely on CO₂ assimilation it provides an unequivocal measure of the actual efficiency with which carbon is assimilated in low light. This suggests large losses of potential crop carbon uptake could be avoided if \( \Phi_{\text{CO}_2,\text{max,app}} \) was maintained with canopy depth (Table 3 and Fig. 4).

Compared with the sun leaves of the upper canopy, lower canopy leaves showed several traits typical of shade acclimation: reduced \( A_{\text{sat}} \), reduced dark respiration (\( R_d \)), and in the case of \( M. \times \) giganteus a significant increase in absorptance (\( \alpha \)) (Table 2). While these changes fit with expectations of shade
acclimation (Boardman, 1977; Givnish, 1988; Björkman, 1981), loss of 14–29% of efficiency of photosynthesis in low light ($\Phi_{\text{CO2max,abs}}$) does not. A loss of low-light photosynthetic efficiency in shaded leaves was not seen in field crops in two separate studies of wheat (Hoyaux et al., 2008; Beyschlag et al., 1990). This parallels studies of non-crop plants. Young upper sun and old lower shade leaves of the semi-arid arborescent C3 monocot Beaucarnea stricta Lem. and the C3 wet forest understory fern Davallia bullata Wail. ex Hook. showed identical values of $\Phi_{\text{CO2max,abs}}$, suggesting no loss of efficiency of photosynthesis in shade, while $A_{\text{sat}}$ was decreased by >70% in both species (Long et al., 1993). Similarly, no decline in $\Phi_{\text{CO2max,abs}}$ was seen between sun and shade leaves of a mangrove forest (Suwa and Akio, 2008) or in wild oat growing in a wheat canopy (Beyschlag et al., 1990).

Lack of shade acclimation has been observed in C4 plants: in the NADP-ME monocot Z. mays and NAD-ME dicot Amaranthus retroflexus L. cultivated in controlled environment cabinets, growth in high vs. low light had no effect on $\Phi_{\text{CO2max,abs}}$. The same was seen in the NADP-ME dicot Euphorbia forbesii Sheriff. and the mixed NAD-ME and NADP-ME dicot Gomphrena globosa L. when grown in a greenhouse either in full sunlight or under a 90% shade cloth (Ehleringer and Pearcy, 1983). $\Phi_{\text{CO2max,app}}$ was unchanged in the PCK monocot Panicum maximum Jacq. grown in a greenhouse either in full sunlight or under layers of shade cloth (Ludlow and Wilson, 1971). The NAD-ME dicot Amaranthus cruentus L. not only maintained $\Phi_{\text{CO2max,app}}$ when grown under shade cloth, but also showed evidence of positive acclimation in terms of decreased bundle sheath leakiness (Tazoe et al., 1981).

Results are from the canopy positions indicated in Fig. 1. Statistically significant difference (Student’s t test) between upper and lower canopy for each species at $P<0.1$ is indicated by #; at $P<0.05$ by *, at $P<0.001$ by **, and at $P<0.0001$ by ***: in the case of a significant difference the higher of the pair is written in bold.

### Table 2. Mean values and standard error of light-saturated net leaf CO$_2$ uptake ($A_{\text{sat}}$), maximum quantum yield of CO$_2$ assimilation on an absorbed light basis ($\Phi_{\text{CO2max,abs}}$), maximum quantum yield of CO$_2$ assimilation on an incident light basis ($\Phi_{\text{CO2max,app}}$), maximum quantum yield of CO$_2$ assimilation corrected for PSII quantum efficiency on an incident light basis ($\Phi_{\text{CO2max,app,PSII}}$), leaf dark respiration ($R_d$), and leaf fractional light absorptance ($\alpha$) for upper and lower canopy leaves of Miscanthus × giganteus Greef et Deu. and Zea mays L.

| Species          | $A_{\text{sat}}$ $(n=24–27)$ | $\Phi_{\text{CO2max,abs}}$ $(n=24–27)$ | $\Phi_{\text{CO2max,app}}$ $(n=24–27)$ | $\Phi_{\text{CO2max,app,PSII}}$ $(n=15)$ | $R_d$ $(n=24–27)$ | $\alpha$ $(n=24–27)$ |
|------------------|-------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|--------------------|---------------------|
| Upper canopy     |                               |                                         |                                         |                                         |                    |                     |
| Miscanthus × giganteus | 27.6*** 5.29                | 0.058*** 0.0078                       | 0.049*** 0.0066                       | 0.058** 0.0085                       | 1.27*** 0.39       | 0.851 0.021         |
| Lower canopy     |                               |                                         |                                         |                                         |                    |                     |
| Miscanthus × giganteus | 16.1 2.97                 | 0.041 0.0097                          | 0.039 0.0084                          | 0.044 0.0097                          | 0.43 0.42          | 0.873* 0.028        |
| Z. mays          |                               |                                         |                                         |                                         |                    |                     |
| Upper canopy     |                               |                                         |                                         |                                         |                    |                     |
| Miscanthus × giganteus | 42.3*** 10.8               | 0.053# 0.010                         | 0.048* 0.009                         | 0.054# 0.0049                       | 1.76*** 0.36       | 0.906 0.016         |
| Lower canopy     |                               |                                         |                                         |                                         |                    |                     |

![Fig. 2](https://academic.oup.com/jxb/article-abstract/68/2/335/2932218)

**Fig. 2.** (A) Response of net CO$_2$ assimilation ($A$) to incident photon flux ($Q$). (B) Strictly light limiting phase of the response of $A$ to leaf absorbed photon flux ($Q_{\text{abs}}$), corresponding to $Q=40–140$ μmol m$^{-2}$ s$^{-1}$. Results correspond to the upper and lower canopy of Miscanthus × giganteus Greef et Deu. and Zea mays L., at the positions indicated by Fig. 1. Open symbols represent the measured mean ±1 SE at a given photon flux for upper canopy leaves and closed symbols lower canopy leaves. Replicate numbers of plants are as given in Table 2. Lines are the best-fit regressions to the original data points. Dashed lines represent upper canopy leaves, and solid lines lower canopy leaves.
et al., 2008). Clearly there is a well-documented ability for a wide diversity of C₄ species to maintain maximum quantum yields when growing under artificial neutral density shade. This is seen in all three major C₄ subtypes (NADP-ME, NAD-ME, PCK) and an intermediate (NAD-ME/NADP-ME), and in both monocots and dicots, suggesting there is no inherent limitation of C₄ photosynthesis at low light.

In contrast, the leaves that became progressively shaded as other leaves formed above them in situ in the current study suffered a decrease in \( \Phi_{\text{CO}_2\text{max,abs}} \) and \( \Phi_{\text{CO}_2\text{max,app}} \). This has not been reported previously, but given the large numbers of leaves examined here, almost 100, it is clearly a statistically proven feature of these production stands of Z. mays and M. × giganteus. As noted above, maximum quantum yields of C₄ species, including Z. mays, do not decline when \( Q \) is reduced with shade cloth. This suggests that some other feature of the lower canopy causes the loss observed under the shade of other leaves in a field setting.

Because of the development pattern of these crops, shade leaves were several weeks older than those in which the ligule had just emerged at the top of the canopy. Could age be a determining factor? In our greenhouse study of M. × giganteus in which shading of leaves was prevented as new leaves were formed above them, there was no loss of \( \Phi_{\text{CO}_2\text{max,abs}} \), even at 60 days. This indicates that the loss is not due to age or leaf position on the stem, but rather a direct response to shading by other leaves or canopy position (Fig. 3). \( \Phi_{\text{CO}_2\text{max,abs}} \) measured in the greenhouse was generally greater than in the field, possibly because the greenhouse has slightly lower light and the environment is more constant and more humid (Table 2 and Fig. 3). This may help avoid cumulative damage that can accrue in the harsher and more variable field environment, for example following cooler mornings coupled with high light exposure (Baker et al., 1989; Farage et al., 2006). Clearly, this manipulation needs to be attempted in field conditions, but at a minimum this experiment demonstrates that the loss is not due to chronological age. Notably, the \( \Phi_{\text{CO}_2\text{max,abs}} \) observed in this protected environment of 0.072 mol mol\(^{-1}\) (Fig. 3) is almost identical to \( \Phi_{\text{CO}_2\text{max,app,PSII}} \).

**Fig. 3.** Maximum quantum yield of CO₂ assimilation on an absorbed light basis (\( \Phi_{\text{CO}_2\text{max,abs}} \)) with days after emergence of the leaf ligule in Miscanthus × giganteus Greef et Deu. Leaves were artificially maintained in unshaded conditions to separate aging from decrease in light quantity and quality, as would otherwise occur with sequential production of leaves above as a canopy develops. Each bar is the mean of six plants (±1 SE).

**Fig. 4.** Modeled canopy CO₂ assimilation (\( A_c \)) for a Miscanthus × giganteus Greef et Deu. canopy (LAI=5) based on actual measurements of weather and canopy geometry at the site of the stands in Illinois. (A) Predicted variation of canopy CO₂ assimilation per unit ground area (\( A_c \)) across a single day (DOY 167, mid-June). This assumes for scenario 1 no decline in \( \Phi_{\text{CO}_2\text{max,app}} \) or \( A_{\text{sat}} \) from top to bottom of the canopy (●), for scenario 2 the measured decline in \( \Phi_{\text{CO}_2\text{max,app}} \) but not \( A_{\text{sat}} \) (○), for scenario 3 the measured decline in \( A_{\text{sat}} \) but not \( \Phi_{\text{CO}_2\text{max,app}} \) (▲), and for scenario 4 the measured decline in both \( \Phi_{\text{CO}_2\text{max,app}} \) and \( A_{\text{sat}} \) (▲). (B) Daily canopy assimilation per unit ground area across the entire month of June, where symbols are as in (A). The integrated total predicted for June for each scenario is given in Table 3.
measured for Z. mays grown under similar controlled greenhouse conditions (Yin et al., 2011, 2014).

Although $A_{sat}$ and $\Phi_{CO_2,max,abs}$ will decline in the lower leaves of plant canopies at the onset of leaf senescence (Ono et al., 2001; Niinemets et al., 2015; Niinemets, 2016a,b; Pons, 2016), the high values for leaf fractional light absorbance ($\alpha$) indicate that leaves measured here in the lower canopy were still healthy and not senescent when they were measured. Relative to the upper canopy, $\alpha$ of lower canopy leaves was maintained in Z. mays, and significantly increased in M. × giganteus (Table 2). This increase was small, but this is not surprising given that $\alpha$ in the upper canopy was already high and close to the maximum reported for healthy green leaves across a range of species (Long et al., 1993).

Decline in $A_{sat}$ as leaves in canopies become shaded is commonly associated with the nitrogen economy of the plant, i.e. remobilizing nitrogen from major sinks, notably Rubisco, to provide nitrogen to upper canopy leaves (Evans, 1993; Osborne et al., 1998; Niinemets et al., 2015; Niinemets, 2016). This is seen in both C$_3$ and C$_4$ canopies (Anten et al., 1995). However, theoretical analysis of the proteins lost in this process suggested that this remobilization, while lowering $A_{sat}$, should not lower $\Phi_{CO_2,max,abs}$ (Hikosaka and Terashima, 1995).

Generally, measurements were more variable in the lower canopy compared with the upper canopy, and in Z. mays compared with M. × giganteus, although these differences were small (Table 2). Greater variability of the lower canopy could be explained by variation of leaf insertion height throughout the duration of the experiment, where leaves measured in the middle of the growing season, at peak LAI, were exposed to lower light intensities. Z. mays transitioned from vegetative to reproductive growth during the course of these measurements, while M. × giganteus remained in the vegetative phase.

Another possible cause of decreased $\Phi_{CO_2,max,app}$ and $\Phi_{CO_2,max,abs}$ is the altered light quality of the lower canopy. Light here is enriched in green and far-red relative to red and blue. Our measurements were made with a single spectral distribution of light based on blue and red LEDs. Although chlorophyll absorbs most strongly in the blue and red, at the high chlorophyll concentrations of healthy leaves, there is little difference in the absorptivity of green and red light or in their direct effect on quantum efficiency of CO$_2$ assimilation (McCree, 1972). However, altered light quality, in particular the ratio of red to far red light, is known to play a major role in phytochrome mediated shade avoidance responses of plants (Casal, 2013; Pons, 2016). Far-red to red ratios are increased about four-fold in T. aestivum and Z. mays canopies at the depth at which 80% of total light has been intercepted (Sattin et al., 1994). While this would not be represented when shade is simulated by growing plants in reduced light or under neutral density shade cloth, as in studies described previously, plants growing in a forest understory do experience this altered light composition. *In situ* measurements of the understory shrub *E. forbesii* gave a high $\Phi_{CO_2,max,app}$ of 0.053, exceeding that of co-occurring C$_3$ species and allowing them to achieve similar photosynthetic carbon gain (Pearcy and Calvin, 1983). While this shows that the decline observed here is not inherent in C$_4$ photosynthesis, *E. forbesii* is taxonomically distant from the grasses used in our study and belongs to a completely independent line of C$_4$ evolution (Sage et al., 2011). Additional experimentation would be necessary to determine whether light quality causes a decline in $\Phi_{CO_2,max,app}$ and $\Phi_{CO_2,max,abs}$ in C$_4$ crops.

Leaves of species adapted to high light conditions may lack the plasticity to effectively acclimate to shade conditions, particularly in C$_4$ plants, which show reduced plasticity in changing light environments when compared with C$_3$ plants (Sage and McKown, 2006; Niinemets et al., 2015; Niinemets, 2016b). While fast-growing grasses such as M. × giganteus are highly plastic in their remobilization of N when compared with other canopy-forming plants, this should primarily impact $A_{sat}$ and not $\Phi_{CO_2,max,abs}$ (Anten et al., 1998; Hikosaka and Terashima, 1995; Niinemets et al., 2015, Niinemets, 2016b).

In addition, developmental effects unique to C$_4$ physiology such as bundle-sheath leakage can cause negative acclimation to low light (Kromdijk et al., 2008; Niinemets et al., 2015).

Increasing stand density through combined efforts of breeding and agronomy has been a key factor in recent increases in yields of Z. mays (Duvick, 2005; Liu and Tollenaar, 2009; Li et al., 2011). M. × giganteus has been selected as an emerging high production C$_4$ bioenergy crop in part for its ability to be grown at high stem densities (Heaton et al., 2010). These trends toward higher stem densities and LAI will result in an ever-increasing proportion of crop carbon gain contributed over a day by shaded leaves. The findings here suggest that loss of efficiency of light-limited photosynthesis with shade, may result in a diminishing rate of return with further planting density increases, unless means are found to maintain $\Phi_{CO_2,max,app}$ within the canopy. The importance of shade photosynthesis is evident in our results: maintaining $\Phi_{CO_2,max,app}$ from top to bottom (scenario 3) would improve canopy carbon gain ($A_c$) more than twice as much as maintaining $A_{sat}$, and without the need for additional leaf nitrogen (scenario 2, Table 3). Although decline in $A_{sat}$ with shading appears almost universal and a key factor in stand nitrogen use efficiency, advances in bioengineering might soon provide means, paralleling ‘stay-green’, that prevent this decline. However, as noted above, the gain in carbon assimilation would be small compared with maintenance of $\Phi_{CO_2,max,app}$.  

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### Table 3. Modeled canopy CO$_2$ assimilation ($A_c$) integrated over the month of June for a Miscanthus × giganteus Gref et Deu. canopy (LAI=5) based on actual measurements of weather and canopy geometry at the site of the stands in Illinois

This assumes for scenario 1 no decline in $\Phi_{CO_2,max,app}$ or $A_{sat}$ from top to bottom of the canopy, for scenario 2 the measured decline in $\Phi_{CO_2,max,app}$ but not $A_{sat}$, for scenario 3 the measured decline in $A_{sat}$ but not $\Phi_{CO_2,max,app}$, and for scenario 4 the measured decline in both $\Phi_{CO_2,max,app}$ and $A_{sat}$, n.a., not applicable.

| Scenario | 1 | 2 | 3 | 4 |
|----------|---|---|---|---|
| $A_c$ (mol m$^{-2}$) | 39.1 | 35.3 | 37.5 | 34.0 |
| Increase in $A_c$ over scenario 4 (%) | 15% | 4% | 10% | n.a. |
Indirect evidence of this limitation in this clade of C₄ grasses may come from a comparison of two productive perennial grasses. M. × giganteus is recognized as a highly productive bioenergy crop, a quality often related to its use of C₄ photosynthesis (Heaton et al., 2010). However, a paradox here is the fact that the Mediterranean C₃ grass Arundo donax appears equally, if not more productive, when the two crops are grown side by side. Arundo donax produces an equally dense canopy, but shows a high Φₐₜₘₜₜ, which may explain what has until now appeared a paradox (Webster et al., 2016). Indeed, shade acclimation is of greatest importance in crops such as these, where dense canopies are formed (Niihemitas, 2016b).

Only single genotypes of the two species were considered here. Z. mays is the most important crop globally in terms of total grain production and Miscanthus species appear the most productive of the emerging perennial C₄ temperate bioenergy crops (Heaton et al., 2010; Long et al., 2015a). Sorghum (Sorghum bicolor L. Moench) and sugarcane (Saccharum officinarum L.) are the next most important C₄ crops after Z. mays in terms of area planted and value. Both are closely related to Miscanthus as revealed by recent genomic analysis, and like the more distantly related Zea are all within the tribe Andropogoneae. They form part of a monophyletic branch of evolution of C₄ NADP-ME genera (Swaminathan et al., 2010, 2012). This close relationship suggests that the other major C₄ crops, i.e. sorghum and sugarcane, might suffer the limitation observed here. Why could this apparent Achilles heel be present?

The ancestors of maize and Miscanthus appear to have existed in very open habitats, where water and nutrient deficiencies would have limited leaf area. There may therefore have been little evolutionary pressure for maintenance of photosynthetic efficiency in shade conditions. Clearly a next step will be to examine within species variability in diversity panels to identify possible breeding resources and establish the taxonomic breadth of this loss of Φₐₜₘₜₜ. If the mechanisms underlying this loss are unraveled then this may open the way to bioengineer maintenance of Φₐₜₘₜₜ with canopy depth in these crops. An up to 10% increase in the productivity of some of the world’s most important crops would seem to make this a target well worth pursuing.

It is estimated that the world may need to double production of primary foodstuffs, of which maize is the largest single component, by 2050 (Tilman and Clark, 2015). Since the approaches used in the Green Revolution are reducing their biological limits, identifying new opportunities to increase genetic yield potential will be critical (Long et al., 2015b; Kromdijk et al., 2016). Understanding the cause of the decline in Φₐₜₘₜₜ and its extent will be necessary to determine whether this apparent Achilles heel to an otherwise most important group of crops can be avoided and a substantial gain in productivity achieved.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Box plot of the maximum quantum yield of CO₂ assimilation on an incident light basis, calculated as in Yin et al. (2014) on measurements where fluorescence data were available (n=14–15 per species and canopy position)
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