PHOTOSYNTHETICA 34 (2): 265-279, 1997

Rapid and straightforward estimates of photosynthetic characteristics using a portable gas exchange system

R. PARSONS*, J.D.B. WEYERS, T. LAWSON and I.M. GODBER

Department of Biological Sciences, University of Dundee, Dundee, DD1 4HN, U.K.

Abstract

Procedures are described for estimating photosynthetic characteristics using a portable infra-red gas analysis (IRGA) system. Once the effects of stomatal limitation on CO_2 assimilation have been established, up to ten parameters of photosynthesis can be estimated for a single leaf within 2 h, including: photosynthetic efficiency and capacity on both photon and CO_2 bases; compensation irradiances and CO_2 compensation concentrations; and light and dark respiration rates. These measurements can be made in the laboratory, glasshouse or field with relative ease. Methods for obtaining near instantaneous ("snapshot") measurements of leaf photosynthesis are also described, using carefully pre-set conditions within the leaf cuvette. Representative results are shown for *Phaseolus vulgaris* L. Important aspects of the procedure's experimental design, assumptions made in the analysis, and limitations of this approach are analysed.

Received 14 August 1996, *accepted* 15 November 1996.

*Fax: +01382 344275; e-mail: r.parsons@dundee.ac.uk

Abbreviations: APAR, absorbed photosynthetically active radiation; C_a, concentration of CO_2 outside leaf; C_i, substomatal CO_2 partial pressure; *CIRAS*, combined infra-red gas analysis system; Γ, CO_2 compensation concentration; G_l, G_{lCO2} composite leaf conductance to water vapour and CO_2, respectively; g_l, leaf conductance to water vapour (one surface only); G_c, cuticular conductance to water vapour; I_c, compensation irradiance; IRGA, infra-red gas analyser; J_{max}, the radiant energy saturated rate of electron transport; $P_{max\text{-}CO2}$, maximum rate of photosynthesis with CO_2 saturating; $P_{max\text{-}i}$, maximum rate of photosynthesis with irradience saturating; P_N, net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; PAR, photosynthetically active radiation; R_d, total dark respiration; R_{day}, dark respiration rate in the light; R_l, total rate of CO_2 production by photorespiration and dark respiration in the light; R_n, dark respiration rate in the dark; R_p, photorespiration rate; RuBPCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; τ, the specificity factor of RuBPCO; T_a, threshold for stomatal limitation of photosynthesis; TPU, the rate of triose phosphate utilisation; V_{cmax}, maximum rate of carboxylation by RuBPCO; V_o, the oxygenation rate of RuBPCO.

Acknowledgements: We wish to thank Prof. Warwick B. Silvester for both theoretical and practical assistance in developing the methods described. We also wish to thank Professors Janet I. Sprent, John A. Raven and John R. Hillman for their critical assistance, Dr Stan D. Wullschleger for clarifying aspects of the analysis of P_N/C_i curves, and Mr Simon Ogston for assistance with non-linear regression techniques.

Additional key words: conductance; infra-red gas analysis; irradiance; photorespiration; photosynthesis; portable IRGA; P_N/C_i; respiration; stomata.

Introduction

The availability of modern photosynthesis measuring instruments based on miniaturised IRGAs, gas supply systems and microprocessor technology allows rapid and straightforward analysis of gas exchange characteristics of leaves (Parkinson *et al.* 1980, Pearcy *et al.* 1991). At the simplest level, this equipment provides instant measures of *in vivo* photosynthetic rates, leaf conductance, and estimates of C_i. However, the capability of these systems to control the irradiance, CO_2 and H_2O environment of the leaf allows the determination of a far greater range of characteristics, such as photosynthetic efficiencies, capacities, and compensation values. This information is potentially valuable in many areas of study: not only for observations on a range of species or cultivars, but also to investigate the effects of experimental treatments.

While methods for estimating individual gas exchange parameters have been described (Pearcy *et al.* 1991, Hall *et al.* 1993), there appears to be no source which provides a standardised protocol which minimises the time taken to determine a wide range of photosynthetic information while optimising accuracy and precision. The aim of this report is to describe such a method. The procedures were developed for a *CIRAS-1* photosynthesis system (*PP Systems*, Hitchin, U.K.), fitted with a broad-leaf cuvette (area = 250 mm^2), and values were analysed after automatic up-loading to a *QuattroPro* spreadsheet. However, the method described is readily amenable to other systems. With suitable equipment, the results can be obtained in the laboratory, glasshouse or field. We illustrate the method by describing its use to characterise photosynthesis in leaves of *P. vulgaris* L. While our approach does not cover all methods of interpretation, it provides an important experimental framework an outline of which should be familiar to those estimating gas exchange parameters.

Materials and methods

Plants: Seeds of *P. vulgaris* L. *cv.* Hardy (Nickerson-Zwaan, Barendrecht, the Netherlands) were soaked overnight in running tap water, then planted individually in 100 mm diameter pots containing *Levington's Universal* compost (*Fisons*, Ipswich, U.K.). Plants were kept in a glasshouse where the temperature was maintained above 15 °C at night and rarely exceeded 30 °C during the day; supplementary lighting was given from 08:00 to 23:00 h by Na-vapour lamps [about 300 μmol(photon) m^{-2} s^{-1} PAR at canopy height]. Plants were watered *via* a capillary matting system. Experiments were carried out on the primary leaves of seedlings aged two to three weeks. Immediately prior to use, plants were transferred to the laboratory. Normally, the pre-equilibrated leaf cuvette was attached without delay, but if a dark pre-treatment was required to close stomata, plants were placed under a cardboard box before use.

Instrument and instrument set up: The *CIRAS-1* IRGA was set up and calibrated according to the manufacturer's instructions. The *CIRAS-1* instrument was fitted with a broad leaf cuvette (exposed area 250 mm^2), an integral air supply unit, a *PP Systems* illumination unit, and a *Sharp PC3000* microcomputer with a 1 MB RAM card. Gas flow rates to the cuvette were set at 5 $cm^3\ s^{-1}$, boundary layer resistance was determined to be 0.28 $m^2\ s\ mol^{-1}(H_2O)$, and leaf temperatures were estimated using the instrument's facility to calculate leaf energy balance. A transmission coefficient of 0.15 was used (following the instructions of the manufacturers). The experiments were carried out on a bench in a laboratory allowing temperature control to be maintained by the building's air conditioning. In other environments, where these experiments may be conducted, such as the field and glasshouses, care must be taken to minimise temperature changes as many of the parameters determined are dependent on temperature. We recorded temperature, CO_2 and H_2O concentrations, and other parameters regularly in a notebook during the experiments, and this action frequently alerted us to potential problems allowing immediate correction before the results were influenced.

Data transfer and analysis: The parameters of photosynthesis were initially recorded by the *CIRAS*. For analysis and manipulation, values were transferred *via* a 'laplink' program from the *Sharp* microcomputer to a *QuattroPro* for *Windows v5.0* spreadsheet on a PC. Values are routinely incorporated into a *pro-forma* spreadsheet file containing parameter headings and units, values and the equations used to re-calculate and check those values produced by the *CIRAS* (described in the *CIRAS* User Manual and by Long and Hällgren 1993). The incorporation of these calculations into the spreadsheet allows the recalculation of the retrieved values after the measurements have been obtained; this may be important where data collection have been found to be inaccurate due to the machine or the parameter settings being incorrect (*e.g.*, inaccurate leaf area estimates, see later).

The values from the spreadsheet can be easily manipulated to produce accurate and presentable graphs of the results. Once templates have been established, the generation of graphs for later data sets is considerably simplified. A simplified version of this spreadsheet has been made available at the World Wide Web site http:www\dundee.ac.uk\bioscience.

Irradiances at the leaf surface and after passage through the leaf (Table 1) were determined by positioning the *CIRAS* PAR sensor at the position the leaf would occupy in the cuvette and measuring the radiation transmitted through each filter. To determine absorbed radiation, a similar procedure was carried out with the PAR sensor immediately below the lower leaf surface. Radiation reflected by the leaf surface was not determined: although the quality of this radiation will have altered at reflection, some of the radiation will be reflected back onto the leaf because of the design of the light reflector.

Table 1. Conversion of PAR to APAR at the irradiances [μmol(photon) m^{-2} s^{-1}] used. The cuvette base was detached from the window and light source, the PAR detector was clamped just below the position that a leaf would occupy. Readings were then taken with and without the leaf in position.

PAR	APAR	APAR/PAR
84	79	94.1
179	169	94.4
194	180	92.8
212	198	93.4
256	238	93.0
344	319	92.7
388	352	90.7
509	469	92.1
564	521	92.4
729	671	92.0
1598	1448	90.6

Results and discussion

Sequence of experimental procedures: The sequence used to determine the photosynthetic characteristics of a new subject leaf is outlined in Fig. 1. This flow chart shows how we first determine the potential of stomatal limitation of photosynthesis and then go on to determine the response of the leaf to light, estimate the respiration rates, and measure the response of assimilation to internal CO_2 concentration. We present below methods of both data collection and analysis for each of these experimental procedures. The order of experiments is designed to streamline the reliable collection of data and allows direct estimates of many important characteristics of photosynthesis to be made.

Determination of the potential of stomatal limitation of photosynthesis: The IRGA was turned on and the required conditions were set up within the leaf chamber. These conditions were allowed to equilibrate, and the machine's calibrations were checked. In the first experiment, a leaf from a plant previously darkened for 1 h was clamped into the leaf chamber and the composite leaf conductance recorded as the stomata opened (Fig. 2). With *P. vulgaris*, G_l increased to a maximum of 300-350 mmol(H_2O) m^{-2} s^{-1} after about 30 min. Other species may show a faster or slower stomatal response, and some might show signs of photoinhibition at an irradiance equivalent to full sunlight (*e.g.*, if P_N starts to decline with time), in which case a lower irradiance should be used and values showing signs of photoinhibition should be rejected, for the purpose of constructing the P_N/G_l curve. To establish a usable threshold for the stomatal limitation of photosynthesis at full irradiance and atmospheric CO_2 concentrations, the values obtained were plotted in the form of a P_N/G_l curve (Fig. 2). Stomata will continue to limit assimilation up to a conductance where $C_a = C_i$. The threshold for stomatal limitation on photosynthetic assimilation is

termed T_a. In most leaves this conductance is never reached, and researchers must accept that if conductances are maintained at a level that does not overly restrict assimilation (up to 10 % of maximum assimilation), then the results provide a fair estimation of photosynthetic parameters. In the experimental leaf used here, the maximum conductance was 250 μmol(H_2O) m^{-2} s^{-1}.

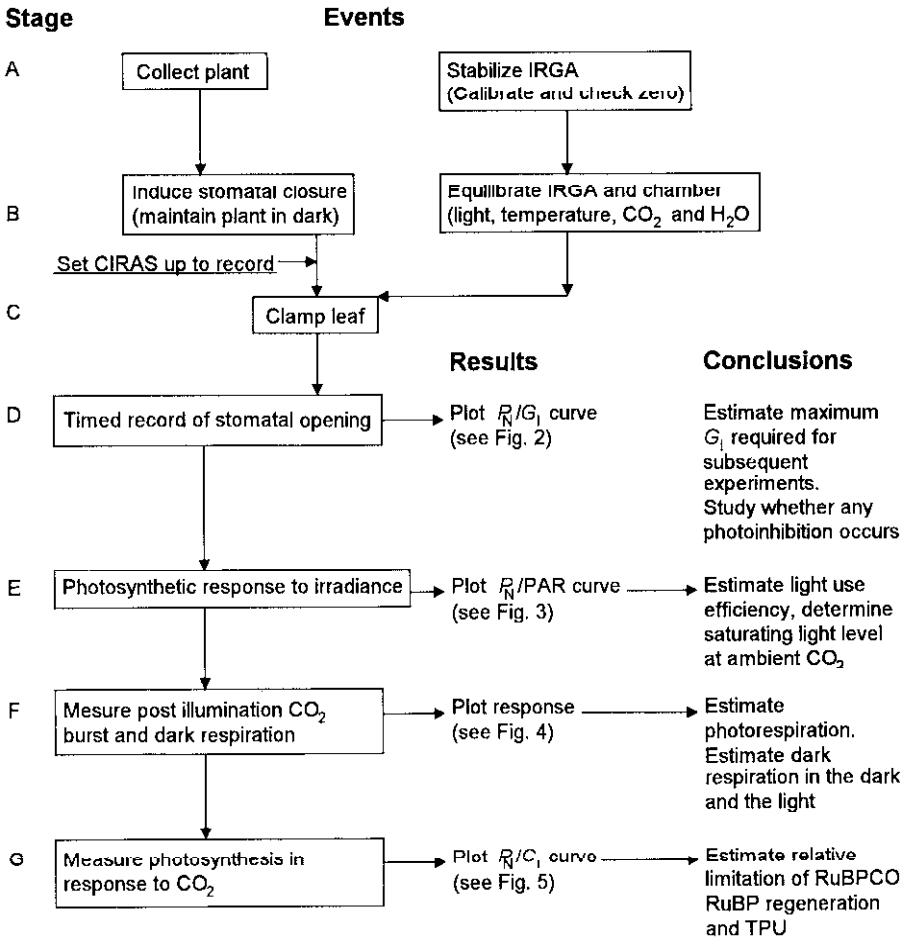

Fig. 1. Flowchart showing main procedures for determining photosynthetic characteristics using a portable IRGA.

If it is unclear whether the upper value of G_l obtained is close to the maximum possible, a theoretical calculation of G_s can be made using the formula in Table 3.4 of Weyers and Meidner (1990). This requires estimates of stomatal frequency and dimensions which may be obtained from epidermal strips, silicone rubber impressions or hand sections. Assuming the composite cuticular conductance (G_c) to be insignificant, a calculation assuming circular pores of diameter equal to pore length should approximate the maximum value of G_l.

$$G_s = \frac{\text{effective diffusion coefficient} \times \text{stomatal frequency} \times \text{pore area}}{\text{pore depth} \times \text{"end correction"}}$$

The values of G_l may be converted to composite conductance to CO_2 by dividing by 1.6 (Farquhar and Sharkey 1982), a simple matter using a spreadsheet. Strictly speaking, the value of G_l should first be corrected for G_c to provide a measure of G_s, but since G_c is generally less than 3 % of maximum values of G_s (Weyers and Meidner 1990, Jones 1992), neglecting this should not cause a large error. However, for further analysis during data collection, G_l is the value displayed by the machine and therefore the one we recommend for routine judgements of how much stomata are limiting P_N.

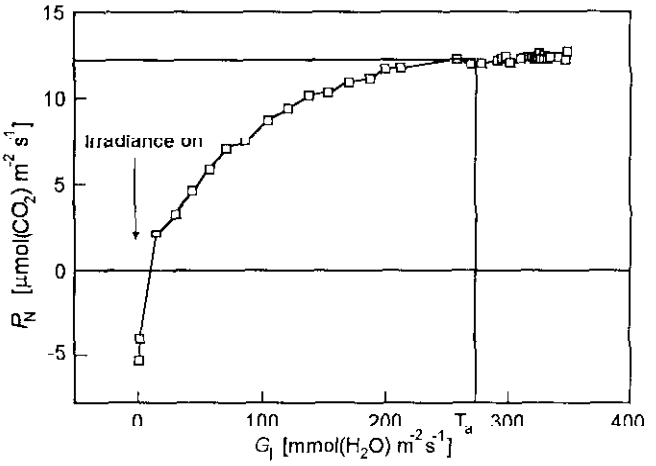

Fig. 2. A P_N/G_l curve estimating a threshold for stomatal limitation of assimilation. The curve was obtained out using a *Phaseolus vulgaris* leaf which had been previously placed in the dark for 1 h. Cuvette conditions were: irradiance = 2028 μmol(photon) m^{-2} s^{-1}; temperature = 25 °C; water vapour concentration 2.02 kPa, and $[CO_2]$ = 35.4 Pa. The threshold for stomatal limitation of photosynthesis (T_a) was estimated by the method described in the text.

Carrying out this experiment forces the operator to consider the potential stomatal limitation of photosynthesis. The results obtained allow a great deal of information to be assessed including (*a*) the limitation of G_l on assimilation rate; (*b*) the detection of photoinhibition; (*c*) the speed of stomatal responses. In further experiments to determine photosynthesis rates in light (Fig. 3), it is essential that G_l is equal or greater than T_a; however, when determining photosynthetic responses to C_i (see Fig. 5), this is not important. If it proves difficult to maintain the necessary high values of G_l for this experiment or elsewhere, it may help (*1*) to ensure that warm temperatures and high humidities are used; (*2*) to carry out experiments early in the day so that stomata do not start to close due to their endogenous rhythm; and (*3*) to ensure that plants do not receive a water stress (deficit or excess) prior to use.

Constructing the P_N/PAR curve: These curves, also termed "*A*/Q" curves (see, *e.g.*, Lawlor 1993), are used to determine the effects of PAR on photosynthetic rates. The protocol shown in Fig. 1 was used, ensuring that G_l equalled or exceeded T_a. It is valuable to ensure steady-state photosynthesis under high PAR before commencing and essential to ensure that P_N readings have stabilised before taking each reading.

With the *CIRAS-1* instrument operating in the mode that determines leaf temperature by energy balance, the new PAR value must be entered correctly before taking a reading, but this can be avoided if a thermistor is used to measure leaf temperature

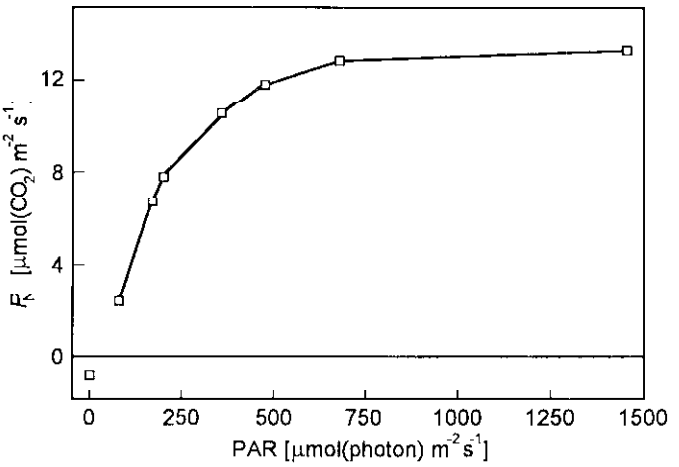

Fig. 3. Construction of the P_N/PAR curve. Chamber conditions were: mean temperature = 23.6 °C, water vapour concentration 2.07 kPa, and $[CO_2]$ = 35.5 Pa. The P_N/PAR curve constructed using the steady state P_N values from the time course except for dark (see text) and APAR values derived from Table 1. The experiment was carried out with G_l above T_a throughout, and according to Fig. 2, photoinhibition was not significant over the time period used.

directly. Ideally, the precise order of PAR values used should be randomised to avoid stomatal closure due to decreasing irradiance. In practice, it may be convenient to compromise this goal in favour of speed and not to repeat the reading at highest irradiance, 2028 μmol(photon) m^{-2} s^{-1}, but to incorporate it as the penultimate PAR followed by a dark treatment. This sequence of treatments allows respiration rates to be estimated (see Fig. 4). The number of PAR values used may also be reduced

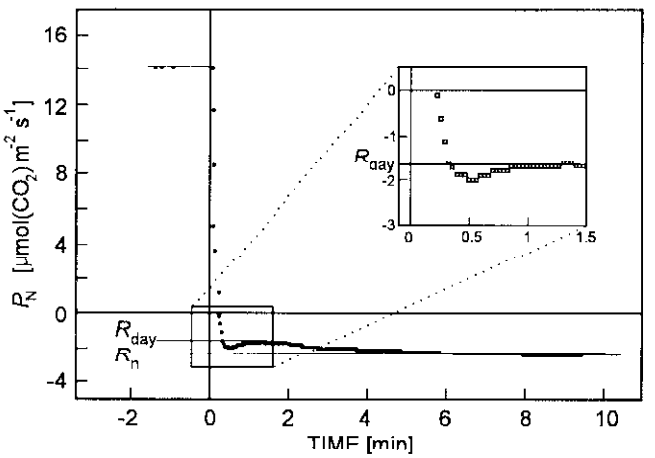

Fig. 4. Methods of estimating respiration rates. CO_2 release from a leaf following transfer to darkness at time 0. Photorespiration is demonstrated by the post-irradiation burst of CO_2, R_{day} was estimated as the minimum value of dark respiration following the peak of CO_2 release, and R_n as the subsequent value of dark respiration.

for speed; however, a minimum of four values in the range 0-200 μmol(photon) m^{-2} s^{-1} appears desirable to minimise error in estimates of photosynthetic efficiency based on linear regression on these points.

A P_N/PAR straight line can then be constructed using the raw values directly; or values of the absorbed PAR (APAR) can be substituted for PAR using values determined after the main experimental sequence (see Fig. 1). Table 1 shows typical APAR:PAR ratios for the *P. vulgaris* leaf, but once again, a separate set of determinations will be necessary for different species or cultivars and probably for different treatments within an experiment and different leaves on a plant. From the P_N/PAR curve (Fig. 3), the following can be derived: (*1*) P_{max} from the upper asymptote of the hyperbolic response curve; (*2*) the compensation PAR (I_c) as the x-intercept; and (*3*) the photosynthetic efficiency on a quantum basis as the slope of the curve just above the x-axis; values estimated from Fig. 3 are given in Table 2.

Table 2. Estimates of photosynthetic characteristics for a primary leaf of *Phaseolus vulgaris*.

Photosynthetic characteristic	Symbol	Value and units
Threshold for stomatal limitation of photosynthesis	T_a	250.0 μmol(H_2O) m^{-2} s^{-1}
Dark respiration rate in the dark	R_n	2.3 μmol(CO_2) m^{-2} s^{-1}
Dark respiration rate in the light	R_{day}	1.6 μmol(CO_2) m^{-2} s^{-1}
Photosynthetic capacity at full sun, 360 g(CO_2) m^{-3}	P_{max-i}	13.2 μmol(CO_2) m^{-2} s^{-1}
Comensation irradiance	I_c	31.9 μmol(photon) m^{-2} s^{-1}
Photosynthetic efficiency (quantum basis)	Φ	0.049 μmol(CO_2) μmol^{-1}(photon)
Photosynthetic capacity at maximum [CO_2], full sun	P_{max-CO_2}	20.6 μmol(CO_2) m^{-2} s^{-1}
CO_2 compensation concentration, full sun	Γ	7.55 Pa
Photosynthetic efficiency (mol CO_2 basis)		0.75 μmol(CO_2) $mmol^{-1}$(CO_2)

Determining leaf respiration: In any attempt to estimate rates of photosynthesis, it is important that the significance of leaf respiration is understood. Photorespiration in C_3 plants (light-dependent release of CO_2) may typically range from 0.1 at 10 °C to 0.3 at 40 °C of the photosynthesis rate (Leegood 1995), and is strongly influenced by factors such as type of plant, temperature, water stress, and CO_2/O_2 concentrations. Dark respiration (R_d), *i.e.*, mainly TCA cycle activity, occurs both in the light (R_{day}) and dark (R_n), and R_{day} is normally lower than R_n (Lawlor 1993). To obtain estimates of R_{day} and R_n and to demonstrate the occurrence of photorespiration (R_p) we recommend following the CO_2 release from a leaf immediately after the light is turned off. This method utilizes the ability of portable IRGAs to resolve CO_2 concentrations rapidly and accurately. A leaf is maintained in the cuvette at high irradiance, high humidity (>80 %), ambient concentrations of CO_2 and temperature, and is left for at least 10 min to allow pools of metabolites to equilibrate and ensure stomatal changes are minimal. The IRGA is set to collect values every 2 s, and after approximately 3 min, the light is switched off, and the cuvette completely darkened. The subsequent values, collected for 10 to 15 min (Fig. 4), can be used to demonstrate R_p from the post-irradiation release of CO_2 (Dekker 1957, cited in Zelitch 1979) although accurate determination of R_p is complicated by many reactions within leaves which may release or utilise CO_2, including continued ribulose-1,5-bisphosphate carboxylase/oxygenase activity (RuBPCO), PEP carboxylase activity, photorespiration and dark respiration (Laisk and Sumberg

1994). We estimate R_{day} as the minimum CO_2 production which occurs after the light is switched off, and the release of CO_2 from photorespiration has ceased, R_n is the rate of CO_2 production that the leaf stabilizes at in the dark.

Constructing the P_N/C_i curve: These curves present the responses of plants to varying C_i, and this requires photosynthesis values over a wide range of CO_2 concentrations, with constant (saturating) irradiance. Variations in C_i are achieved by altering the external concentration (C_a). Typically, these curves are carried out at saturated irradiance (obtained from the P_N/PAR curve), high humidity (to keep stomata open), and ambient temperature. We recommend following a regime of decreasing and then increasing C_a so that the stomatal changes (predominantly shutting at high CO_2) are minimised. Therefore, a logical time to obtain these results would be at the end of the experimental period. To provide sufficient values for model analysis and parameter predictions, we construct P_N/C_i curves by measuring assimilation at approximately ten CO_2 concentrations. In the experiment shown in Fig. 5, target C_a concentrations were 5, 10, 15, 20, 36, 50, 70, 140, and 160 Pa CO_2. Readings were taken when the photosynthetic rates were stable, *ca.* 2 min after alteration of CO_2. Using the present *CIRAS-1* model with manual CO_2 control, some skill and practice is required to obtain set values of CO_2, but specific concentrations are not required, as actual values obtained will be stored and used for plotting the graph and further analysis. Other systems and the automatic *CIRAS* instruments allow the automatic generation of particular C_a. The results obtained (Fig. 5) were plotted as a measured photosynthetic rate against a calculated C_i.

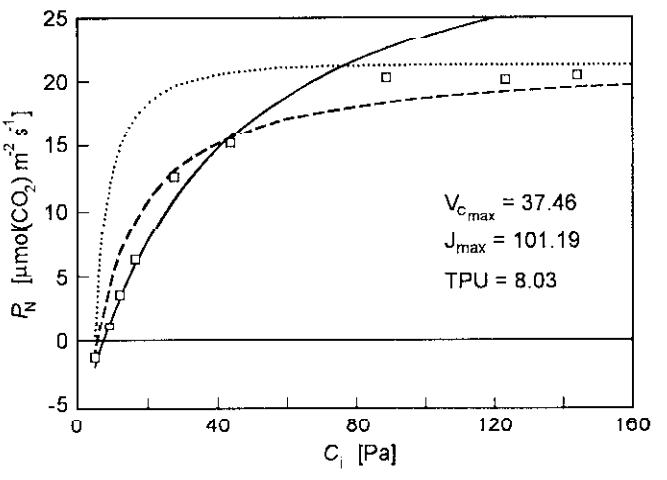

Fig. 5. Construction of the P_N/C_i curve. Chamber conditions were PAR = 2028 µmol(photon) m^{-2} s^{-1}; temperature = 23.7 °C; water vapour concentration = 2.1 kPa. The leaf was exposed to each CO_2 partial pressure for 3-4 min. The P_N/C_i curve was constructed using the steady state P_N values and C_i values calculated by the *CIRAS* (for algorithm, see Materials and methods). Points represent the actual values recorded, the unbroken line represents the model W_c, the dashed line represents the model W_j, and the dotted line represents the model W_p.

We have also used a specialist gas mixing device (Parsons *et al.* 1992) and an automatic gas sampler to allow rapid and repeatable determination of P_N/C_i curves

(results not shown). The C_i in the leaf is calculated by the instrument as a function of the boundary layer conductance, stomatal conductance, external concentration of CO_2, and the assimilation rate (Caemmerer and Farquhar 1981, *CIRAS* User Manual, Hall *et al.* 1993). This estimation of C_i requires accurate measurement of each of these parameters and may represent, to an inexperienced investigator, a derived value with a precision that cannot be determined.

Analysis of P_N/C_i curves: From the P_N/C_i curve it is possible to derive details of the saturation rates of photosynthesis at high C_i as well as estimates of the biochemical and metabolic processes involved. Initially from the response curve, three important results can be obtained: (*1*) The CO_2 saturated rate of photosynthesis [μmol(CO_2) m^{-2} s^{-1}], as the asymptotic rate obtained on the graph. (*2*) The carboxylation efficiency [μmol(CO_2) m^{-2} s^{-1} Pa^{-1}], which is a measure of the ability of the plant to assimilate CO_2 *via* RuBPCO. This is calculated by the slope of the initial curve when P_N is zero. Carboxylation efficiency is dependent on the O_2 concentration and is typically 2-2.5 μmol m^{-2} s^{-1} Pa^{-1} in 2 kPa O_2 and 1-2 μmol m^{-2} s^{-1} Pa^{-1} in 21 kPa O_2 (Lawlor 1993). (*3*) The CO_2 compensation concentration [Pa] can initially be estimated visually from the curve, as the point where the curve cuts the x axis when y is equal to zero. This is when there is no net flux of CO_2.

Following these initial calculations, further parameters can be calculated following the model proposed by Farquhar *et al.* (1980), as subsequently modified by Caemmerer and Farquhar (1981), Sharkey (1985), Harley and Sharkey (1991), and Harley *et al.* (1992). These authors suggest that the P_N/C_i response consists of two phases (Long and Hällgren 1993). The first of these phases is the initial response below C_i concentrations of 20 Pa; here, ribulose bisphosphate (RuBP) is saturated, and RuBPCO activity limits carboxylation. The slower rise of the curve beyond its inflection point represents the second phase. The higher C_i present within this phase result in the limiting factor being the supply of RuBP. This model can be used to provide estimations of the maximum rate of carboxylation by RuBPCO (V_{cmax}), the PAR-saturated rate of electron transport (J_{max}) and the rate of triose phosphate utilisation (TPU) which indicates the availability of inorganic P for the Calvin cycle (Sharkey 1985). In the calculation of these parameters according to the model, the following equation is used to express the relationship between assimilation rate and internal CO_2. This relies on the concept that it is a minimum of any of the three factors; RuBPCO activity (W_c), RuBP regeneration (W_j), and regeneration of inorganic phosphate (W_p) which limits CO_2 assimilation. That is:

$$P_N = \frac{\{1 - 0.5\ O\}}{\tau\ C_i} \times \text{minimum of } \{W_c,\ W_j,\ W_p\} - R_{day}$$

R_{day} refers to the release of CO_2 in the light by processes other than photorespiration (Brooks and Farquhar 1985), and may be estimated using the modelling equations below or the experimental procedure described previously.

When the rate of carboxylation is solely limited by the activity of RuBPCO, carboxylation can be described by the equation:

$$W_c = (V_{cmax}\, C_i)/[C_i + K_c\,(1 + O/K_o)]$$

where K_c and K_o, respectively, are the Michaelis-Menten constants of RuBPCO for CO_2 and O_2, and O is the concentration of oxygen in the stroma [Pa]. The conditions of this limitation can be imposed by low C_i concentrations (<20 Pa) and high irradiance [>1500 μmol(photon) m^{-2} s^{-1}]. This limitation is shown by line W_c in Fig. 5.

When electron transport limits photosynthesis by the regeneration of RuBP, carboxylation rate can be expressed by the following equation:

$$W_j = \frac{J\,C_i}{[4\,(C_i + O/\tau)]}$$

J is the potential rate of electron transport, τ represents the specificity factor for RuBPCO (Jordan and Ogren 1984). The factor 4 represents the fact that four electrons will generate sufficient ATP and NADPH to regenerate RuBP (Farquhar and Caemmerer 1982). Carboxylation limited by the regeneration of inorganic P can be described by the expression

$$W_p = 3\ TPU + 0.5\ V_o = 3\ TPU + \frac{V_c\ 0.5\ O}{C_i\,\tau}$$

where V_o represents the rate of oxygenation of RuBPCO. Using the Farquhar *et al.* (1980) model, based on these equations, valuable information of the biochemical limitation to photosynthesis can be obtained by applying the model to the basic P_N/C_i curve obtained experimentally (see Fig. 5).

Non-linear regression techniques should be used to make estimations of V_{cmax}, J_{max} and TPU from the P_N/C_i curves obtained through gas analysis (Harley *et al.* 1992, Wullschleger 1993). V_{cmax} is estimated from C_i values below 20 Pa to ensure that assimilation is limited solely by the amount and activity of RuBPCO. From this initial part of the model a value of V_{cmax} and R_{day} is estimated, which can then be incorporated into the model to evaluate J_{max} and TPU. The TPU evaluation should only be attempted on those curves that show a TPU limitation, indicated by a saturation of the curve (Wullschleger 1993). Various studies have shown that these parameters obtained from the P_N/C_i curves are modified by treatments such as elevated CO_2 concentrations (*e.g.*, Harley *et al.* 1992) and nitrogen limitation (Pettersson and McDonald 1994).

Obtaining near instantaneous measurements of P_N and G_s: One frequent use of portable photosynthetic equipment is to take "snapshot" readings of photosynthesis and conductance. Carrying out the experiments described here will enhance understanding of the particular leaf physiology of a plant and will allow instrument conditions to be set to closely match those occurring around the leaf just before measurement is made. The irradiance should be adjusted to match that of C_a set to a value close to the concentration the plant was grown at, and H_2O content set so that the final humidity at the leaf surface is maintained (allowing for changes in the boundary layer caused by clamping of the leaf in a stirred chamber). When these

conditions are correctly set, users may find that stable results are obtained very quickly (1-2 min), and that drift over time caused by changes in stomatal opening and/or changes in CO_2 assimilation are minimised. The speed at which chamber conditions equilibrate with leaf conductance is demonstrated in Fig. 6, which also shows drift induced by changes in stomatal aperture, due to (a varying input of) CO_2 concentration. When conditions are correctly set and drift minimised, the photosynthetic rate and conductance of a large number of leaves can be measured and recorded efficiently.

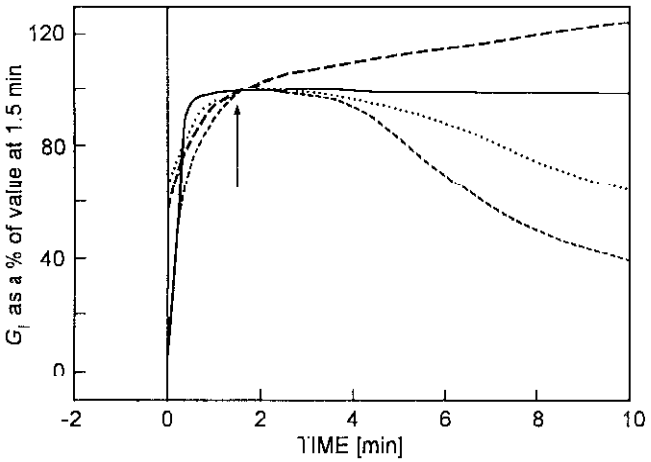

Fig. 6. Determination of when to take 'snapshot' readings. Stabilisation of the *CIRAS* instrument. Readings were captured every 2 s for 5 min followed by readings at 1 min intervals. The effects of input CO_2 concentrations [Pa] on leaf conductance was monitored. The arrow indicates the inferred stabilisation time of the instrument at 1-2 min. Each curve represents individual leaves.

Errors in the measurement of leaf area: The calculations used to obtain the photosynthesis values in the *CIRAS* all use the value of leaf area, either directly or indirectly. It is therefore important to obtain an accurate estimation of leaf area. This problem does not arise in leaves which are broad enough to fill the leaf cuvette chamber, but errors may occur when using plants with small or pinnate leaves. Here, an accurate estimation of the leaf area enclosed within the leaf chamber must be used. To illustrate the errors that can occur if inaccurate estimations of the leaf area are made, a P_N/C_i curve for *Sesbania rostrata*, a legume with pinnate leaves, was obtained and the results recalculated using the spreadsheet method discussed previously, using underestimates and overestimates of the actual leaf area. The percentage error for CO_2 assimilation and C_i at each of the points recorded is presented in Table 3. When assaying leaves that do not fill the chamber, we routinely collect the values using a visual estimate of leaf area, and following the experiment, the leaf is removed and its area measured accurately, and the results recalculated using the spreadsheet.

Coping with stomatal heterogeneity: An important consideration in the determination of leaf conductance is the distribution and uniformity of stomata on the leaf measured. For dorsiventral leaves, stomata may be present on one side (adaxial or abaxial) or on both sides (sometimes in equal numbers, but many with an unequal distribution, normally with a higher frequency on the abaxial surface). Leaf cuvettes

that enclose both sides of the leaf measure the cuticular and stomatal conductances of both sides at the same time, *i.e.*, G_l. The determination of conductance on one particular side of the leaf requires that measurements are taken from that side only and may allow an operator to determine conductances for the abaxial and adaxial surfaces of the leaf. However, workers should be aware that patchy stomatal closure (as occur in response to some treatments - Terashima *et al.* 1986, Downton *et al.* 1988) will result in the inaccurate calculation of C_i (Laisk 1983, Mansfield *et al.* 1990) because of the apparent reduction of leaf area for both evaporation rate and photosynthesis, and the curvilinear nature of P_N/C_i curve. Attempts to apply equations based on total leaf area to leaves where only certain sectors are active would clearly give erroneous "averaged" values (Cheeseman 1991). Due to heterogeneity in both stomatal density and gas exchange, users should ensure that values obtained on replicate leaves should be carried out on a similar position on the leaf lamina (Weyers *et al.* 1997). This is especially important when using "snapshot" readings to make comparisons among treatments.

Table 3. The percent errors obtained when the leaf area [cm^2] is inaccurately input into the IRGA by values of up to ± 0.4 cm^2, based on a leaf with an area of 1.4 cm^2.

Leaf area [cm^2]	P_N [$\mu mol(CO_2)\ m^{-2}\ s^{-1}$]	Error [%]	C_i [Pa]	Error [%]
actual (1.4)	24.2	-	29.2	-
+0.1	22.6	6.7	29.1	0.23
-0.1	26.1	7.1	29.3	0.23
+0.2	21.2	12.5	29.1	0.43
-0.2	28.3	14.3	29.4	0.57
+0.3	20.0	17.6	29.0	0.61
-0.3	30.9	21.4	29.5	0.93
+0.4	18.9	22.2	28.9	0.77
-0.4	34.0	28.6	29.6	1.37

Instruments: The portable photosynthesis measuring system used for this report was a *CIRAS-1* IRGA. Similar systems are also manufactured by *ADC* (*Analytical Development Co.*, Hoddesdon, U.K.), *Walz Mess- und Regeltechnik* (Effeltrich, Germany), *LI-COR* (Lincoln, U.S.A.) and *CID* (Vancouver, Canada). Each of these instruments generally has the capability to measure absolute and relative CO_2 and H_2O partial pressures, temperature, irradiance, and atmospheric pressure. Photosynthetic measurements are achieved by using specialised leaf cuvettes to enclose a gas space around a part or the whole of a leaf, and measuring the changes in gas concentrations that occur as gas flows at known rates through the chamber. The instruments utilise a microprocessor-based data storage and calculation system which allows near-instantaneous determination of conductance and assimilation rates. While the method described is based on the *CIRAS* IRGA, it can be easily modified for various similar equipment.

References

Brooks, A., Farquhar, G.D.: Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Estimates from gas-exchange measurements on spinach. - Planta **165**: 397-406, 1985.

Caemmerer, S. von, Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. - Planta **153**: 376-387, 1981.

Cheeseman, J.M.: PATCHY: Simulating and visualizing the effects of stomatal patchiness on photosynthetic CO_2 exchange studies. - Plant Cell Environ. **14**: 593-599, 1991.

Downton, W.J.S., Loveys, B.R., Grant, W.J.R.: Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. - New Phytol. **108**: 263-266, 1988.

Farquhar, G.D., Caemmerer, S. von: Modelling of photosynthetic response to environmental conditions. - In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (ed.). Physiological Plant Ecology II. Pp. 549-587. Springer-Verlag, Berlin - Heidelberg - New York 1982.

Farquhar, G.D., Caemmerer, S. von, Berry, J.A.: A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. - Planta **149**: 78-90, 1980.

Farquhar, G.D., Sharkey, T.D.: Stomatal conductance and photosynthesis. - Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C., Long, S.P. (ed.). Photosynthesis and Production in a Changing Environment - A Field and Laboratory Manual. - Chapman and Hall, London - Glasgow - New York - Tokyo - Melbourne - Madras 1993.

Harley, P.C., Sharkey, T.D.: An improved model of C_3 photosynthesis at high CO_2: Reversed O_2 sensitivity explained by lack of glycerate reentry into the chloroplast. - Photosynth. Res. **27**: 169-178, 1991.

Harley, P.C., Thomas, R.B., Reynolds, J.F., Strain, B.R.: Modelling photosynthesis of cotton grown in elevated CO_2. - Plant Cell Environ. **15**: 271-282, 1992.

Jones, H.G.: Plants and Microclimate: A Quantative Approach to Environmental Plant Physiology. - Cambridge University Press, Cambridge 1992.

Jordan, D.B., Ogren, W.L.: The CO_2/O_2 specificity of ribulose 1,5-*bis*phosphate carboxylase/oxygenase. Dependence on ribulosebisphosphate concentration, pH, and temperature. - Planta **161**: 308-313, 1984.

Laisk, A.: Calculation of leaf photosynthetic parameters considering the statistical distribution of stomatal apertures. - J. exp. Bot. **34**: 1627-1635, 1983.

Laisk, A., Sumberg, A.: Partitioning of the leaf CO_2 exchange into components using CO_2 exchange and fluorescence measurements. - Plant Physiol. **106**: 689-695, 1994.

Lawlor, D.W.: Photosynthesis: Molecular, Physiological and Environmental Processes. - Longman Group, Harlow 1993.

Leegood, R.C.: Effects of temperature on photosynthesis and photorespiration. - In: Smernoff, N. (ed.): Environment and Plant Metabolism. Flexibility and Acclimation. Pp. 45-62. Bios Scientific Publishers, Oxford 1995.

Long, S.P., Hällgren, J.-E.: Measurement of CO_2 assimilation by plants in the field and the laboratory. - In: Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C., Long, S.P. (ed.): Photosynthesis and Production in a Changing Environment - A Field and Laboratory Manual. Pp. 129-167. Chapman and Hall, London - Glasgow - New York - Tokyo - Melbourne - Madras 1993.

Mansfield, T.A., Hetherington, A.M., Atkinson, C.J.: Some current aspects of stomatal physiology. - Annu. Rev. Plant Physiol. Plant mol. Biol. **41**: 55-75, 1990.

Parsons, R., Sprent, J.I., Raven, J.A.: A simple open flow system used to measure acetylene reduction activity of *Sesbania rostrata* stem and root nodules. - J. exp. Bot. **43**: 595-604, 1992.

Parkinson, K.J., Day, W., Leach, J.E.: A portable system for measuring the photosynthesis and transpiration of graminaceous leaves. - J. exp. Bot. **31**: 1441-1453, 1990.

Pearcy, R.W., Ehleringer, J., Mooney, H.A., Rundel, P.W.: Plant Physiological Ecology. Field

Methods and Instrumentation. - Chapman and Hall, London 1991.

Pettersson, R., McDonald, A.J.S.: Effects of nitrogen supply on the acclimation of photosynthesis to elevated CO_2. - Photosynth. Res. **39**: 389-400, 1994.

Sharkey, T.D.: Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. - Bot. Rev. **51**: 53-105, 1985.

Terashima, I., Sakaguchi, S., Hara, N.: Intra-leaf and intracellular gradients in chloroplast ultrastructure of dorsiventral leaves illuminated from the adaxial or abaxial side during their development. - Plant Cell Physiol. **27**: 1023-1031, 1986.

Weyers, J.D.B., Lawson, T., Peng, Z.Y.: Variation in stomatal characters at the whole leaf level. - In: van Gardingen, P.R., Foody, G.M., Curren, P.J. (ed.): Scaling Up. In Press. Cambridge University Press, Cambridge 1997.

Weyers, J.D.B., Meidner, H.: Methods in Stomatal Research. - Longman Group, Harlow 1990.

Wullschleger, S.D.: Biochemical limitations to carbon assimilation in C_3 plants - A retrospective analysis of the A/C_i curves from 109 species. - J. exp. Bot. **44**: 907-920, 1993.

Zelitch, I.: Photorespiration: Studies with whole tissues. - In: Gibbs, M., Latzko, E. (ed.): Photosynthesis II. Pp. 353-367. Springer-Verlag, Berlin - Heidelberg - New York 1979.