Leaf photosynthesis and respiration of three bioenergy crops in relation to temperature and leaf nitrogen: how conserved are biochemical model parameters among crop species?

S. V. Archontoulis1,2, X. Yin1, J. Vos1, N. G. Danalatos2 and P. C. Struik1,*

1 Centre for Crop Systems Analysis, Plant Sciences Group, Wageningen University, Wageningen, The Netherlands
2 Laboratory of Agronomy and Applied Crop Physiology, Department of Agriculture, University of Thessaly, Volos, Greece

* To whom correspondence should be addressed. E-mail: paul.struik@wur.nl

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Abstract

Given the need for parallel increases in food and energy production from crops in the context of global change, crop simulation models and data sets to feed these models with photosynthesis and respiration parameters are increasingly important. This study provides information on photosynthesis and respiration for three energy crops (sunflower, kenaf, and cynara), reviews relevant information for five other crops (wheat, barley, cotton, tobacco, and grape), and assesses how conserved photosynthesis parameters are among crops. Using large data sets and optimization techniques, the C3 leaf photosynthesis model of Farquhar, von Caemmerer, and Berry (FvCB) and an empirical night respiration model for tested energy crops accounting for effects of temperature and leaf nitrogen were parameterized. Instead of the common approach of using information on net photosynthesis response to CO2 at the stomatal cavity ($A_n - C_i$), the model was parameterized by analysing the photosynthesis response to incident light intensity ($A_n - I_{inc}$). Convincing evidence is provided that the maximum Rubisco carboxylation rate or the maximum electron transport rate was very similar whether derived from $A_n - C_i$ or from $A_n - I_{inc}$ data sets. Parameters characterizing Rubisco limitation, electron transport limitation, the degree to which light inhibits leaf respiration, night respiration, and the minimum leaf nitrogen required for photosynthesis were then determined. Model predictions were validated against independent sets. Only a few FvCB parameters were conserved among crop species, thus species-specific FvCB model parameters are needed for crop modelling. Therefore, information from readily available but underexplored $A_n - I_{inc}$ data should be re-analysed, thereby expanding the potential of combining classical photosynthetic data and the biochemical model.

Key words: $A - I_{inc}$ curves, acclimation, bioenergy crops, crop modelling, day and night respiration, electron transport rate, leaf nitrogen, photosynthesis, Rubisco carboxylation, temperature.

Introduction

In conventional crop modelling leaf photosynthesis is calculated from net photosynthesis light response curves ($A_n - I_{inc}$; see symbols explanation in Table 1) at ambient atmospheric CO2 level using empirical functions (e.g. SUCROS; Goudriaan and van Laar, 1994). In the context of better understanding biological processes and exploring the impact of climate change, recent crop models (e.g. GECROS; Yin and van Laar, 2005), 3D models (e.g. Evers et al., 2010), or terrestrial ecosystem models (e.g. LPJmL; Beringer et al., 2011) calculate photosynthesis based on the mechanistic model of Farquhar, von Caemmerer, and Berry (Farquhar et al., 1980; the FvCB model hereafter).

The FvCB model describes photosynthesis as the minimum of the Rubisco-limited rate and the electron transport-limited rate. The key parameters of the model are the maximum Rubisco carboxylation rate ($V_{cmax}$), the maximum electron transport rate ($J_{max}$), and the mitochondrial day respiration ($R_d$). These biochemical parameters are influenced both by the physiological status of a leaf such as the amount of leaf nitrogen per unit area ($N_a$) (e.g. Harley et al., 1992) and by short- and long-term changes of...
environmental variables such as temperature, light (e.g. Hikosaka, 2005), CO₂ (e.g. Makino et al., 1994), and drought (e.g. Galmes et al., 2007).

Usually, the FvCB parameters are obtained by analysis of net photosynthesis response to CO₂ at the stomatal cavity (Aₙ–Cₚ) (e.g. Sharkey et al., 2007) or by combining Aₙ–Cₚ and Aₙ–Iinc curves (e.g. Braune et al., 2009) or by combining these curves with chlorophyll fluorescence measurements (Yin et al., 2009). Obviously, to parameterize the FvCB model, information on Aₙ–Cₚ is predominantly considered to be essential, and an ongoing discussion is mainly focused on improving the methods of analysing these Aₙ–Cₚ curves (Ethier et al., 2004; Sharkey et al., 2007; Gu et al., 2010).

In the context of forward crop modelling typically for predictions at the ambient CO₂ level, the FvCB model is used to project leaf photosynthetic rates in response to both temporal (diurnal and seasonal) and spatial (within a crop canopy) variation in light intensity. This implies that in the context of inverse modelling, important FvCB model parameters Jmax and Vcmax should and can be estimated from Aₙ responses to Iinc This would reflect better the tradition whereby crop modellers describe leaf photosynthesis from its response to light intensity (e.g. Goudriaan, 1979), in contrast to the tradition that photosynthesis physiologists study gas exchange measurements mainly across various levels of CO₂ (e.g. von Caemmerer and Farquhar, 1981). In fact, the FvCB model can be parameterized from analysis of Aₙ–Iinc data alone (Niinemets and Tenhunen, 1997; Kosugi et al., 2003), but so far there is no information about the accuracy of Jmax and Vcmax parameters derived from such an analysis. If Jmax and Vcmax estimates derived from analysis of Aₙ–Iinc are similar to those obtained from the common Aₙ–Cₚ analysis or combined analysis of Aₙ–Cₚ and Aₙ–Iinc curves, it may generate an opportunity to reduce empiricism in crop models by using readily available Aₙ–Iinc data. Therefore, the first objective of this study is to explore this opportunity by parameterizing the FvCB model using Aₙ–Iinc data.

In the light of current trends for a parallel increase in food and energy production from crop species in the context of climate change, the use of the FvCB-based simulation models together with an urgent need to feed these models with photosynthetic and respiration parameters has been increased (e.g. Beringer et al., 2011). Compared with the rich information found for trees in the literature, there are only a few reports on Jmax, Vcmax, Rₙ, and night respiration (Rd) parameters in relation to environmental and management factors for economically important crop species (e.g. Müller et al., 2005; Braune et al., 2009; Yin et al., 2009) and these are virtually lacking for new bioenergy species. Therefore, the second objective of this study is 3-fold: (i) to provide new information on photosynthesis and respiration for three Mediterranean energy crops (Helianthus annuus, sunflower; Hibiscus cannabinus, kenaf; and Cynara cardunculus, cynara); (ii) to summarize existing information for five major cash crops (wheat, barley, cotton, tobacco, and grape); and (iii) to assess how conserved FvCB parameters are among crop species to better assist modellers in this exploitation.

Sunflower, kenaf, and cynara crops were chosen because these crops have great potential to increase bioenergy production in the Mediterranean region (Archontoulis et al., 2010a, b; Danalatos and Archontoulis, 2010). In addition,
The chosen crops cover a wide range of bioindustrial applications (biodiesel, bioethanol, heat, and electricity) and fit into different cropping strategies (short or long growing period, cultivation with or without irrigation, etc.). Sunflower is widely grown in the Mediterranean region, but kenaf and cynara cultivation is still in the experimental phase; relevant information for crop modelling is currently being accumulated for these crops, including vertical distribution of light and nitrogen within crop canopies (Archontoulis et al., 2011). Photosynthetic gas exchange studies for sunflower have been reported (e.g. Connor et al., 1993), but there are only a few for kenaf (Muchow, 1990; Cosentino et al., 2004) and none for cynara.

The present analysis focuses on the FvCB parameters in response to temperature and $N_d$ for these bioenergy crops. This is because earlier studies on $V_{cmax}$ and $J_{max}$ temperature dependencies showed great species-to-species variability (Leuning, 2002; Medlyn et al., 2002a), and because $N_d$ is linearly related to Rubisco content that drives $CO_2$ fixation (Makino et al., 1994), reflects leaf dynamics well (leaf age, rank; Archontoulis et al., 2011), and comprises a reference index for scaling photosynthetic $CO_2$ assimilation from leaf to canopy levels (de Pury and Farquhar, 1997). Among bioenergy crops, the perennial cynara has long annual growth cycles (~10 months each; Archontoulis et al., 2010a). Given the numerous reports together with their diverse findings on photosynthetic and respiratory acclimation to growth environment (Atkin et al., 2005; Ow et al., 2008; Yamori et al., 2005, 2010; Silim et al., 2010), seasonal acclimation effects on photosynthesis and respiration for the cynara crop are also investigated.

**Materials and methods**

**Literature data for $A_{c-C_0}$ versus $A_{c-V_{cmax}}$ curves**

The first objective of this study was to compare $V_{cmax}$ and $J_{max}$ estimates derived either from $A_{c-C_0}$ or from $A_{c-V_{cmax}}$ curves. For this, published data from Yin et al. (2009) for *Triticum aestivum* (cv. Minaret) were used. All relevant parameter values required to fit the FvCB model to the $A_{c-C_0}$ or $A_{c-V_{cmax}}$ data set were available, therefore avoiding any statistical artefact in $V_{cmax}$ and $J_{max}$ estimation. Wheat measurements (four replicates; all at 25°C) were conducted on leaves with different $N_d$ status (15 sets of $A_{c-C_0}$ and 15 of $A_{c-V_{cmax}}$ curves), allowing the comparison of $J_{max}$ and $V_{cmax}$ estimates to be made over a wide range of their values. For more information about the measurements, see Yin et al. (2009).

**Energy crop species and study site**

Sunflower (cv. Panter), kenaf (cv. Everglades 41), and cynara (cv. Biango avario) crops were grown in different sections of the same field (for details, see Archontoulis et al., 2011) in central Greece (39°25′43.4″ N, 22°05′09.7″ E, 105 m asl) for 3 years (2007–2009). The site has a Mediterranean climate with cold/wet winters and warm/dry summers (Supplementary Fig. S1 available at JXB online). The soil was loamy, classified as Aquic Xerofluvent, with a shallow groundwater table (1.8–2.8 m below the surface during May). In general, crops grown at that site produce much higher biomass yields than crops grown on dry soils (e.g. Archontoulis et al., 2010b). During summer, sunflower and kenaf crops were frequently irrigated at intervals of 4–6 d according to potential evapotranspiration (for site-specific calculations, see Danalatos and Archontoulis, 2010) while cynara was irrigated only a few times, when necessary during May–June but not during November–April (see precipitation in Supplementary Fig. S1).

**Gas exchange measurements and experimental protocol**

Leaf gas exchange (GE) measurements were implemented *in situ* in fully expanded leaves using a portable open gas exchange system with a 6.25 cm$^2$ clamp-on leaf chamber (ADC, LCi/LCpro+, Bioscientific Ltd, Hoddesdon, UK). $CO_2/H_2O$ exchanged by the leaf was measured using an infrared gas analyser in a differential mode. The system allowed for an automated microclimate control in the leaf chamber. Before each measurement, attached leaves were adapted for 10–45 min to chamber conditions, depending on leaf age, time of the day, and season. Daytime GE measurements were taken within 1–2 d after irrigation and during morning hours to ensure no water stress and to avoid midday depression of photosynthesis. Night-time GE measurements were initiated 30–45 min after sunset and lasted for 4–5 h each time.

To parameterize the model, a common experimental protocol was applied per species, including four different sets of GE measurements. In all sets, $CO_2$ concentration was kept at 380±5 μmol mol$^{-1}$. The first set aimed to determine the response of net photosynthesis ($A_o$) to incident light ($I_{inc}$). Accordingly, at fixed leaf temperature and measured $N_d$, $A_o$ was determined in 11 $I_{inc}$ steps (2000, 1500, 1000, 500, 250, 200, 150, 100, 50, 20, and 0 μmol photons m$^{-2}$ s$^{-1}$); in total, 76 curves were constructed. Adaptation time to each $I_{inc}$ level was ~5 min, except for $I_{inc}$=0 where it was >10 min; 3–5 replicated $A_o$ measurements were taken at each $I_{inc}$ step to ensure stability and precision of measurements. Given that the examination of steady-state photosynthesis takes considerable time and that GE measurements should be done within a limited time frame in order to avoid stress conditions (see above), the response of $A_o$ to leaf temperature (set II) was determined at three $I_{inc}$ levels: 450, 900, and 1800 μmol photons m$^{-2}$ s$^{-1}$. $N_d$ was also determined. At each $I_{inc}$, leaf temperature was increased or decreased up to 10°C from the ambient temperature in steps of 2–4°C and replicated $A_o$ measurements were recorded every 5 min. To establish the relationship between net photosynthesis and $N_d$ (set III), it was necessary to evaluate leaves with as wide an $N_d$ range as possible. So, in addition to earlier sets, $A_o$ measurements were done at saturated $I_{inc}$ (1600–1800 μmol photons m$^{-2}$ s$^{-1}$) on leaves from different insertion heights in the canopy, from different growth stages, and from plots with different $N_d$ status. Per leaf (~180 leaves assessed), 5–10 measurements were taken at leaf temperature close to the ambient temperature.

To obtain direct measurements of the mitochondrial respiration occurring in the night ($R_d$), the response of $R_d$ to temperature was investigated (set IV). Leaf temperature increased or decreased up to 10°C from the ambient temperature in small steps of 1–2°C, and replicated $R_d$ measurements were recorded every 4 min. Measurements were done on leaves with (as much as possible) variable $N_d$.

To validate the models, GE measurements obtained from the same genotypes growing in the same site during summer 2005 and 2006 (set V) were used. Sunflower and kenaf GE measurements were collected using similar techniques and time frames to those described for sets I–IV. In cynara, a different protocol was followed. The external unit that controls chamber microclimate was removed to obtain measurements under real ambient conditions. Measurements were recorded every 4–8 min, while climatic variables were continuously changing following 24 diurnal trends, thereby providing a data set to assess whether the FvCB model can predict $A_o$ under real fluctuating field conditions.

The wide range of measuring temperature used (15–40°C) unavoidably resulted in variation in vapour pressure difference (VPD). An effort was made to reduce that variation by keeping humidity high at high temperature. In most cases, VPD was maintained below 3 kPa to prevent stomatal closure (Bernacchi et al., 2001). Although VPD was sometimes above 3 kPa at the
highest temperatures, the stomatal conductance for H₂O vapour was not less than 0.30 mol m⁻² s⁻¹ (as in Yamori et al., 2005).

All measured Aₙ data were corrected for the CO₂ respired under the gasket surface (total 4 mm width; R. Newman, personal communication) following the common approach of Pons and Welschen (2002). All GE characteristics were re-calculated according to von Caemmerer and Farquhar (1981), for example to provide the Cₛ values that are required as input to the FvCB model (see below). In addition, the number of replications and observations were increased to reduce the measurement noise, especially when low CO₂ exchange rates were measured (e.g. respiration).

The portion of the leaf used for measurements was cut and its area was measured with a Li-Cor area meter. The leaf material was then weighed after drying at 70 °C to constant weight and its total nitrogen concentration was measured using the Kjeldahl method. From these measurements, the leaf nitrogen content Nₘ (g N m⁻²) was calculated.

**Model and its parameterization**

The FvCB model predicts Aₙ (µmol CO₂ m⁻² s⁻¹) as the minimum of two processes (see Fig. 1), the Rubisco carboxylation-limited rate (Aₜ) and the RuBP regeneration- or electron transport-limited rate (Aᵢ):

\[ Aₙ = \min(Aₜ, Aᵢ) \]  \hspace{1cm} (1)

Rubisco-limited photosynthesis is calculated as a function of maximum carboxylation capacity (V_emax, µmol CO₂ m⁻² s⁻¹):

\[ Aₜ = \frac{V_{\text{max}}(Cᵢ - Γᵢ)}{Cᵢ + K_{m₁}(1 + O/K_{µ₁})} - R_d \]  \hspace{1cm} (2)

where Cᵢ (µbar) and O (µbar) are the intercellular partial pressures of CO₂ and O₂, respectively, K_m₁ (µbar) and K_m₂ (µbar) are the Michaelis–Menten coefficients of Rubisco for CO₂ and O₂, respectively, and Γᵢ (µbar) is the CO₂ compensation point in the absence of R_d (day respiration in µmol CO₂ m⁻² s⁻¹), which comprises mitochondrial CO₂ release occurring in the light other than photorespiration (von Caemmerer et al., 2009).

There are various equations to describe the rate of photosynthesis when RuBP regeneration is limiting (Farquhar and von Caemmerer, 1982; Yin et al., 2004). The most widely used form is given by:

\[ Aᵢ = \frac{J(Cᵢ - Γᵢ)}{4Cᵢ + 8Γᵢ} - R_d \]  \hspace{1cm} (3)

where J (µmol e⁻ m⁻² s⁻¹) is the photosystem II electron transport rate that is used for CO₂ fixation and photorespiration. J is related to the amount of incident photosynthetically active irradiance (I_inc; µmol photons m⁻² s⁻¹) by:

\[ J = \frac{(κ_{2L₂}I_{\text{inc}} + J_{\text{max}} - \sqrt{(κ_{2L₂}I_{\text{inc}} + J_{\text{max}})^2 - 4θJ_{\text{max}}κ_{2L₂}I_{\text{inc}}})}{2θ} \]  \hspace{1cm} (4)

where J_{max} (µmol e⁻ m⁻² s⁻¹) is the maximum electron transport rate at saturating light levels, θ is a dimensionless convexity factor for the response of J to I_inc, and κ_{2L₂} (mol e⁻ mol⁻¹ photons) is the conversion efficiency of I_inc into J at limiting light levels (Yin and Struik, 2009a; Yin et al., 2009). The formulation of Equations 2 and 3 assumes instantaneous mesophyll conductance (g_m) for CO₂ transfer to chloroplasts, so that Cᵢ is used as the proxy for the chloroplast CO₂ level (C₂). There is increasing evidence that g_m might be low enough to allow a significant drawdown of Cᵢ from Cᵢ in most species (Warren, 2004; Flexas et al., 2008). However, based on the available GE data, it was risky to evaluate g_m (Pons et al., 2009; von Caemmerer et al., 2009; Yin and Struik, 2009b), hence the forms of Equations 2 and 3 had to be used, as in the earlier studies (e.g. Medlyn et al., 2002; Kosugi et al., 2003). Omitting g_m in the analysis means

![Fig. 1. Main panel: typical net photosynthesis light response curve (Aₙ-I_inc) at ambient CO₂ concentration. Curve regions for the Rubisco carboxylation-limited rate (Aₜ-limited, Equation 2; solid line) and the electron transport-limited rate (Aᵢ-limited, Equation 3; dotted line) are indicated. Usually, Aₜ-limitation occurs above 1500 µmol photons m⁻² s⁻¹; however, it is also possible that the entire Aₙ-I_inc curve is described as Aᵢ-limited. Inset panel: representative portion of the Aₙ-I_inc curve used in calculations of the day respiration (R_d), night respiration (R_n), and apparent quantum yield (Φ_{CO₂LL}). R_d and Φ_{CO₂LL} were calculated from linear regression analysis to open circles while the filled circle represents the value of the R_n. For details, see the Materials and methods.](https://example.com/f1.png)
that an appropriate consideration is needed in choosing values of the Rubisco kinetic constants (see below).

The temperature responses of respiration and of Rubisco kinetic properties (K\text{mc} and K\text{mo}) are described using an Arrhenius function (Equation 5) while the temperature responses of V\text{emax} and J\text{max} were explored using a peaked Arrhenius function (Equation 6); both functions were normalized with respect to their values at 25 °C:

\[
X = X_{25} \exp \left[ \frac{E_i(T - 25)}{298R(T + 273)} \right]
\]

where \( T \) is the leaf temperature (°C); \( X_{25} \) is the value of each parameter at 25 °C (\( R_{225}, K_{mc25}, K_{mo25}, V_{emax25}, \) and \( J_{max25} \)); \( E_i \) is the activation energy of each parameter (\( E_{R}, E_{V}, E_{Kc}, E_{V}, \) and \( E_i \) in J mol\(^{-1}\)); \( D_i \) is the deactivation energy for \( J_{max} \) and \( V_{emax} \) (\( D_i \) and \( D_i \) in J mol\(^{-1}\)); \( S_i \) is the entropy term for \( J_{max} \) and \( V_{emax} \) (\( S_i \) in J K\(^{-1}\) mol\(^{-1}\)); and \( R \) is the universal gas constant (=8.314 J K\(^{-1}\) mol\(^{-1}\)). Given that Equation 5 is a special case of Equation 6, F-tests were performed to determine whether Equation 6 described temperature responses of \( V_{emax} \) and \( J_{max} \) significantly better than did Equation 5. When Equation 6 was overparameterized, as often observed in the literature (Dreyer et al., 2001; Medlyn et al., 2002a), then \( S_i \) was fixed at 650 J K\(^{-1}\) mol\(^{-1}\) (Harley et al., 1992).

Rubisco kinetic properties are generally assumed constant among C\(_3\) species (von Caemmerer et al., 2009). However, values of these constants and their temperature dependency reported in the literature vary appreciably, so the choice of Rubisco parameters is a matter of considerable uncertainty (Dreyer et al., 2001). In this work, similar to many other reports (e.g. Medlyn et al., 2002a; Müller et al., 2005), Rubisco parameters reported by Bernacchi et al. (2001) were selected because these values (i) were estimated from \textit{in vivo} measurements without disturbance of the leaf; and (ii) were derived using the \textit{C}r-based FvCB model and hence are compatible with the present analysis assuming an infinite \( g_{\text{in}} \) (see above). The parameter values are: \( K_{mc25}=404.9 \) Jbar; \( K_{mo25}=278.4 \) mbar; \( E_{Kmc}=79.430 \) J mol\(^{-1}\); and \( E_{Kmo}=36.380 \) J mol\(^{-1}\) (Table 1). Furthermore, using these values, the temperature dependence of \( \Gamma^* \) was calculated as (Yin et al., 2004):

\[
\Gamma^* = 0.50 \frac{K_{mc}}{K_{mo}} \left[ \exp (-3.3801 + \frac{5220}{298R(T + 273)}) \right]
\]

where the factor 0.5 is mol CO\(_2\) released when Rubisco catalyses the reaction with 1 mol O\(_2\) in photospiration. The term in the brackets was derived using Bernacchi et al. (2001) parameters for temperature dependence of maximum carboxylation and oxygenation rates of Rubisco.

The basal capacity of \( R_{225}, V_{emax25}, \) and \( J_{max25} \) is linearly related to \( N_a \) (Harley et al., 1992; Hirose et al., 1997; Müller et al., 2005; Braune et al., 2009):

\[
R_{225} = x_R(N_a - N_b)
\]

\[
V_{emax25} = x_V(N_a - N_b)
\]

\[
J_{max25} = x_J(N_a - N_b)
\]

where \( x_R \) (\( \mu \)mol CO\(_2\) g\(^{-1}\) N s\(^{-1}\)), \( x_V \) (\( \mu \)mol CO\(_2\) g\(^{-1}\) N s\(^{-1}\)), and \( x_J \) (\( \mu \)mol g\(^{-1}\) N s\(^{-1}\)) are the slopes for \( R_{225}, V_{emax25}, \) and \( J_{max25} \), respectively, and \( N_b \) (g N m\(^{-2}\)) is the minimum value of \( N_a \) at or below which \( A_c \) is zero. In principle, this \( N_b \) is practically impossible to measure and its estimation depends on the statistical methods used and on the available data sets. For instance, different \( N_b \) estimates were found when different data sets were examined (\( A_c \) or \( V_{emax} \) or \( J_{max} \); e.g. Harley et al., 1992; Müller et al., 2005; Supplementary Table S1 at JXB online) or when \( N_b \) was estimated simultaneously with other parameters in optimization procedures or when different equations (linear or non-linear) were applied to the same data set (Niinemets and Tenhunen, 1997). Given the simplicity required in modelling and the lack of biological interpretation of different \( N_b \) values for the same species, a unique \( N_b \) value (per species) was determined beforehand from direct assessments of \( A_c \)– \( N_a \) plots. Then this estimate was used as input parameter.

There is some evidence that the activation energy for respiration (\( E_R \)) depends on the position of the leaf in the canopy (Bolstad et al., 1999; Griffin et al., 2002) and perhaps \( E_R \) is also associated with \( N_a \) since a close relationship between leaf canopy position and \( N_a \) usually exists (Archontoulis et al., 2011). This was tested by assuming a linear relationship between \( E_R \) and \( N_a \):

\[
E_{R_a} = E_{R_0} + a_{R_a} N_a
\]

and it was checked whether the slope parameter \( a_{R_a} \) differed significantly from zero.

So far, temperature and nitrogen relationships for \( R_d \) have been described, as extensive GE measurements during the night period were available. However, the FvCB requires estimates for \( R_d \), which is much more difficult to measure. To estimate \( R_d \), regression analysis was applied to the linear sections of the \( A_n–I_{fuc} \) curves for each species (Fig. 1, inset; Kok method; Sharp et al., 1984). From this analysis, \( R_d \) was calculated as the y-axis intercept of the linear regression and the corresponding \( R_d \) was estimated as the mean of the \( R_d \) values at 0 μmol photons m\(^{-2}\) s\(^{-1}\). Additionally, the apparent quantum efficiency at limiting light (\( \Phi_{Q2LL} \)) on incident light was calculated from the slope of the regression. The \( I_{fuc} \) range for this regression analysis was typically 20–150 μmol m\(^{-2}\) s\(^{-1}\) (Fig. 1, inset), while in a few cases the \( I_{fuc} \) range was slightly different, especially for data sets obtained at high temperatures. The estimated \( R_d \) was then related to \( R_a \) as:

\[
R_d = R_X \times (R_n - a_R)
\]

where \( R_X \) and \( a_R \) are the slope and the x-axis intercept of the linear model, respectively. By assuming that activation energies for \( R_d \) and \( R_a \) are similar and taking into account the precise quantification of \( R_d \) based on a large data set, the temperature and nitrogen dependencies of \( R_d \) can be calculated from combining Equations 5, 8, 11, and 12. This approach allows \( R_d \) values to be estimated for sets II and III (see above) where \( I_{fuc} \) exceeds 350 μmol m\(^{-2}\) s\(^{-1}\), for which it was not possible to use the Kok method for estimating \( R_d \).

Summary of parameters and statistics

The basic equations of the FvCB model, Equations 1–4, capture the response of \( A_c \) to \( C_i \) and to \( I_{fuc} \). Coupled with auxiliary temperature (Equations 5–7) and nitrogen (Equations 8–12) equations, the model also quantifies leaf photosynthesis and respiration (\( R_d \) and \( R_n \) in response to these environmental variables. Data from sets I–IV were analysed using step-wise optimization procedures. Per crop, 16 parameters were estimated following the order: step 1, \( N_b \); step 2, \( \lambda_R, E_{Kmc}, E_{Kmo} \); step 3, \( R_d, a_R, \) step 4, \( I_{fuc}, E_{R_a}, E_{R_s}, S_{i} \); step 5, \( K_{mc} \); step 6, \( K_{mo} \); \( E_{V}, D_{i}, S_{j}, \) and 0 (see the Results). Inputs to the model are: \( C_i, I_{fuc} \) leaf temperature, and \( N_a \). So, just like using \( A_c–C_i \) curves, using \( A_c–I_{fuc} \) data to calculate FvCB model parameters (e.g. \( V_{emax} \)) also requires \( C_i \) as an input to the model, meaning that any (short-term) change in stomatal aperture during the \( A_c–I_{fuc} \) measurements will have been reflected in the values of \( C_i \) and thus have little effect on the calculation of the FvCB parameters. For a similar reason, the direction of changing \( I_{fuc} \) levels for measuring \( A–I_{fuc} \) curves will also have little impact on parameter estimation (see Yin et al., 2011).

For each step, regression fitting was carried out using the GAUSS method in PROC NLIN of SAS (SAS Institute Inc.). To investigate seasonal effects of acclimation on photosynthesis and
respiration rates of cynara, data sets were split into two periods: a cold period with low light from November to April and a warm period with high light from May to June (Supplementary Fig. S1 at JXB online). Then, dummy variables (Z1=1 and Z2=0 for warm and Z1=0 and Z2=1 for cold periods, respectively) were introduced into the regression analysis to separate for the effects. A dummy variable was also used to best estimate the $N_b$ parameter (see the Results).

The goodness of model fit was assessed by calculating $r^2$ and the relative mean root square error ($r$RMSE). A sensitivity analysis was also performed. Model predictions were validated against independent data sets (set V).

Results

$V_{cmax}$ and $J_{max}$ estimates from $A_{n}$–$C_i$ and/or $A_{n}$–$I_{inc}$ curves

$V_{cmax}$ and $J_{max}$ were estimated for wheat, from either $A_{n}$–$I_{inc}$ or $A_{n}$–$C_i$ curves alone or from the combined data of the two curves. The following parameters were set as inputs to the model (see Equations 1–4 and 7): $K_{mc25}$ and $K_{m25}$ from Bernacchi et al. (2001); and $R_{d25}$, $R_{a25}$, $K_{DLL}$, and $\theta$ per set of data from Yin et al. (2009). $V_{cmax}$ and $J_{max}$ were successfully estimated simultaneously in 40 out of the 45 cases (15 sets×3 methodologies). In five cases, it was not possible to estimate $V_{cmax}$ from $A_{n}$–$I_{inc}$ curves because in these cases the entire curve was $A_i$ limited (Fig. 1). Then we first calculated $V_{cmax}$ directly from Equation 2 with observed $C_i$ as input for simple substitution using data points where $I_{inc}$ >1500 µmol photons m$^{-2}$ s$^{-1}$, and secondly by setting $V_{cmax}$ as an input to the model, the $J_{max}$ parameter was estimated again. To be consistent, results for all $A_{n}$–$I_{inc}$ curves were presented following the two-step approach, because estimates from both approaches were very close.

Figure 2 illustrates $V_{cmax}$ and $J_{max}$ estimates from $A_{n}$–$C_i$ and from $A_{n}$–$I_{inc}$ curves versus the combination of those curves. As expected, $V_{cmax}$ and $J_{max}$ estimates obtained from $A_{n}$–$C_i$ curves were almost identical to the estimates based on the combined data ($r^2=0.97–0.99$). However, it was found that $A_{n}$–$I_{inc}$ curves alone also provided sufficient estimates ($r^2=0.91–0.93$) and thus can be considered as an alternative to predominant $A_{n}$–$C_i$ curves to parameterize the FvCB model. In fact, regression lines in Fig. 2 were matching across a very wide range of $V_{cmax}$ and $J_{max}$ values. Even in cases where photosynthetic response to light were entirely $A_i$ limited (Fig. 1), $V_{cmax}$ estimates obtained from either $A_{n}$–$I_{inc}$ or $A_{n}$–$C_i$ data were close (Fig. 2). The slight discrepancy of the estimates at high $V_{cmax}$ and $J_{max}$ values (Fig. 2) caused a lower $r^2$ for the $A_{n}$–$I_{inc}$ compared with the $A_{n}$–$C_i$ estimates.

Step-wise estimation of model parameters for bioenergy crops

Step 1: $N_b$ estimation

Measured light-saturated $A_{n}$ ($A_{n}$max) responded non-linearly to increasing $N_a$ in all tested crops (Fig. 3; $r^2 > 0.81$; $P < 0.001$). An effect of temperature was detected in this relationship only at high $N_a$ (Fig. 3). To estimate the $N_b$ value properly from these plots a dummy variables approach was used, in order to obtain a unique $N_b$ estimate per crop, while allowing the equation to vary with different temperatures (optimum versus non-optimum temperature ranges; Fig. 3). Derived parameters are listed in Table 2. $N_b$ values for all crops were close to 0.4 g N m$^{-2}$, while the lack of $N_a$ data below 0.7 g m$^{-2}$ caused a high standard error of the $N_b$ estimate (Table 2).

Step 2: $R_n$ in relation to temperature and $N_a$

By combining Equations 5, 8, and 11, $R_n$ parameters were estimated (Table 3). In cynara, an additional seasonal effect was found, with significantly higher $R_n$ rates for the winter/cold- compared with the summer/warm-growing leaves (Fig. 4). Incorporation of this effect into the model improved $r^2$ from 0.68 to 0.72. Of the two $R_n$ parameters, temperature sensitivity ($E_{Ra}$) was significantly ($P < 0.01$) affected by season, but the slope of the $R_n$–$N_a$ relationship ($\chi_R$) was not ($P=0.263$); thus, a common $\chi_R$ value was calculated (Table 3). The $R_n$ models’ goodness of fit was satisfactory ($r^2 > 0.72$; $r$RMSE < 0.28 across species).

Step 3: relationship between $R_{ld}$ and $R_n$

Plotting $R_{ld}$ versus $R_n$ gave a good linear relationship with no significant differences among species ($P=0.225$; Fig. 5;
Table 3. Analysis showed that mitochondrial respiration was inhibited by ~28% in the light. The observed x-axis intercept ($a_R = 0.39$) differed significantly from zero ($P = 0.0039$), indicating that $R_n$ and $R_d$ were not entirely proportional (Fig. 5). Additionally, no effect of $N_a$ ($r^2 = 0.01; P = 0.67$) but a significant effect of temperature ($r^2 = 0.18; P = 0.008$) was found on the $R_d/R_n$ ratio, showing that the ratio approached unity at high temperatures. Similarly, the $R_n/A_{n,\text{max}}$ ratio—ranging from 7% to 11% across bioenergy species—was insensitive to changes in $N_a$ ($P > 0.05$), but increased significantly with increasing temperature ($r^2 = 0.62; P < 0.01$; data not shown).

Table 2. Estimates (SE in parentheses) of the non-linear equation used to describe data illustrated in Fig. 3

| Species     | Symbol | $A_{n,\text{max}}$ | $c$   | $N_b$ |
|-------------|--------|---------------------|-------|-------|
| Sunflower   | Filled (26–34 °C) | 36.6 (2.48) | 1.19 (0.195) | 0.387 (0.078) |
|             | Open   | 26.4 (1.45) | 1.65 (0.313)  |
| Kenaf       | Filled (27–35 °C) | 35.8 (2.18) | 1.29 (0.269) | 0.390 (0.126) |
|             | Open   | 29.2 (2.12) | 1.45 (0.334)  |
| Cynara      | Filled (22–31 °C) | 36.4 (2.41) | 1.08 (0.191) | 0.416 (0.097) |
|             | Open   | 23.9 (2.16) | 1.22 (0.725)  |

* a Symbols in Fig. 3.

* b The confidence limits for $N_b$ are: 0.231–0.541, 0.139–0.640, and 0.225–0.608 for sunflower, kenaf, and cynara, respectively ($P = 0.05$).

Table 3. The relationships of $V_{c\text{max}}$ to temperature and $N_a$ were quantified by fitting Equations 2 and 5–12 to data obtained at high light levels ($I_{\text{inc}} \approx 1500 \mu\text{mol} \text{ m}^{-2} \text{s}^{-1}$) to ensure that $A_n$ is limited only by Rubisco. All required parameters ($\chi_v$, $E_v$, $D_v$, and $S_v$) were well estimated. Across species, there were small differences in $\chi_v$ (<12%; Table 3), and large differences in temperature sensitivities >30 °C (Fig. 6a; including other crops). Sunflower temperature sensitivity was best described by the peaked Arrhenius equation ($r^2 = 0.736; P < 0.001$; Table 3), showing an optimum...
Table 3. Estimates (SE in parentheses) of parameters used to describe temperature and nitrogen sensitivities of photosynthesis and respiration rates in three bioenergy crops

For cynara, when significant differences between warm and cold seasons were found, two estimates are given. For units see Table 1.

| Parameter | Sunflower | Kenaf | Cynara-warm | Cynara-cold |
|-----------|-----------|-------|-------------|-------------|
| $R_n$     | 0.609 (0.006) | 0.954 (0.015) | 0.775 (0.009) |             |
| $E_{r,c}$ | 117 912 (1814) | 100 740 (3250) | -10 900 (5617) | 146 956 (4281) |
| $E_{e,c}$ | -23 346 (770) | -15 743 (1455) | 33 040 (2490) | -26 640 (1858) |
| $n$ [night] | 2492 | 1403 | 3212 |             |
| $r^2$     | 0.799 | 0.793 | 0.724 |             |
| $R_d/R_n$ |           |       | 0.843 (0.040) |             |
| $b_n$     |           |       | 0.390 (0.107) |             |
| $a_n$     |           |       |             |             |
| $V_{c,max}$ | 73.8 (0.94) | 66.7 (0.92) | 65.2 (0.62) |             |
| $E_a$     | 53 688 (1631) | 61 812 (1402) | 190 831 (33 853) |             |
| $D_r$     | 205 638 (355) | 0 | 158 486 (30 907) |             |
| $S_r$     | 650$^c$ | 0 | 550 (108.2) |             |
| $\kappa_{2LL}$ | 0.255 (0.018) | 0.278 (0.013) | 0.314 (0.014) | 0.419 (0.011) |
| $\theta$  | 0.607 (0.027) | 0.627 (0.023) | 0.847 (0.011) |             |
| $n$ [day]$^b$ | 1366 | 2042 | 2334 |             |
| $r^2$     | 0.928 | 0.909 | 0.916 |             |
| Ratio     | $J_{max}/V_{c,max}$ | 1.95 | 1.83 | 1.53 | 1.41 |
|           | $R_d/V_{c,max}$ | 0.0067 | 0.0103 |     | 0.0085 |

$^a$ Warm period— from early May to end of June; cold period— from November to mid-April; see supplementary Fig. S1 at JXB online.

$^b$ Number of data used in the analysis.

$^c$ Fixed value (see the Materials and methods).

$^d$ Alternatively the following parameters: $E_i=28 149$, $D_i=474 614$, and $S_i=1482$ (with a temperature optimum of 41.7 °C) gave equal temperature sensitivities but values were rejected due to a high standard error of the estimate.

$^e$ Normalized to 25 °C.

temperature for $V_{c,max}$ at 38.7 °C (calculated from Equation A1 in the Appendix). For kenaf and cynara no optimum temperature was observed within the measurement range tested (18-41 °C; Fig. 6a). To explore any acclimation of $V_{c,max}$ to growth environments in cynara, the model was allowed to estimate different parameters for two contrasting seasons. No significant effect of the growing season on $\chi_v$ (65.8 versus 64.3; $P=0.094$) or on $E_a$, $D_r$, and $S_r$ parameters ($P=0.247$) was found, meaning little seasonal $V_{c,max}$ acclimation.

Step 5: $\kappa_{2LL}$ in relation to temperature and $N_a$

$\kappa_{2LL}$ was estimated indirectly from $\Phi_{CO2LL}$ information (see Equation A2). Correlations of $\kappa_{2LL}$ with temperature, light, and nitrogen were investigated afterwards. The results indicated poor correlations with $N_a$ ($r^2=0.26$, $P=0.025$), leaf temperature ($r^2=0.19$, $P=0.104$), and the combination of the above ($r^2=0.44$, $P < 0.01$; data not shown). However, better relationships were obtained when $\kappa_{2LL}$ was regressed against seasonal temperature ($r^2=0.40$, $P=0.004$) and radiation data ($r^2=0.34$, $P=0.003$), showing a long-term $\kappa_{2LL}$ acclimation. This became clearer when average $\kappa_{2LL}$ values per crop and per growth environments were considered (Fig. 7). These findings were supported fairly well by literature data (Fig. 7). Based on this analysis, average $\kappa_{2LL}$ values per species were considered in further analyses (including acclimation effect for cynara, Table 3).

Step 6: $J_{max}$ in relation to temperature and $N_a$

All $J_{max}$ temperature sensitivities (except kenaf; Table 3) were best described using Equation 6. Across species, $J_{max}$ temperature sensitivity was highly variable (Fig. 6b including other crops), while the maximum $J_{max}$ was obtained at lower temperature than the maximum $V_{c,max}$ (temperature optimum of 32, 42, and 33 °C for sunflower, kenaf, and cynara, respectively; Fig. 6). As a result, there was a decreasing trend of the $J_{max}/V_{c,max}$ ratio with increasing temperature (Fig. 8). For cynara, a significant ($P < 0.05$) temporal change was found for the $\chi_j$ parameter (Table 3). $\chi_j$ showed a larger variability (36% change) than $\chi_v$ (12% change) among species and growth environments studied (Table 3). The parameter $\theta$ was lower for sunflower (0.60) and higher for cynara (0.84), but close to the commonly used value of 0.75 in all cases. All these differences (including temperature and nitrogen sensitivities) among species and growth environments became smaller when the $J_{max}/V_{c,max}$ ratio was plotted against leaf temperature (Fig. 8).

Sensitivity and validation analysis

To investigate the uncertainty introduced into the estimates by the chosen Rubisco kinetic parameters, the initial values of Bernacchi et al. (2001) were increased or decreased by 20% and optimization procedures were repeated. Not
surprisingly, a maximum change was obtained in the estimated $V_{cmax}$, whereas the remaining parameters were less affected (<5%; data not shown). Given that even the maximum change in $V_{cmax}$ was ~11% in response to a 20% change, the parameter estimates were quite stable despite the uncertainties in values of Rubisco kinetic constants. A further analysis showed that the predicted $A_n$ was sensitive to a 20% decrease in $\chi_v$ and $\chi_p$, whereas its sensitivity to other changes was weak (Fig. 9).

Lastly, the models were validated against independent data sets (Fig. 10). Predictions versus observations for sunflower and kenaf were satisfactory ($r$RMSE < 0.15; Fig. 10a, b). For cynara the FvCB model was tested using measurements from a series of 24 h diurnal cycles (Fig. 10c), where stress conditions were unavoidably present (data sets outside the calibration range). In general, predictions were close to actual measurements, except for those data obtained from 14:00 h to 18:00 h, where a systematic overestimation was detected (Fig. 10c). The FvCB model responded to lowering temperature in late afternoon by increasing $A_n$; however, actual measurements indicated that the photosynthetic apparatus could not recover so quickly from the ‘photosynthesis midday depression’. The failure in predicting the depression and its after-effect during the recovery hours (Fig. 10c) might be attributed to the ‘steady-state’ character of the FvCB model. These results suggest that prediction of diurnal photosynthesis for species grown in the Mediterranean region requires more detailed approaches in which $g_{recovery}$ functions for $A_n$ (midday depression), and the effects of leaf water potential should be included (see Tuset et al., 2003; Vico and Porporato, 2008; Yin and Struik, 2009a).

Discussion

Use of $A_n$–$I_{inc}$ curves to parameterize the FvCB model

The FvCB model parameters, $J_{max}$ and $V_{cmax}$ in particular, have been predominantly estimated from $A_n$–$C_i$ data sets (Harley et al., 1992; Medlyn et al., 2002a). The value of $A_n$–$C_i$ curves for parameterizing the FvCB model is confirmed (Fig. 2). It was also shown that $V_{cmax}$ and $J_{max}$ can be estimated sufficiently well by an appropriate analysis of $A_n$–$I_{inc}$ data alone ($r^2$=0.91–0.93; Fig. 2). Unlike $J_{max}$, $V_{cmax}$ cannot always be estimated from $A_n$–$I_{inc}$ curves; that is when the entire curve is $A_j$ limited (Fig. 1). This is often observed in field crops (e.g. cotton; Wise et al., 2004). Actually, Boote and Pickering (1994) used only the $A_j$ equation of the FvCB model to calculate leaf photosynthesis in their canopy photosynthesis model. For the purpose of using the complete FvCB model, the two-step approach is proposed to estimate both $V_{cmax}$ and $J_{max}$ from $A_n$–$I_{inc}$ data. This is in line with the approach of Niinemets and Tenhunen (1997), but in contrast to that of Kosugi et al. (2003) and Müller et al. (2005) who assumed a fixed $J_{max}/V_{cmax}$ ratio of 2.1 at 25°C (based on Wullschleger, 1993) in their analyses. This assumption does not allow for the flexibility of the ratio as observed for different species or for the same species when grown under different environments, thereby introducing many uncertainties in parameter values (see Fig. 8 and discussion below).

The present results indicated that information from $A_n$–$I_{inc}$ curves has been underexplored. Use of $A_n$–$I_{inc}$ curves has an additional advantage in that data of $A_n$–$C_i$ curves may be uncertain due to CO₂ leakage during gas exchange
measurements when CO₂ set point values are either below or above the ambient air CO₂ level (Flexas et al., 2007). Crop modellers used to measure \( A_n - I_{inc} \) curves under an ambient CO₂ condition, upon which an empirical model for light-response curves is parameterized. Provided that values of \( C_i \) across \( I_{inc} \) levels are properly monitored, re-analysing readily
available $A_n$–$I_{inc}$ data to parameterize the FvCB model will strengthen photosynthesis calculations in crop models. This would expand the potential of combining classical photosynthetic data and the biochemical FvCB model to assess the impact of climate change on crop production and to examine options of bioenergy production under a changing climate.

On the other hand, caution should be exercised that use of $A_n$–$I_{inc}$ data sets does not allow the model to account for the TPU (triose phosphate utilization)-limited rate, the third limitation added by Sharkey (1985) to the FvCB model. TPU limitation sets an upper limit to the maximum photosynthetic capacity and is usually observed at high CO$_2$ and low O$_2$ levels (e.g. Wise et al., 2004), although many studies still ignore this limitation (e.g. Wohlfahrt et al., 1999). The limitation, if it occurs, can be easily identified, at the high end of $A_n$–C$_i$ curves, versus the Rubisco limitation that can be identified at the low end of $A_n$–C$_i$ curves. In the present study, where essentially $A_n$–$I_{inc}$ curves were used, it was not possible to detect this limitation, because both Rubisco and TPU limitations, if any, will occur at the high end of $A_n$–$I_{inc}$ curves. This is certainly the disadvantage of using $A_n$–$I_{inc}$ curves to parameterize the FvCB model. Fortunately, the present light response curves were obtained under ambient CO$_2$ conditions, so any TPU limitation, if it exists, can be assumed to be negligible under these measurement conditions. In the future climate where the ambient CO$_2$ level is expected to increase, the TPU limitation will be more likely to occur. Therefore, use of $A_n$–$I_{inc}$ curves to estimate FvCB model parameters needs to be tested across high CO$_2$ levels and a broad range of other environmental variables in order to decide how conserved these parameters are.

Below the effects of temperature, $N_a$, and season on photosynthesis and respiration parameters, all derived from the current $A_n$–$I_{inc}$ data for three bioenergy crops, are discussed. The present findings will be compared with those reported for the crops wheat, barley, cotton, tobacco, and grapevine based on $A_n$–C$_i$ or combined $A_n$–C$_i$ and $A_n$–$I_{inc}$ data sets, with attention to any conserved nature in these parameters among species.

Night and day respiration parameters: $\chi_{R0}$ $E_{R,0}$ $E_{R,0}'$ $b_{R0}$ and $a_R$

This study is among few in the literature providing direct $R_n$ measurements, underlining the great importance of respiration in carbon budgets (Valentini et al., 2000). The present estimates for $\chi_{R0}$ (range: 0.61–0.95 μmol CO$_2$ g$^{-1}$ N s$^{-1}$; Table 3) agree well with previous reports for crops (Hirose et al., 1997; Reich et al., 1998; Müller et al., 2005; Braune et al., 2009), but current values are almost double compared with those for trees (Bolstad et al., 1999; Griffin et al., 2002). The temperature sensitivity for respiration ($E_{R,n}$) was significantly correlated with $N_a$ in all species (Equation 11; Table 3), indicating that respiration in leaves with high $N_a$ values (young/sun leaves) was less sensitive to changes in temperature, while leaves with lower $N_a$ values were more sensitive (senescence/shade leaves). Griffin et al. (2002) and Bolstad et al. (1999) working with tree leaves that were positioned in different canopy layers—also having different $N_a$ values—found temperature sensitivities similar to those in the present study, while Turnbull et al.
For cotton, Harley et al. (1992) reported a simple temperature-sensitive \( R_n \) model for leaves with variable \( N_a \). The present analysis indicated that it is useful to calculate both \( R_n \) components as a function of \( N_a \) (e.g. Fig. 4; across all species, \( r^2 \) scaled from 0.53 to 0.77). The component \( R_{25} \) accounted for 27% and \( E_{Rn} \) for the other 5% of this improvement in \( r^2 \). However, the remaining unexplained variability in night data sets (see \( r^2 \) in Table 3; Fig. 4) means that apart from \( N_a \), other factors should be explored.

Unlike for \( R_n \), it is difficult to measure \( R_d \) directly as such measurements require sophisticated methodologies (e.g. Haupt-Herting et al., 2001; Pinelli and Loreto, 2003; Pärnık and Keerberg, 2007). Its value is empirically estimated indirectly using various methods (for a comparison see Yin et al., 2011), or is commonly fixed as 1% of \( V_{c,max} \) or as 50% of \( R_m \) (de Purry and Farquhar, 1997; Wohlfahrt et al., 1998; Medlyn et al., 2002a; Kosugi et al., 2003; Braune et al., 2009). Here, application of the Kok method (Sharp et al., 1984) indicated a 28% reduction in \( R_d \) compared with \( R_n \), an estimate which is positioned at the lowest reported range (light inhibition range: 24–90%; Buckley and Adams, 2011, and references therein).

Rubisco and electron transport parameters: \( N_{Dv} \), \( \chi_v \), \( \chi_o \), \( k_{2LL} \), \( \theta \), \( E_v \), \( E_p \), \( D_v \), and \( D_j \)

The present findings for \( N_b \) (Fig. 3) along with published data support the idea that this threshold value for photosynthesis is not affected by temperature (Sage and Pearcy, 1987; Makino et al., 1994; Niinemets and Tenhunen, 1997), \( CO_2 \) (Harley et al., 1992; Hirose et al., 1994), or irradiance levels (Makino et al., 1997). Excluding the statistical bias that usually exists in \( N_b \) estimations (see the Materials and methods) it is believed that a common \( N_b \) is 0.3–0.4 g N m\(^{-2}\) for C\(_3\) crop species (excluding legume crops; Supplementary Table S1 at JXB online). For use in modelling, it was shown that a ±20% change in the \( N_b \) value resulted in a <5% change in the predicted \( A_n \) (Fig. 9).

The relationships between \( A_n,max \) and \( N_a \) at near-optimum temperature ranges for sunflower, kenaf, and cynara (Fig. 3) agreed well with several non-legume C\(_3\) species (Supplementary Fig. S2 at JXB online). The observed decline in \( A_n,max \) at high temperature (Fig. 3; \( N_a >2 \) g N m\(^{-2}\)) is associated with \( g_m \) (Bernacchi et al., 2002) and/or \( V_{c,max} \) and \( J_{max} \) limitations of photosynthesis (Fig. 6). \( N_a \) and leaf temperature explained >81% of the temporal (seasonal) and spatial (within a crop canopy) variation in \( A_n,max \) values (Fig. 3). The remaining unexplained variability might be due to leaf adaptation to different microenvironments created by \( CO_2 \) and light gradients within crop stands (Buchmann and Ehlinger, 1998; Archontoulis et al., 2011). This may have an additional impact on \( J_{max} \) and \( V_{c,max} \) estimates and their ratio.

Nevertheless, the observed consistency among \( A_n,max-N_a \) plots (Supplementary Fig. S2 at JXB online) along with the similar \( \chi_v \) estimates for sunflower, kenaf, cynara, cotton, wheat, and barley (range: 60–82 µmol CO\(_2\) g\(^{-1}\) N s\(^{-1}\); Table 3;
Harley et al., 1992; de Pury and Farquhar, 1997; Müller et al., 2005, 2008; Braune et al., 2009) suggests that \( \chi_a \) is very conserved for this plant group (\( A_{n,\text{max}} = 30-35 \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \); Supplementary Fig. S2).

Unlike \( \chi_s \), \( \chi_j \) for the same group was highly variable (90–165 \( \mu \text{mol e}^{-} \text{ g}^{-1} \text{ N s}^{-1} \)). However, the parameter \( \chi_j \) (which determines \( J_{\text{max,2LL}} \); Equation 10) is not independent of, but interrelated to, the values of \( \kappa_{2LL} \) and \( \theta \) (see Equation 4). This means that use of constant \( \kappa_{2LL} \) and \( \theta \) values across species and environments will bias \( J_{\text{max}} \) estimates and therefore the \( \text{J}_{\text{max}}/V_{\text{cmax}} \) ratio. Among sunflower, kenaf, and cynara, \( \chi_j \) varied by 36%, \( \kappa_{2LL} \) by 39%, and \( \theta \) by 28%, but in different directions (Table 3). When \( \kappa_{2LL} \) was fixed to 0.3 and \( \theta \) to 0.7 (commonly assumed values; de Pury and Farquhar, 1997; Medlyn et al., 2002a), the \( \chi_j \) variation among crops and growing environments became smaller (15%), and the \( \text{J}_{\text{max}}/V_{\text{cmax}} \) ratio less variable.

The present analysis showed that variation in the electron transport rate among bioenergy crops followed changes in environmental conditions during growth (Supplementary Fig. S1 at JXB online), with higher \( J \) rates for cynara in low light (<700 \( \mu \text{mol m}^{-2} \text{s}^{-1} \); winter period) and higher \( J \) rates for sunflower and kenaf in high light conditions (>700 \( \mu \text{mol m}^{-2} \text{s}^{-1} \); summer period; Table 3, Equation 4). This is consistent with recent findings for tobacco (Yamori et al., 2010) where plants grown under low light enhanced the efficiency of light acquisition while those grown under high light enhanced the capacity of light utilization, through changes in chlorophyll contents, the chlorophyll \( \alpha/b \) ratio, and cytochrome \( f \) and Rubisco contents.

In studies of Wullschleger (1993), Dreyer et al. (2001), and Medlyn et al. (2002a) the \( \kappa_{2LL} \) was fixed as a constant at 0.18, 0.24, and 0.30, respectively, across species, crop stages, and environments. However, Yin et al. (2009) directly demonstrated a positive relationship between \( \kappa_{2LL} \) and \( N_a \), which was confirmed by the results of a model curve-fitting procedure (Müller et al., 2005; Braune et al., 2009; Yamori et al., 2010).

In Fig. 7, \( \kappa_{2LL} \) information for eight crops is summarized and this large variation is interpreted in the light of long- or short-term response to temperature or irradiance. Across species, the highest \( \kappa_{2LL} \) values were found in crops grown under long-term low irradiance and temperature conditions (Fig. 7a, c). To understand this, it is necessary to underline the components of the \( \kappa_{2LL} \) parameter (see Equation A3 derived by Yin et al., 2004, 2009; Yin and Struik, 2009a; also see equation 6 in Niinemets and Tenhunen, 1997). The fraction of \( I_{\text{inc}} \) absorbed by the leaf photosynthetic pigments (parameter \( \beta \) in Equation A3) is affected by long-term changes in light and temperature through its changes in leaf morphology. Leaves grown at high temperature are generally thinner, with a lower ability to absorb light (Poorter and Evans, 1998; Yamori et al., 2005), therefore providing a reasonable explanation for the observed \( \kappa_{2LL} \) reduction with increasing temperature. On the other hand, leaves grown at high irradiance are thicker (Niinemets and Tenhunen, 1997), indicating that \( \kappa_{2LL} \) variation is much more complex and still not fully understood. Nonetheless, caution should be exercised when modelling canopy photosynthesis based on the sun/shade approach (de Pury and Farquhar, 1997; Yin and van Laar, 2005) because \( \kappa_{2LL} \) increases with increasing \( N_a \) (Fig. 7d), while \( \kappa_{2LL} \) also increases with decreasing light (Schultz, 2003; shade leaves which generally have low \( N_a \) values; Fig. 7b).

The normalized temperature functions of \( V_{\text{cmax}} \) and \( J_{\text{max}} \) were variable across crops (Fig. 6), particularly above 30°C, in line with Leuning (2002). This means that the assumption used in crop modelling, a unique \( A_n \) response to temperature across crop species, is inappropriate when photosynthesis is calculated by the FvCB model. In the case of no available data, it is suggested that researchers as a first approximation use \( V_{\text{cmax}} \) and \( J_{\text{max}} \) temperature parameters from species that belong to the same family (see Fig. 6; cotton and kenaf belong to Malvaceae; sunflower and cynara to Asteraceae).

The \( \text{J}_{\text{max}}/V_{\text{cmax}} \) ratio provides an estimate of the relative activities of RuBP regeneration and Rubisco carboxylation, and incorporates both temperature and \( N_a \) effects. This study confirms (Table 3) the generally reported \( \text{J}_{\text{max}}/V_{\text{cmax}} \) value of 2.0±0.5 (Wullschleger, 1993; Poorter and Evans, 1998; Bunce, 2000; Leuning, 2002; Medlyn et al., 2002a). However, this ratio should not be considered constant in absolute terms. \( V_{\text{cmax}} \) is dependent on the Rubisco parameters used (up to 11% change; see also Medlyn et al., 2002a) and \( J_{\text{max}} \) is affected by the assumed \( \kappa_{2LL} \) and \( \theta \) values used (see earlier discussion). For instance, grape showed a much higher \( \text{J}_{\text{max}}/V_{\text{cmax}} \) ratio compared with other crops (Fig. 8). Apart from the effect of species, there are two possible artefacts causing this: the different Rubisco parameters used in that study (Schultz, 2003) and the lower grape \( \kappa_{2LL} \) values compared with the other crops (Fig. 7b). Also use of \( C_l \) instead of \( C_c \) affects this ratio. Thus approaches (e.g. Kosugi et al., 2003; Müller et al., 2005) that fix the \( \text{J}_{\text{max}}/V_{\text{cmax}} \) ratio at a constant value to parameterize the FvCB model should receive critical evaluation.

**Seasonal effects on photosynthesis and respiration in cynara**

Direct interpretation of the seasonal effects on \( A_n \) and \( R_n \) for cynara is difficult because both the climate (Supplementary Fig. S1 at JXB online) and the plant stage are different, with new and old leaves being present (Archontoulis et al., 2010a; Searle et al., 2011). \( R_n \) acclimated to cold and warm environments to a larger extent than did \( A_n \) (Table 3; Fig. 4). This is consistent with previous studies (Yamori et al., 2005; Ow et al., 2008; Silim et al., 2010).

The nature of \( R_n \) acclimation is variable within and among plant species, and it is usually related to changes in \( E_{\text{Rn}} \) and/or to changes in \( R_{n25} \) (Atkin et al., 2005; Searle et al., 2011). Given that \( E_{\text{Rn}} \) did not change between seasons (\( P=0.269; \) Table 3) and that the measured winter leaves had higher \( N_a \) values than the summer leaves (on average 2.48 versus 1.53 g N \text{ m}^{-2}; see also Fig. 7d), this indicates that basal capacity, \( R_{n25} \), plays an important role in this acclimation. Secondly, \( E_{\text{Rn}} \) was also higher during winter
periods. Apparently, cynara follows an ‘acclimation type II’ (Atkin et al., 2005) where the overall elevation of the  \( R_n \)–
temperature response was affected by season and growth stage (Fig. 4).

Among FvCB parameters analysed, seasonal effects were found on two electron transport parameters,  \( \chi_d \) and  \( \kappa_{2LL} \) (Table 3 and earlier discussion), and none related to  \( V_{cmax} \). Literature information on  \( A_n \) acclimation is diverse among studies (Wilson et al., 2000; Medlyn et al., 2002a; Bernacchi et al., 2003; Hikosaka, 2005; Yamori et al., 2005; Braune et al., 2009; Silim et al., 2010). As far as is known, only Wilson et al. (2000) reported both  \( \chi_d \) and  \( \chi_e \) seasonal changes in trees, while Braune et al. (2009) found only  \( \chi_d \) variation for barley as in the present study. For cynara, the normalized  \( V_{cmax} \) and  \( J_{max} \) temperature functions were slightly changed between seasons, in line with other field studies (Medlyn et al., 2002b; Schultz, 2003), but in contrast to growth chamber studies (Bernacchi et al., 2003; Yamori et al., 2005; Ishikawa et al., 2007; Braune et al., 2009) where plants were grown only at different temperatures. The fact that this study assessed leaves with different  \( N_L \) status may be a reason, but an inconsistency between actual field and controlled chamber studies is obvious.

The  \( J_{max}/V_{cmax} \) ratio has been reported to be either sensitive or insensitive to growth temperature (see discussion by Hikosaka et al., 2005), growth irradiance (Poorter and Evans, 1998; Yamori et al., 2010), and seasonal changes (Bunce, 2000; Medlyn et al., 2002b). The present results suggest that cynara regulates the balance between RuBP regeneration and Rubisco carboxylation to maintain the  \( J_{max}/V_{cmax} \) ratio almost constant (change <8%; Table 3) across seasons and growth stages.

Conclusions

This study provides new information on photosynthesis and respiration rates for three bioenergy crops, sunflower, kenaf, and cynara. It provides an alternative way to parameterize the FvCB model from  \( A_n-I_{inc} \) data, instead of using  \( A_n-C_i \) data that are more expensive to obtain. It was shown that major FvCB model parameters,  \( V_{cmax} \) and  \( J_{max} \), derived from either  \( A_n-C_i \) or  \( A_n-I_{inc} \) analysis, are very close (\( r^2=0.92 \)). Present models can predict photosynthesis under varying levels of  \( C_i \),  \( I_{inc} \), temperature, and leaf nitrogen, and can estimate night respiration under varying levels of temperature and leaf nitrogen, for the three bioenergy crops. Comparisons of FvCB model parameters among sunflower, kenaf, cynara, cotton, wheat, barley, tobacco, and grapevine indicated that only a few parameters were conserved. This means that in order to feed crop models properly, species-specific FvCB model parameters are needed. In this context, readily available  \( A_n-I_{inc} \) data—that have been underexplored—can assist in this respect. By combining classical photosynthetic data and the biochemical model, the potential of crop growth models to assess the impact of climate change on crop production and to examine options of bioenergy production under a changing climate is enlarged. Further research is needed to quantify reliably the effects of photosynthetic acclimation and diurnal midday depression identified in this study.

Supplementary data

Supplementary data are available at JXB online.

Figure S1. Average monthly temperatures, radiation, and precipitation at the experimental site (period: 2007–2009). Sunflower measurements were taken from July to August; kenaf measurements from July to September, and cynara measurements from November to June.

Figure S2. Reported relationships between light-saturated net assimilation rate at ambient CO\(_2\) concentration and at near-optimum temperature (\( A_{n,max} \) in \( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \)) and leaf nitrogen content (g N m\(^{-2}\)) for C\(_3\) crops (a), C\(_3\) legume crops and trees (b), and C\(_4\) crops (c). (d) An average relationship for C\(_3\) and C\(_4\) crops.

Table S1. Reported  \( N_L \) values (minimum leaf nitrogen for photosynthesis, in g N m\(^{-2}\)) for various species.

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Appendix

1. Estimating the optimum temperature from the peaked Arrhenius equation

The optimum temperature for  \( V_{cmax} \) or  \( J_{max} \) in Equation 6 is given by the following equation (Medlyn et al., 2002a):

\[
T_{opt} = \frac{D_n}{S_n - R \cdot \ln\left( \frac{E_n}{P_n-E_n} \right)} \quad (A1)
\]

2. The relationship between  \( \kappa_{2LL} \) and  \( \Phi_{CO2LL} \)

By dividing both parts of Equation 3 by  \( I_{inc} \) and re-arranging, the efficiency of incident light conversion into e\(^{-} \),  \( \kappa_{2LL} \), can be calculated mathematically from  \( \Phi_{CO2LL} \):

\[
\Phi_{CO2LL} = \frac{A_n + R_d}{I_{inc}} \bigg|_{I_{inc}=0} = \frac{\kappa_{2LL}}{4C_i + 8T_s} \frac{C_i - T_s}{C_i}
\]

\[
\Leftrightarrow \kappa_{2LL} = \frac{\Phi_{CO2LL} \cdot 4C_i + 8T_s}{C_i - T_s} \quad (A2)
\]

This approach was also used by Niinemets et al. (2001), but lacks any further interpretation.
3. Components of parameter $\kappa_{2LL}$

Yin et al. (2004) described a generalized stoichiometric equation for $A_i$, where the linear photosystem II (PSII) electron transport rate $(J)$ was replaced by the total electron transport rate passing PSII $(J_2)$ and fractions of the total $e^-$ flux passing PSI (cyclic $(f_{cyc})$ and pseudocyclic $(f_{pseudo})$ pathways). Again, under low light conditions, dividing $J$ by $I_{inc}$ yields $\kappa_{2LL}$ as follows (Yin and Struik, 2009a; Yin et al., 2009):

$$\kappa_{2LL} = \frac{J}{I_{inc}} = \frac{J_2}{I_{inc}} \left( 1 - \frac{f_{pseudo}}{1 - f_{cyc}} \right) = \rho_2 \beta \Phi_{2LL} \left( 1 - \frac{f_{pseudo}}{1 - f_{cyc}} \right)$$

(A3)

By definition, the variable $J_2$ can be replaced by the term $\rho_2 \beta \Phi_{2LL} I_{inc}$, where $\rho_2$ is the fraction of absorbed irradiance partitioned to PSII (usually assumed to be 0.5), $\beta$ is the fraction of $I_{inc}$ absorbed by the leaf photosynthetic pigments, and $\Phi_{2LL}$ is the PSII $e^-$ transport efficiency under limiting light.

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