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ORIGINAL ARTICLE

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Photosynthetic light response curves determined with the leaf oxygen electrode: minimisation of errors and significance of the convexity term

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Abstract From photosynthetic studies on a range of monocotyledonous (C-3 and C-4) and dicotyledonous (C-3) plants using a leaf oxygen electrode, we conclude the following. (i) A non-linear model [J.H.M.Thornley (1976) Mathematical models in plant physiology, Academic Press, London; B. Marshall and P.V. Biscoe (1980) J Exp Bot 31:29–39] significantly better describes the photosynthetic light response curve [rate of photosynthesis (*P*) versus incident photosynthetic photon flux density (*I*)] than the frequently used linear hyperbolic model [E.I. Rabinowich (1951) Photosynthesis and related processes, vol 2, Wiley, New York]. (ii) When used at the recommended CO_2 partial pressures ($C_a = 1–5$ kPa), CO_2 supply saturates the photosynthesis rate in the C-3 dicot *Phaseolus coccineus* L. but not in the C-3 monocot *Hordeum vulgare* L.. (iii) Fits using a linear hyperbolic model for *P* versus *I* produce relatively large and statistically significant errors (≈60%) in the estimation of P_{max} and quantum efficiency (α) if C_a is not > 5 kPa. (iv) The convexity term, θ, incorporated into the non-linear models for *P* versus *I* appears to reflect the limitation placed on the carboxylation processes by the supply of CO_2 to the chloroplast stroma. Therefore, the use of a non-linear model providing an estimate of θ should be encouraged, as it is likely to provide information on the physiological status of plants.

Keywords Convexity term · *Hordeum* (photosynthesis) · Light response curve · *Phaseolus* (photosynthesis) · Photosynthesis · *Zea* (photosynthesis)

Abbreviations C_a: CO_2 partial pressure of leaf chamber (air) · *I*: incident photosynthetic photon flux density (PPFD) · IRGA: infrared gas analyser · LRC: light response curve · *P*: photosynthesis rate · *P*n: net photosynthesis rate · *P*g: gross photosynthesis rate · α quantum efficiency · *R*d: dark respiration rate · θ: convexity term.

Introduction

Photosynthesis rates are often used as measures of the well being of plants and so their determination is important in many disciplines of plant science research. Frequently, one of two methods is used to determine photosynthesis rates. In laboratories where the main focus of interest is photosynthesis, infrared gas analysers (IRGAs) are routinely used as they have excellent sensitivity and are versatile. However, IRGAs and their accompanying gas mixing/delivery systems are expensive, and are, therefore, not the choice for the majority of plant biologists whose primary interests lie outside of photosynthesis. Consequently, the leaf O_2 electrode, which is relatively inexpensive and simple to operate, has gained wide acceptance in many laboratories where photosynthesis rates are only occasionally measured.

The leaf O_2 electrode is a closed-chamber system that was first introduced by Delieu and Walker (1981). A detached leaf, or leaf piece, is sealed in a chamber containing an atmosphere that should saturate the CO_2 requirement for photosynthesis (> 1 kPa partial pressure CO_2). As there is no simple way of regulating CO_2 levels

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during an experiment, photosynthesis rates (P) are usually measured as a function of incident light intensity (I), i.e. light response (P versus I) curves (LRCs), are often measured.

Several methods can then be used to determine important photosynthetic parameters such as the apparent quantum yield of photosynthesis (α'), the maximum photosynthesis rate ($P_{\max}$) and the dark respiration rate (Rd). Often, a linear hyperbolic relationship between P and I is assumed (e.g. Rabinowich 1951; see Eq. 1), and α' is estimated by forcing a linear regression line through the first few low-light data points. Such practice can incur large errors because (i) the initial curve may not be truly linear, (ii) only a few data points are used in the regression, and (iii) the values of P and I associated with these data points are small and so errors of measurement are comparatively large. Similarly, $P_{\max}$ is estimated by forcing a line parallel to the I axis through a few data points considered to lie on the asymptote, and this may also incur errors as, again, only a few data points are used, and true light saturation may not have been achieved. An alternative approach that is often used relies on transformation of the linear hyperbolic data to give a linear function (e.g. plots of I/P versus I; $\alpha' = 1/\text{intercept}$, $P_{\max} = 1/\text{gradient}$). In this case it is argued that errors are reduced as all of the data points are used to estimate α' and $P_{\max}$.

Several workers, however, have pointed out that Pg (gross photosynthesis rate) versus I plots rarely conform to a linear hyperbolic model such as that given in Eq. 1. Often, a non-linear (quadratic) function (see Eq. 2), which allows for the often-observed sharp discontinuity between the initial near-linear slope (α') and the final near-linear asymptote ($P\text{g}_{\max}$), better describes LRCs. This discontinuity provides an additional photosynthetic parameter θ, or the convexity term, which can vary between a value of 0 and 1. When $\theta = 0$, the curve of Pg versus I degenerates into a linear hyperbolic function; when $\theta = 1$, the relationship converts to a Blackman response (for a comprehensive review of the non-linear models, see Thornley 1976).

Several quadratic models have been developed, and in all cases these models provide a better fit to the data than linear models. However, there is some confusion over the significance of θ, and in some cases this has resulted in a misuse of the model, or a misinterpretation of the data.

Initially the quadratic models were derived from simple mechanistic principles (Rabinowich 1951; Chartier 1966, 1970) and incorporated terms for the conductance of CO_2 across the leaf to the site of carboxylation and the rate of C-3 cycle turnover. For example, Thornley (1976), and later Marshall and Biscoe (1980), who extended the model to describe the Pn (net photosynthesis rate) versus I relationship, viewed θ as a parameter that expresses the relative limitations placed on Rubisco turnover by two processes (see Eqs. 2 and 3, respectively). These limitations are the supply of NADPH, ATP and ribulose 1,5-bisphosphate (the 'biochemical processes'), and the supply of CO_2 (the 'physical processes'), which is controlled by the CO_2 diffusion gradient and conduction pathways into the leaf. When $\theta = 0$, only the biochemical processes are limiting; when $\theta = 1$, only the physical processes are limiting. Clearly, in these derivations, θ is a function of the efficiency of the carboxylation processes.

However, this view of the physiological significance of θ has been challenged (Terashima and Saeki 1983; Leverenze 1987, 1988; Vogelman et al. 1989). These groups have provided evidence that both leaf development and leaf architecture can cause changes in the light gradient penetrating into green tissues, and proposed that these give rise to changes in θ. More recent work at near-ambient CO_2 levels has suggested that changes in the intensity and direction of illumination during growth can affect θ, but also provide evidence for a strong dependence of θ on atmospheric CO_2 partial pressures (C_a; Ogren 1993; Ogren and Evans 1993; Palmquist et al. 1994).

Given the ambiguity surrounding the functional significance of θ, many workers have preferred to use a non-linear quadratic model to provide a better fit to the data, and then to ignore the value of θ. In these cases, θ is merely used as a fitting factor and no functional significance is assigned to it. Some workers prefer to go even further and ensure that the magnitude of θ exerts no influence on that of α'; in such cases, α' is usually estimated separately using linear regression, whilst $P_{\max}$ is derived from the non-linear model (e.g. Leith and Reynolds 1987; Stirling et al. 1994; Cannell and Thornley 1998). It should be noted that from a purely theoretical viewpoint, θ exerts no influence on the magnitude of either α (at $I = 0$) or $P_{\max}$ (at $I = \infty$), but in practice regression through a finite number of data points will generate some dependence.

Most of the studies mentioned above have investigated the LRCs of samples at ambient CO_2 partial pressures (300–700 Pa), and there is very little published data on the behaviour of the P versus I response at the higher levels commonly used with the leaf oxygen electrode ($\approx$1–5 kPa). In our laboratory, we have observed that the LRCs from a range of monocotyledonous and dicotyledonous species, determined using the leaf O_2 electrode, rarely conform to either a linear hyperbolic ($\theta = 0$) or a Blackman ($\theta = 1$) relationship. Therefore, a non-linear quadratic formulation such as that proposed by Thornley (1976) or Marshall and Biscoe (1980) may provide a better description of the dependence of P on I. The observation that a linear hyperbolic response ($\theta = 0$) is rarely observed with leaf O_2 electrodes when the recommended chamber CO_2 partial pressures are used ($C_a < 5$ kPa) may be interpreted in two ways. One explanation is that CO_2 is saturating (as claimed by Delieu and Walker 1981) but that θ is unrelated to the CO_2 supply (as claimed by Terashima and Saeki 1983; Leverenze 1987, 1988; Vogelman et al. 1989). Alternatively, CO_2 supply limits photosynthesis when C_a is < 5 kPa, and θ is indeed a function of CO_2 supply (as claimed by Thornley 1976; Marshall and Biscoe 1980). Whichever is the case, it is clear that methods for the extraction of $P_{\max}$ and α' from P versus I curves based on an assumption of a linear hyperbolic relationship ($\theta = 0$) are incorrect and will incur errors that may be unacceptably large.

In this paper we report the results from a series of experiments designed to investigate fully the nature of the LRCs determined with leaf O_2 electrodes. Barley, bean (both C-3) and maize (C-4) plants were used to study the relationship between θ and C_a, and the accuracy of the models describing Pn versus I plots was assessed. Firstly, we show that changes in chamber C_a have a very strong and significant effect on θ. Second, we show that serious errors arise when $P\mathrm{n}_{\max}$ and α' are determined from data assuming a linear hyperbolic relationship at the recommended levels of C_a, and we go on to make suggestions on how to minimise these errors.

Materials and methods

Plant material

Seeds of barley (*Hordeum vulgare* L. cv. Golden Promise), scarlet runner bean (*Phaseolus coccineus* L. cv. Scarlet Emperor), and maize (*Zea mays* L. cv. Kelvedon Glory), were germinated on moistened filter paper and incubated in the dark for 5 days in a growth cabinet (19 ± 2 °C). Seedlings, selected for uniformity, were transplanted singly in 15-cm (barley and bean) or 18-cm (maize) plastic pots containing Levington potting compost and located in a growth cabinet maintained at 19 ± 2 °C, which provided a relative humidity between 60 and 70% and a photoperiod of 16 h (130 µmol quanta m^{-2} s^{-1}).

Photosynthetic measurements

Photosynthesis rates were determined from measurements of O_2 evolution using the Hansatech LD2 leaf-disc electrode system (Hansatech, King's Lynn, Norfolk, UK) designed by Delieu and Walker (1981, 1983).

All measurements were made at a constant temperature (25 ± 0.05 °C). Illumination of the chamber was provided by a 50-W, 24°-dispersion, halogen dichroic lamp (Osram), emitting through a water bath to remove infrared heat. Neutral-density filters (Balzers, Liechtenstein) were used at the surface of the chambers to attenuate the light to the desired levels (0–1,363 µmol quanta m^{-2} s^{-1}). Fresh leaf samples were cut (6–10 cm^2) just prior to use and placed in the thermostatted chamber. Chamber volumes were then determined using the volume injection method (Delieu and Walker 1981). Gas mixtures containing the desired CO_2 partial pressures (1, 5 and 10 kPa) were produced using cylinders of compressed air and 10% CO_2 in compressed air. The mixtures were humidified at room temperature (ca. 20 °C) by bubbling through water traps before passing to the leaf chambers. The chambers were purged with the desired gas mixture at 100 ml min^{-1} for 3 min to equilibrate before sealing the valves. All measurements were made as follows. Samples were initially irradiated at 150 µmol quanta m^{-2} s^{-1} for approximately 5 min, and then left to dark-adapt for at least 15 min until a steady rate of dark respiration was attained. The chamber was then flushed for 3 min with fresh gas, sealed, and the sample then exposed to a new light level until a steady-state rate of O_2 evolution was reached. The chamber was flushed with fresh gas before measurement at each new light level; generally, illumination was altered sequentially, from dark to high light.

Data analyses

Two models were used in this study to calculate photosynthetic parameters.

The linear hyperbolic model

This model was proposed by Rabinowich (1951), and predicts that at high CO_2 partial pressures, photosynthesis rates are governed by light intensity. Three parameters are included: $P_{\max}$ (gross or net), the maximum rate of photosynthesis; α, the quantum efficiency; and Rd, the dark respiration rate.

$$P = \frac{P_{max}\alpha I}{P_{max} + \alpha I} \tag{1}$$

where P is the photosynthesis rate, $P_{\max}$ is the maximum rate of (gross or net) photosynthesis, α is the quantum efficiency at low irradiance, and I is the irradiance (photosynthetic photon flux density, PPFD). The gross and net photosynthesis rates (Pg and Pn, respectively) are related as $Pg = Pn + Rd$, where Rd is the rate of dark respiration.

The non-linear model

Thornley (1976) pointed out that the observed light response curve is better described by a quadratic function, viz:

$$\theta Pg^2 - (Pg_{\max} + \alpha I)Pg + (Pg_{\max}\alpha I) = 0 \tag{2}$$

where θ is the convexity term. Marshall and Biscoe (1980) extended this model to describe Pn versus I viz:

$$\theta Pn^2 - (Pg_{\max} + \alpha I - \theta Rd)Pn + (\alpha I(Pg_{\max} - (1-\theta)Rd) - RdPg_{\max} = 0 \tag{3}$$

$P\mathrm{n}_{\max}$ is calculated as $P\mathrm{n}_{\max} = P\mathrm{g}_{\max} - (1-\theta)R\mathrm{d}$ (Marshall and Biscoe 1980). When θ is zero, Eq. 3 degenerates into Eq. 1; when it is unity, Eq. 3 describes a Blackman curve. Equation 3 is a quadratic (second-order polynomial) of the form

$$y = ax^2 + bx + c = 0 \tag{4}$$

that can be solved for its roots using

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \tag{5}$$

Equation 5 was used to solve for $P\mathrm{g}_{\max}$, α, θ and Rd using the non-linear Solver routine in Microsoft Excel version 5 (conjugate method, central derivatives, quadratic estimate, scaled); solutions were found by minimising $\sum x^2$ calculated for the observed and fitted P values by successive iteration. Values for $P\mathrm{n}_{\max}$ were calculated using the relationships given above. In addition, the linear hyperbolic model (Eq. 1) was fitted to the same data sets using both an iterative method (Solver) and linear regression techniques after transformation to a linear form. An Excel 5.0 spreadsheet for solving P versus I plots using Eqs. 1, 2 and 3, and instructions, can be downloaded from http://www.gla.ac.uk/ibls/bmb/pjd/lrc.html.

Analysis of variance was performed using MINITAB's General Linear Model (version 10).

Results

The relationships between incident light intensity and net photosynthetic oxygen evolution when bean, barley and maize leaves were exposed to 1, 5 and 10 kPa partial pressure CO_2 are presented in Fig. 1. We determined by

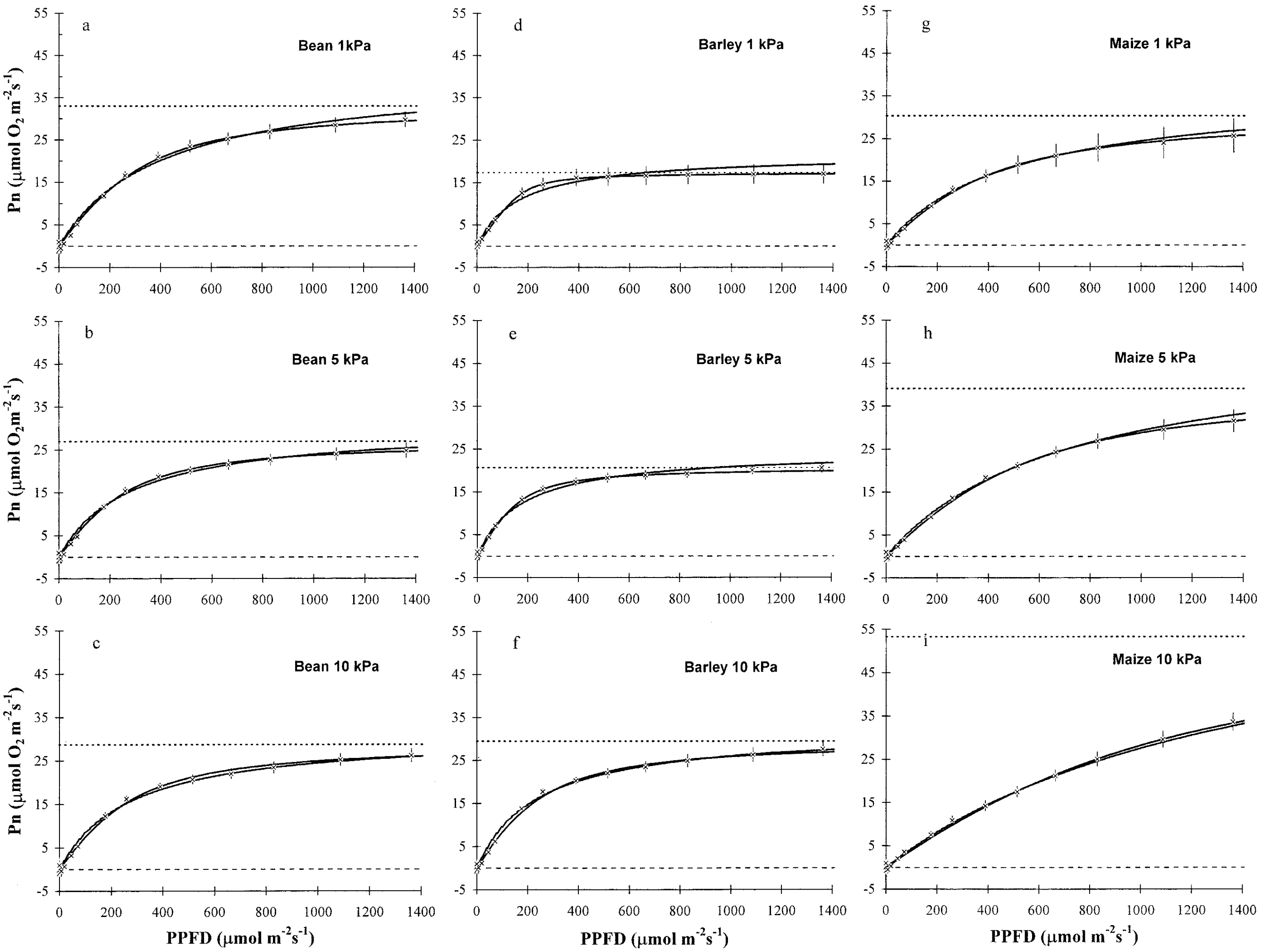

Fig. 1 Photosynthetic light response curves for bean (*Phaseolus coccineus*; **a–c**), barley (*Hordeum vulgare*; **d–f**) and maize (*Zea mays*; **g–i**) measured with leaf oxygen electrodes. Samples were taken at random from plants in a growth room and immediately placed in the thermostatted leaf chamber (25 ± 0.05°C) and exposed to the initial light and dark pre-adaptation regime as described in the text. The chambers were flushed for 3 min (100 ml min^{-1}) with fresh humidified gas of 1 kPa (**a, d, g**), 5 kPa (**b, e, h**) or 10 kPa (**c, f, i**) partial pressure CO_2 in air. The chambers were then sealed and exposed to one of the light levels indicated until a steady rate of net oxygen exchange (Pn) was attained. The chambers were then flushed with fresh gas and Pn determined at another light level. Each panel presents the average and standard errors of the pooled data from five separate response curves determined from five separate leaves. Superimposed on these data are the fitted responses: *solid line* non-linear model fit (Eq. 3), *broken line* linear hyperbolic model (Eq. 1)

IRGA that, when used according to the published instructions (Delieu and Walker 1981), C_a could vary between 1 and 5 kPa (data not presented); in this study we chose to use levels that span this range. Each curve in Fig. 1 is the average of five separate plots (± standard errors) that were determined from five separate leaves. Table 1 presents a summary of the differences in $P\text{n}_{\text{max}}$, $P\text{g}_{\text{max}}$, α', θ and Rd for each species at each CO_2 partial pressure.

The effect of C_a on photosynthesis

The effects of changes in C_a on the photosynthetic parameters determined by the non-linear model are presented in Table 1 and Fig. 1. Increasing the CO_2 partial pressure from 1 to 10 kPa produced no significant ($P > 0.05$) increase in $P\text{n}_{\text{max}}$ for bean. In contrast, $P\text{n}_{\text{max}}$ for maize and barley increased significantly ($P < 0.01$) over the same CO_2 concentration range; a similar pattern of change was observed when the effect of C_a on the maximum gross photosynthesis rate was examined (Table 1). Increasing C_a also produced significant ($P < 0.05$) changes in the apparent quantum efficiency, α', for barley and bean, but not for maize. No significant changes ($P > 0.05$) were observed for the dark respiration rates (Rd) for any of the species with increasing CO_2 partial pressures. However, C_a had a dramatic effect on the convexity term θ (Table 1, Fig. 2). Increasing C_a from 1 to 10 kPa produced a significant ($P < 0.01$) linear decrease in θ (from 0.697 to 0.222 for bean, from

Table 1 Summary of the statistical analyses of the effects of CO_2 partial pressures on photosynthetic parameters from bean (*Phaseolus coccineus*), barley (*Hordeum vulgare*) and maize (*Zea mays*). The non-linear model of Marshall and Biscoe (1980) was used to determine values for Pg_{max}, Pn_{max}, α', θ and Rd from each of the 45 light response curves presented in Fig. 1. Analysis of variance was performed on each of these parameters using the GLM routine in Minitab 10.0. Values presented are the normal analysis of variance (ANOVA) probabilities (P); *n.s.* not significant

	Comparisons between CO_2 levels (kPa)	Pn_{max}	Pg_{max}	α'	θ	Rd
Bean	1 vs. 5	n.s.	n.s.	n.s.	0.01	n.s.
	5 vs. 10	n.s.	n.s.	n.s.	0.05	n.s.
	1 vs. 10	n.s.	n.s.	0.05	0.01	n.s.
Barley	1 vs. 5	n.s.	n.s.	0.05	0.01	n.s.
	5 vs. 10	n.s.	n.s.	n.s.	0.01	n.s.
	1 vs. 10	0.01	0.01	n.s.	0.01	n.s.
Maize	1 vs. 5	0.01	0.01	n.s.	0.01	n.s.
	5 vs. 10	0.01	0.01	n.s.	0.01	n.s.
	1 vs. 10	0.01	0.01	n.s.	0.01	n.s.

0.936 to 0.352 for barley and from 0.686 to 0.162 for maize).

Comparison of the models

Between 1 and 5 kPa partial pressure CO_2 (Fig. 1a, b, d, e, g, h), the levels likely to be present in the chambers when leaf O_2 electrodes are used as recommended (DW2 Handbook 1984; Delieu and Walker 1981, 1983), the linear hyperbolic model tended to under-estimate photosynthesis in the 100–500 μmol m^{-2} s^{-1} PPFD light range, and over-estimate photosynthesis in the low- and high-PPFD range. Therefore, one consequence of using the linear model will be the persistent over-estimation of α', the apparent quantum efficiency. For all data sets, the observed data were used to fit both the linear and non-linear models, and in all cases it was found that the linear model gave a poorer fit ($n=45$). An analysis of variance test was performed on the residual errors for each fit and a significant reduction was found with the non-linear model ($P<0.004$).

Figure 3 shows that the discrepancies generated by the two models were most noticeable at lower CO_2 partial pressures. As CO_2 levels were increased, the average residual errors that arose from the linear hyperbolic model decreased, so that in no case at 10 kPa CO_2 was any significant difference ($P>0.05$) observed between the linear and non-linear model.

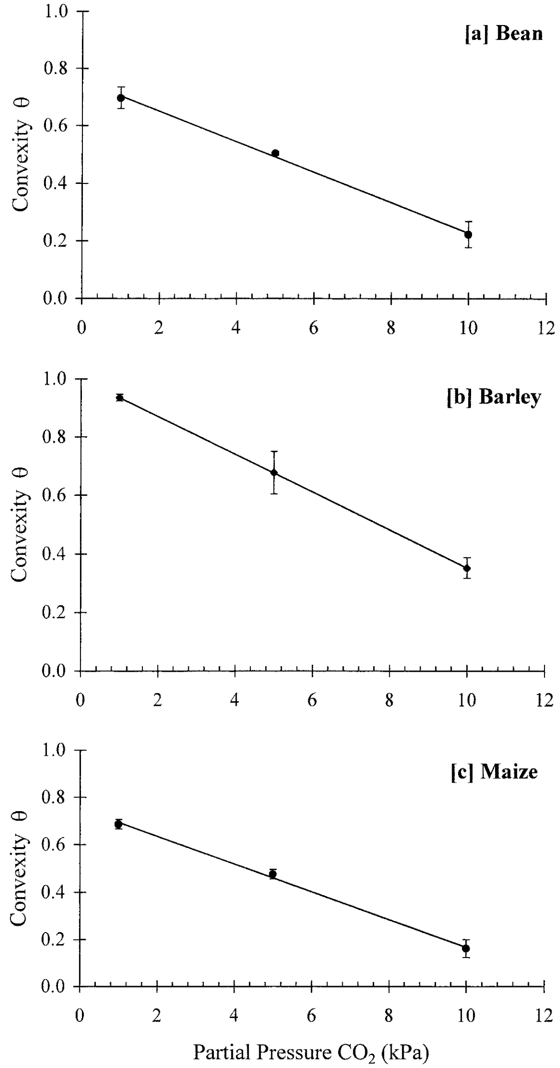

Fig. 2 The effect of CO_2 partial pressures on the convexity parameter, θ, for bean (**a**), barley (**b**) and maize (**c**). Each datum point represents the average (±SE) value from five separate determinations of θ at each CO_2 partial pressure

Discussion

In this report we provide evidence that for all three species studied here, θ is strongly inversely related to external CO_2 partial pressures. These observations are consistent with the assertion originally proposed by Thornley (1976) and Marshall and Biscoe (1980) that θ reflects the relative importance of CO_2 supply to the site of carboxylation in the turnover of the C-3 cycle. For the experiments we report here, leaf pieces were randomly taken from plants in the growth room just prior to measurement, and so the differences in θ cannot be attributed to differences in the developmental patterns and light gradients within leaves.

A dependence of θ on C_a has been reported before (Ogren and Evans 1993). In their experiments θ was estimated from measurements on CO_2 exchange using four different partial pressures of CO_2 ($\approx$20, $\approx$40, 100 and 5,000 Pa) and was shown to decrease as C_a was increased from 20 to 100 Pa. However, on changing C_a from 100 to 5,000 Pa, θ increased from $\approx$0.6 to $\approx$0.95 (Fig. 2 in Ogren and Evans 1993; cf. Fig. 2 presented here). There appears to be some discrepancy regarding the behaviour of θ at high values of C_a, and these

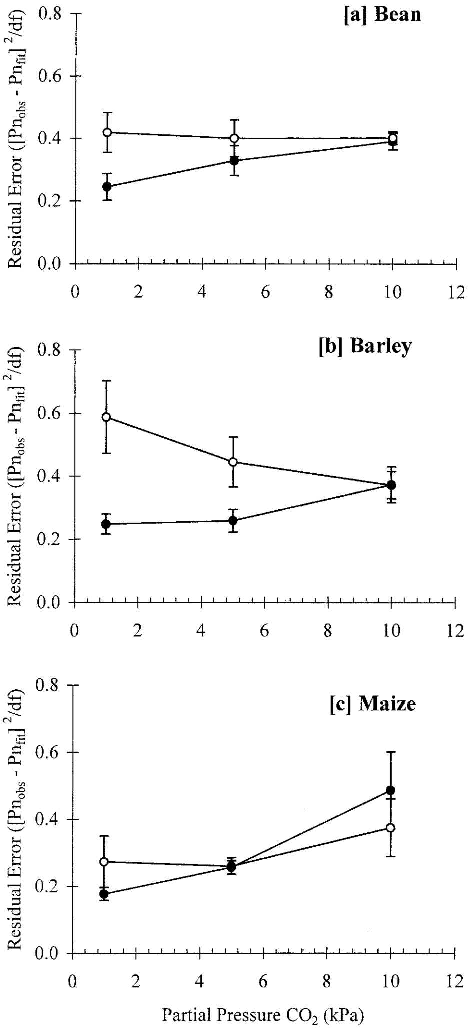

Fig. 3 The effect of CO_2 partial pressures on the linear and non-linear model residual errors for bean (**a**), barley (**b**) and maize (**c**). The residual errors were calculated as the mean sum-of-squares. Each datum point is the average (±SE) calculated from the residual errors of five separate experiments. *Solid symbols* Non-linear model, *open symbols* linear hyperbolic model

differences may arise from differences associated with the plant material used, the range of C_a employed, or the methodology (IRGA versus leaf O_2 electrode).

The residual errors that were generated when curves were fitted to the data using the linear hyperbolic and non-linear models clearly indicate that the latter model provides a better description of the Pn (or Pg) versus I relationship. The data presented here suggest that only at 10 kPa CO_2, as the non-linear model degenerates into the linear form (i.e. $\theta \rightarrow 0$), are the two models equivalent in terms of their accuracy. Therefore, if Pn measurements are made using the CO_2 levels recommended with the manufacturer's instructions (Delieu and Walker 1981, 1983; Hansatech, DW2 Handbook 1984), then the non-linear model should be used. Failure to do this will result in a 50–100% increase in the residual errors, and errors will also be propagated in the estimates of P_{max} and α'. Table 2 presents the average values for Pg_{max} and α' determined from the same data sets by the two models ($n = 45$). It is clear that when C_a is 1 kPa, α' can be significantly over-estimated by the linear model in barley by as much as 60% ($P < 0.001$) although bean (25%, $P = 0.045$) and maize (21%, $P = 0.003$) are also significantly affected. Similarly, Pg_{max} is significantly over-estimated at 1 kPa CO_2 when the linear model is used with bean (28%, $P = 0.015$); a similar pattern was observed with barley (60%, $P = 0.043$) and maize (37%, $P = 0.635$), but with the latter, although large, the differences were not significant. With increasing CO_2 levels, the discrepancies between the values of Pg_{max} and α' decreased and were not significant at 10 kPa CO_2.

Figure 1 shows that the Pn versus I curves of maize are unusual as Pn does not saturate even at high light intensities, and Pn_{max} increases dramatically as CO_2 partial pressure is increased. The reason for this probably lies with the acidification of the mesophyll cell cytoplasm by the action of phosphoenolpyruvate carboxylase. The high partial pressures of CO_2 used with the leaf O_2 electrode are clearly aphysiological and may have led to an irreversible acidification of the cytoplasm. It is clear that if the photosynthesis rates of maize leaves are to be determined using a leaf O_2 electrode, this should be done using low CO_2 partial pressures and the non-linear model.

Our data are consistent with the contention that for CO_2 partial pressures of 1 kPa and above, θ estimates the relative importance of CO_2 diffusion to the site of

Table 2 Comparison of the estimates of Pg_{max} and α' from the linear hyperbolic and non-linear models. In this analysis values of Pg_{max} (μmol O_2 m^{-2} s^{-1}) and α' (dimensionless) were determined using both the linear and non-linear models for each individual data set ($n = 45$). The values presented are the average of the five independent estimates of Pg_{max} and α' (note: the values presented here are slightly different from those presented in Fig. 1; this is due to the different ways in which the data were grouped and analysed). Two-factor analysis of variance was then performed on the data for each species using 'CO_2 partial pressures' and 'Model' as the main factors. Significance: * <0.05, ** <0.01, *** <0.001

	C_a (kPa)	Bean			Barley			Maize		
		Linear	Non-Linear	% Error	Linear	Non-Linear	% Error	Linear	Non-Linear	% Error
Pg_{max}	1	42.00	32.78	28.1^{*}	21.68	13.52	60.4^{*}	40.42	29.55	36.8
	5	32.69	28.03	16.6	24.50	21.29	15.1	51.42	41.37	24.3
	10	33.44	31.54	6.0	36.88	35.64	3.5	54.12	68.40	–20.9
α'	1	0.1111	0.0890	24.8^{*}	0.1473	0.0926	59.1^{***}	0.0805	0.0664	21.1^{**}
	5	0.1146	0.1010	13.5	0.1607	0.1164	38.1	0.0745	0.0704	5.8
	10	0.1213	0.1154	5.1	0.1368	0.1132	20.8	0.0566	0.0494	14.6

carboxylation in the rate of CO_2 fixation. It is therefore likely that under some circumstances θ is a meaningful and significant photosynthetic parameter, and its routine measurement may provide important information on the physiological status of plants. In the experiments we report here, valid comparisons of the CO_2 supply/carboxylation processes (i.e. values of θ) can not be made between species, but comparisons between samples from the same species are possible. This is so because all plants were grown and maintained in similar conditions. Any effects of development on light gradients within leaves were therefore minimised, and all differences in θ can be attributed to changes in the CO_2 supply/carboxylation processes. However, leaves from different species may have different optical properties and so differences in the light gradients within leaves may contribute to the observed value of θ.

In conclusion, to those many investigators using leaf O_2 electrodes we make the following recommendations:

1. When chamber CO_2 partial pressures of < 10 kPa are used, the non-linear quadratic model (Eqs. 2 or 3) should be employed. This general rule should apply for monocotyledonous and dicotyledonous plants. We find the model of Marshall and Biscoe (1980) gives a consistently better fit than that of Thornley (1976), but these differences in residual error were not significant. However, we find the model of Thornley to be more useful as it is less prone to finding local minima solutions.
2. If higher chamber CO_2 partial pressures of around 10 kPa are used, the linear hyperbolic model (Eq. 1) adequately describes the Pn versus I relationship of C-3 monocotyledons and dicotyledons.
3. We do not recommend the use of the leaf O_2 electrode with C-4 plants. The combined effect of high chamber CO_2 levels and endogenous phosphoenolpyruvate carboxylase activity may conspire to acidify the tissue and produce unreliable results.
4. Comparisons on the value of the convexity term, θ, can provide meaningful information on the carboxylation processes. However, reliable comparisons can be made only if factors affecting leaf development can be minimised.

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