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Variation in measured values of photosynthetic quantum yield in ecophysiological studies

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Abstract Photosynthetic efficiency is often quantified as the light-limited, maximum quantum yield in ecophysiological studies. Four published comparative studies report that photosynthetic efficiency varies little among plant species of widely diverse origins, and that quantum yields were near the maximum theoretically attainable value. However, many other published studies contradict this conclusion, reporting quantum yields as low as 30% of those found in the comparative studies. These studies have created the impression that certain plants, particularly wild plants growing outdoors, may have intrinsically low photosynthetic efficiencies. To investigate the validity of these differing interpretations, we compiled quantum yield data from a survey of 30 published studies and compared those with data from the two most comprehensive comparative quantum yield studies. We also included quantum yield observations that we made on ten species. While our data confirm the results of the comparative studies indicating that maximum quantum yield is high and invariant, the literature survey data showed a wide range of quantum yield values. To investigate whether low quantum yield values could be caused by data collection and analysis techniques, we analyzed photosynthetic light-response data. Substantial underestimation of quantum yield could result from including in the calculation data extending beyond the linear region of the photosynthetic light response. In some cases quantum yield measurements can be influenced by changing levels of intercellular CO₂ during measurements. We conclude that many quantum yield values reported in the literature are affected by one

or more of these errors, and the intrinsic efficiency of photosynthesis is mostly invariant among C₃ plants. This emphasizes the importance of the measurement and data analysis protocols in obtaining accurate and reliable quantum yield data.

Keywords Quantum yield · Photosynthesis · O₂ evolution · CO₂ assimilation · Sun/shade

Introduction

The quantum yield of photosynthesis (ϕ) is a measure of photosynthetic efficiency expressed in moles of photons absorbed per mole of CO₂ fixed or O₂ evolved. Depending on the measurement conditions and techniques, ϕ measurements reflect different aspects of the photosynthetic apparatus. The maximum quantum yield (ϕ_{\max}) is measured when photosynthesis is light-limited, a situation diagnosed by a linear relationship between photosynthesis and photosynthetic photon flux density (PPFD). Because this is the most common usage of ϕ in ecophysiological studies, we restrict our discussion to ϕ_{\max} . Theoretically, ϕ_{\max} is 0.125, meaning that 8 moles of photons are required to reduce 1 mole of CO₂ in the absence of photorespiration. Because there is some cyclic photophosphorylation, ϕ_{\max} may be closer to 0.112 in most plants (Long et al. 1993). This does not hold for C₄ plants because of the energy requirements of the CO₂ concentrating mechanism. Hence we further restrict our discussion to C₃ species. A distinction must also be made between quantum yields calculated from O₂ evolution versus CO₂ assimilation data. Since oxygen is evolved earlier in the photosynthetic pathway compared with CO₂ assimilation, ϕ_{\max} calculated from O₂ evolution data (ϕ_{O_2}) is affected by fewer processes than the quantum yield of CO₂ assimilation (ϕ_{CO_2}). Thus, ϕ_{O_2} may be somewhat higher than ϕ_{CO_2} . Because of these differences, it is important to define the measurement techniques and conditions when making ϕ measurements in ecophysiological studies.

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Both ϕ_{O_2} and ϕ_{CO_2} are reduced by photorespiration. Because O_2 is taken up and CO_2 is released by photorespiratory metabolism, ϕ is reduced in proportion with the photorespiration rate. Photorespiration has an additional effect on ϕ_{CO_2} by competing with CO_2 fixation for the products of electron transport (ATP and NADPH). Most gas-phase O_2 evolution measurements are made under 5% CO_2 , which virtually eliminates photorespiration, and thus defines the maximum efficiency with which light energy is converted to reducing power by the thylakoid membrane components of photosynthesis. When making CO_2 exchange measurements in C_3 species, photorespiration can be reduced by making measurements at low (usually 1 or 2%) O_2 . Because ϕ_{CO_2} is measured both with and without photorespiration, we must define operational ϕ_{CO_2} , measured at atmospheric O_2 levels, separate from intrinsic ϕ_{CO_2} , measured at low O_2 levels.

We have found a notable discrepancy among published values of ϕ . Several comparative studies have investigated the variations in ϕ among various species grown under different conditions. In a study of 42 species using a gas-phase O_2 electrode, Björkman and Demmig (1987) found a mean ϕ_{O_2} of 0.108 with less than 5% coefficient of variation (CV). Long et al. (1993) measured maximal ϕ_{CO_2} and found a mean of 0.092 with equally low variance, even among plants from widely divergent families. These two studies confirmed the results of earlier work which had shown that, in the absence of stressful conditions, there is little variation in quantum yield (Ehleringer and Björkman 1977; Osborne and Garrett 1983). Many researchers have measured ϕ in the context of a larger set of experiments and report ϕ values 30–50% lower than the theoretical maximum (Bongi and Long 1987; Ögren and Evans 1993). If quantum yield varies substantially among species, there may be ecolog-

ical costs to photosynthesis that must be considered for many species. On the other hand, if calculated ϕ is easily affected by the methods of data collection and analysis, and much of the variation in ϕ may be explained, then the conclusion of low variation in photosynthetic efficiency reached in the comparative studies would be supported. Thus, an understanding of the basis for variation in ϕ values is critical to the interpretation of ecophysiological photosynthetic studies.

We investigated the variation in ϕ and its potential causes. To determine variation in published ϕ values, we surveyed the literature to compare the variation with that of two comparative ϕ studies (Björkman and Demmig 1987; Long et al. 1993). We also measured the photosynthetic light-response in several species of plants, including both naturally established species growing outdoors and domestic species growing in controlled environments, to determine the variation among a variety of species grown in different conditions. Photoinhibition and changes in alternative electron sinks did not satisfactorily explain the variance in published ϕ values. Because ϕ calculations were sensitive to errors in data collection and analysis, we used photosynthetic light-response data to show that this is the most likely explanation for the reported variance in ϕ .

Materials and methods

Literature survey

To estimate the variance in ϕ across a wide range of photosynthesis studies, we surveyed agricultural and ecophysiological literature. Our final data set contains 58 quantum yield observations for 48 species from 29 references (see Tables 1, 2 for complete results). Since ϕ_{O_2} and ϕ_{CO_2} measurements probe different parts of the photosynthetic system, we separated the data based on measurement technique. Three cases where gas-exchange measure-

Table 1 Values of ϕ calculated from O_2 evolution measurements in the surveyed literature

Species	ϕ_{O_2}	Growth conditions ^d	Reference
<i>Acer saccharum</i> Marshall. ^a	0.0630	F	Naidu and DeLucia 1997
<i>A. saccharum</i> Marshall. ^{a,b}	0.0120	F	Naidu and DeLucia 1998
<i>A. saccharum</i> Marshall. ^{a,c}	0.0230	F	Naidu and DeLucia 1998
<i>Chenopodium abum</i> L.	0.1053	F	Lal and Edwards 1995
<i>Eucalyptus maculata</i> Hook.	0.0543	F	Ögren and Evans 1993
<i>Flaveria cronquistii</i>	0.1080	GH	Lal and Edwards 1995
<i>F. robusta</i>	0.1069	GH	Lal and Edwards 1995
<i>F. pringlei</i>	0.1083	GH	Lal and Edwards 1995
<i>Opuntia basilaris</i> Engelm. & Bigelow	0.0750	GH	Adams et al. 1987
<i>O. basilaris</i> Engelm. & Bigelow	0.0300	F	Adams et al. 1987
<i>Phaseolus vulgaris</i> L.	0.1025	GC	Castonguay and Markhart 1991
<i>P. acutifolius</i>	0.1050	GC	Castonguay and Markhart 1991
<i>Pisum sativum</i> L.	0.0970	GH	Chow et al. 1990
<i>P. sativum</i> L.	0.0800	GH	Chow et al. 1990
<i>P. sativum</i> L.	0.0923	GC	Oquist et al. 1992
<i>P. sativum</i> L.	0.1080	GH	Chow et al. 1990
<i>Quercus suber</i> L. ^b	0.1100	F	Faria et al. 1996
<i>Q. suber</i> L. ^c	0.0875	F	Faria et al. 1996
<i>Q. rubra</i> L. ^a	0.0550	F	Naidu and DeLucia 1997
<i>Q. rubra</i> L. ^{a,b}	0.0320	F	Naidu and DeLucia 1998
<i>Q. rubra</i> L. ^{a,c}	0.0430	F	Naidu and DeLucia 1998
<i>Ricinus communis</i> L.	0.0998	F	Lal and Edwards 1995
<i>Tradescantia albiflora</i> Kunth.	0.0966	GC	Oquist et al. 1992

^a Trees planted in pots on forest floor

^b Shade-adapted leaves

^c Sun-adapted leaves

^d Growth conditions:

GC growth chamber,

GH greenhouse, F field

Table 2 Values of ϕ calculated from CO₂ assimilation measurements in the surveyed literature

Species	ϕ CO ₂	Growth conditions ^c	Reference
<i>Brassica napus</i> L.	0.0550	GH	Farage and Long 1991
<i>B. napus</i> L.	0.0400	F	Farage and Long 1991
<i>Carica papaya</i> L.	0.0488	GH	Marler et al. 1993
<i>Duchesnea indica</i> (Andrzejowski) Focke. ^a	0.0600	F	Osborne et al. 1997
<i>Erythrina poeppigiana</i> (Walp.) O.F.	0.0663	F	Nygren 1995
<i>Fagus grandifolia</i> Ehrh.	0.0426	F	Teskey and Shrestha 1985
<i>Helianthus annuus</i> L.	0.0650		Jacob and Lawlor 1991
<i>Lepechinia calycinia</i>	0.0550	F	Field and Mooney 1983
<i>Liquidambar styraciflua</i> L.	0.0466	F	Teskey and Shrestha 1985
<i>Liriodendron tulipifera</i> L.	0.0550	F	Teskey and Shrestha 1985
<i>Manilkara zapota</i> (L.) von Royen	0.0300	GH	Mickelbart and Marler 1996
<i>Mucuna pruriens</i> L. var. <i>utilis</i> ^b	0.0480	GC	Monson et al. 1992
<i>Olea europaea</i> L. var. <i>europaea</i> cv. Rajo	0.0270	GH	Bongi and Long 1987
<i>Oryza australiensis</i>	0.0649	GH	Yeo et al. 1994
<i>O. glaberrima</i> Stendel	0.0505	GH	Yeo et al. 1994
<i>O. latifolia</i> Derv.	0.0590	GH	Yeo et al. 1994
<i>O. longiglumis</i>	0.0553	GH	Yeo et al. 1994
<i>O. longistaminata</i> A. Chev. & Roehr.	0.0610	GH	Yeo et al. 1994
<i>O. meridionalis</i>	0.0634	GH	Yeo et al. 1994
<i>O. nivara</i>	0.0769	GH	Yeo et al. 1994
<i>O. officinalis</i>	0.0684	GH	Yeo et al. 1994
<i>O. rhizomatis</i>	0.0586	GH	Yeo et al. 1994
<i>O. rufipogon</i> Griffiths	0.0457	GH	Yeo et al. 1994
<i>O. sativa</i> L.	0.0800	GH	Agarie et al. 1996
<i>O. sativa</i> L.	0.0522	GH	Yeo et al. 1994
<i>Pinus sylvestris</i> L.	0.0570	F	Leverenz 1987
<i>P. taeda</i> L.	0.0269	F	Teskey and Shrestha 1985
<i>Prunus cerasus</i> L. cv. Montmorency	0.0266	GH	Beckman et al. 1992
<i>Quercus alba</i> L.	0.0324	F	Teskey and Shrestha 1985
<i>Q. rubra</i> L.	0.0355	F	Teskey and Shrestha 1985
<i>Q. cerris</i> L.	0.0700		Valentini et al. 1995
<i>Salix</i> sp.	0.0500	F	Ogren and Sjostrom 1990
<i>Scirpus olneyi</i> Gray.	0.0650	F	Long and Drake 1991
<i>Simmondsia chinensis</i> (Link.) Schneider	0.0590	GH	Loreto and Bongi 1989
<i>Triticum aestivum</i> L.	0.0820	GC	Dai et al. 1993
<i>T. aestivum</i> L. cv. Avalon	0.0520	GC	Farage et al. 1991
<i>T. aestivum</i> L.	0.0650		Jacob and Lawlor 1991
<i>T. aestivum</i> L. cv. Alexandria	0.0650	GC	Habash et al. 1995
<i>Vaccinium ashei</i> Reade	0.0350	GH	Davies and Flore 1986
<i>Vitis vinifera</i> L.	0.0413	GH	Correia et al. 1990

^a Open top chambers on forest floor

^b Grown and measured at 26°C

^c Growth conditions:
CG growth chamber,
GH greenhouse, F field

ments were made at low O₂ to eliminate photorespiration were not included with the atmospheric (21%) O₂ measurements. For data expressed on an incident-light basis, we assumed an absorptance of 0.85 to convert to absorbed light. While this represents an average absorptance across many species, it does not substantially affect our analysis. Of the 40 observations in Table 2, 14 were converted to an absorbed basis. The mean of these was slightly lower (0.45) than the other 26 (0.57) but the standard deviations were not different between these groups (0.014 vs. 0.013). The assumed absorptance was used in only one case in Table 1. Thus, the effect of the assumed absorptance value seems to be minimal in the final analysis. Data were only taken from controls in each study to avoid including values from plants which may have been photoinhibited or otherwise altered by experimental treatments.

As a baseline for the comparisons, we used two extensive surveys of quantum yield. The first was a survey of 42 species by Björkman and Demmig (1987). Since our study was only concerned with C₃ species, we excluded data for five C₄ species reported in this paper. All measurements in the first study were made with an O₂ electrode (ϕ O₂). The second study was by Long et al. (1993) in which ϕ CO₂ was measured in C₃ species using gas-exchange and a light-integrating-sphere leaf cuvette. In this study, measurements were made at 1% O₂ to eliminate photorespiration.

Quantum yield measurements

We calculated the light-limited quantum yield from CO₂ assimilation measured on eight naturally-established native species growing in the field and two cultivated species grown in artificial conditions (see Table 3 for complete results). Measurements were made on four individuals of each species. Four naturally established species, *Acer saccharum* Marshall., *Aesculus glabra* Willd., *Asarum canadense* L., and *Parthenocissus quinquefolia* (L.) Planchon., were sampled from the understory of a native woodland located 8 km northeast of Urbana, Illinois (40°08'N, 88°09'W). The other native species, *Apocynum androsaemifolium* L., *Helianthus tuberosus* L., *Rhamnus cathartica* L., and *Solidago canadensis* L. grew in an adjacent prairie remnant. All outdoor measurements were made during July and September 1998. Two cultivated species were grown under controlled-environment conditions. Soybean [*Glycine max* (L.) Merr.] plants were grown in a greenhouse in Urbana, Illinois, and Linden bean (*Phaseolus vulgaris* L. var. Linden) plants were grown in a controlled environment cabinet (Model PGW36, Conviron Inc. Winnipeg, Manitoba, Canada) at 27/19°C (day/night) and a 15-h daylength.

Gas-exchange measurements were made using a portable photosynthesis system (model 6400, Li-Cor Inc, Lincoln, Neb., USA). Irradiance within the cuvette was provided by an integrated red-blue light-emitting diode (LED) source (model 6400-02, Li-Cor, Inc.), and the CO₂ mole fraction entering the leaf cuvette (C_e) was

Table 3 Light-limited quantum yields of CO₂ exchange and leaf absorbance of ten species in central Illinois. Gas-exchange measurements were made at 21% O₂ and absorbance was measured afterward on the same leaf. Absorbance values in parentheses were not measured

Species	ϕCO_2	Leaf absorbance	Growth conditions
<i>Acer saccharum</i> Marshall.	0.066±0.002	0.859±0.007	Forest understory
<i>Aesculus glabra</i> Willd.	0.083±0.009	0.865±0.005	Forest understory
<i>Apocynum androsaemifolium</i> L.	0.076±0.003	(0.85)	Open meadow
<i>Asarum canadense</i> L.	0.080±0.000	0.858±0.005	Forest understory
<i>Glycine max</i> (L.) Merr.	0.086±0.001	0.867±0.005	Greenhouse
<i>Helianthus tuberosus</i> L.	0.077±0.002	(0.85)	Open meadow
<i>Parthenocissus quinquefolia</i> (L.) Planchon	0.079±0.003	0.880±0.003	Forest understory
<i>Phaseolus vulgaris</i> L. var. Linden	0.084±0.001	0.832±0.012	Growth chamber
<i>Rhamnus cathartica</i> L.	0.084±0.008	(0.85)	Open meadow
<i>Solidago canadensis</i> L.	0.090±0.006	(0.85)	Open meadow

maintained at 400 $\mu\text{mol mol}^{-1}$. Light-response curves were measured at 15 PPF levels for plants grown in full sunlight and 13 levels for shaded plants. In all cases, ten PPF levels between 0 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were used. Data were logged when the total coefficient of variation parameter was at or below 0.1%, resulting in logging intervals between 3 and 10 min. The chamber dewpoint was maintained above 16°C. Tests on field-grown plants showed that beginning light-response curves at low light resulted in limitation of photosynthesis at high light due to insufficient stomatal opening (data not shown). Therefore, light-response curves were started at high PPF levels. Additional measurements were made on the *P. vulgaris* plants to investigate the role of changing photorespiration during the measurements. Low oxygen measurements were made by providing a mixture of 98% N₂ and 2% O₂ to the inlet of the gas-exchange system. “Constant C_i” measurements were made by controlling the cuvette inlet CO₂ level (C_e) at each measurement point so C_i was maintained at 400±20 $\mu\text{mol mol}^{-1}$.

Leaf absorbance was measured following the gas-exchange measurements. For plants growing in the field, a 9.6-cm² leaf disc was removed and brought to the laboratory in a moist enclosed container. Whole leaves of indoor plants were measured immediately after gas-exchange measurements were completed. Leaf absorbance was calculated from measurements of reflectance and transmittance made with an integrating sphere (model LI-1800-12, Li-Cor, Inc.) and a UV-visible spectroradiometer (model OL752, Optronic Laboratories, Orlando, Fla., USA). Absorbance was calculated relative to the red-blue LED light source.

Calculations

Gas-exchange data were calculated as in von Caemmerer and Farquhar (1981). Quantum yield was calculated from photosynthetic light-response data using linear regression across three or more points where net CO₂ assimilation was linearly related to PPF. Reported values are the mean ϕ of four individuals from each species. To study the effect of nonlinearity in the light-response, ϕCO_2 was calculated from gas-exchange data using successive regression lines fit to between 2 and 14 points of the curve starting with low-PPFD. Individual data points are the mean±1 SE of four observations, and regressions were calculated on the averaged data points. At low irradiance, measurements were sometimes affected by the Kok effect, an increase in respiration rates at low PPF (<20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) which results in a change in the initial slope of the light-response curve (Sharp et al. 1984). This was detected by a sudden change in ϕ at low light. In these cases we excluded the lowest two (0 and 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) points from the analysis.

Results and discussion

Quantum yield values in the literature

Our literature survey shows there is much more variation in reported quantum yield values from photosynthesis

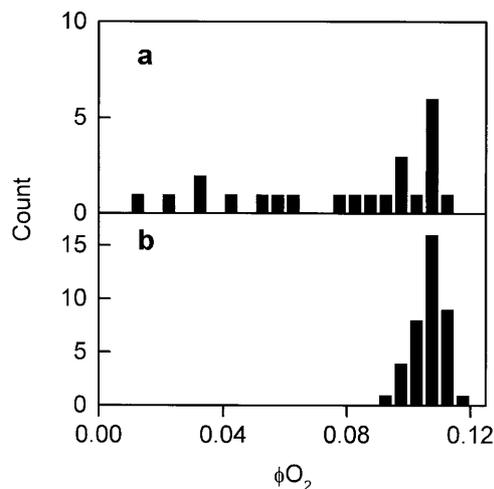


Fig. 1a, b Frequency distributions of quantum yield values measured by O₂ evolution. Data were collected from: **a** 23 observations from the literature survey (see Table 1) or **b** observations of 36 C₃ species published by Björkman and Demmig (1987)

studies compared with comparative ϕ studies. Among the ϕ values measured with an O₂ electrode, the coefficient of variation (CV) was 26% compared with 5% for the study by Björkman and Demmig (1987) (Fig. 1). Several observations of very low ϕO_2 values also resulted in a substantially lower mean photosynthetic efficiency of 0.089 compared with 0.108. A similar discrepancy was seen with the survey of ϕCO_2 in which the 39 observations had a 27% CV compared with 7% in our observations and 2.6% in the study of Long et al. (1993; Fig. 2). The mean ϕCO_2 at atmospheric O₂ was 0.053 in the literature survey compared with 0.081 in our study. Under low O₂ conditions, Long et al. (1993) found a mean quantum yield of 0.093 while the three studies in our literature survey measured at 2% O₂ had a mean ϕCO_2 of 0.085.

The mean ϕO_2 and ϕCO_2 in the literature survey are substantially affected by several very low values. The ϕO_2 distribution was highly skewed with the lowest one-third of the observations strongly biasing the mean and variance. The top half of the observations are within the range of the Björkman and Demmig (1987) dataset with the same mode in each case (Fig. 1). The distribution of

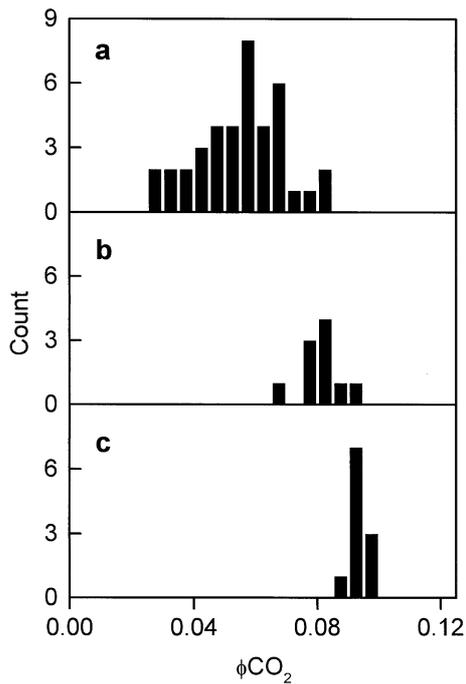


Fig. 2a–c Frequency distributions of quantum yield values measured by CO_2 assimilation. Data were collected from: **a** 39 observations from the literature survey (see Table 2), **b** observations on 10 species measured with gas-exchange, or **c** observations from 11 species measured by Long et al. (1993)

ϕCO_2 observations was more normal with no group of observations that stand out from the rest (Fig. 2a). Thus the approximately 30% lower ϕCO_2 in the literature survey compared with our study represents the majority of the published data from photosynthesis studies.

Causes of low ϕ

Photoinhibition

Low ϕ values often result from photoinhibition, a light-induced reduction in photosynthesis that may either result from photodamage or photoprotective chloroplast processes. These could have been induced by the growth environment of the plants or by the experimental protocol (Groom and Baker 1992). These are most often caused by cool temperatures or excess PPFD (e.g., Bongi and Long 1987; Groom and Baker 1992; Long et al. 1994). For example, Björkman and Demmig (1987) caused the lowest ϕ (<0.06) by subjecting leaves to more than 120 min of over-saturating PPFD. We attempted to exclude from our survey plants that may have been photoinhibited by only including data from control plants in each study. It is possible that photodamage was inadvertently induced during measurements, for example if leaves were stored in the dark between collection and measurements and then immediately given high PPFD. It is not possible to tell from the data presented in any individual study whether or not this occurred. Thus, we can-

not directly determine the extent that photodamage and/or photoprotection may contribute to the high variance in ϕ .

Photorespiration and alternative electron sinks

Several physiological processes divert reducing power from carbon reduction and could account for the observed variation in ϕCO_2 . The largest of these sinks is photorespiration, which usually occurs at 10–30% of the photosynthesis rate at 25°C with 350 $\mu\text{mol mol}^{-1}$ CO_2 and 21% O_2 (Sharkey 1988; Peterson 1990; Yeo et al. 1994). This sink can be eliminated by measuring ϕCO_2 under a reduced (1–2%) O_2 atmosphere. Other “alternative” sinks, including NO_3 and non-photorespiratory oxygen reductions, have been found in some leaves, although they are generally small (Edwards and Walker 1983; Robinson 1988; Cornic and Briantais 1991). Long et al. (1993) conclude that the energy demands of these processes account for the approximately 10% difference between their mean ϕCO_2 and the mean ϕO_2 reported by Björkman and Demmig (1987). Even if photorespiration varied by 100% this could only cause a 30% variation in quantum yield. Since we find greater than 30% variation, even under conditions where photorespiration should vary little, photorespiration cannot explain the variance in ϕ . Likewise, the alternative sinks are generally less than 10% of the total electron sinks and also cannot adequately explain the variance in ϕ .

Measurement technique

Because photoinhibition and alternative electron sinks do not sufficiently explain the observed variance in ϕ , we investigated the influence of photosynthetic data collection and analysis techniques on calculated ϕ values. The calculation of ϕ_{max} was based on the analysis of the photosynthetic light-response curve. This curve has four regions (Leverenz 1987): (1) the Kok effect region, where mitochondrial respiration increases at very low light (usually $<20 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD), affecting net photosynthesis and increasing the slope of the light-response curve; (2) the region where net photosynthesis is linear with increasing PPFD; (3) the transition region between the linear and light-saturated regions; and (4) the light-saturated region. Only the linear region meets the assumptions that light is limiting and respiration is constant for calculating ϕ_{max} . Including points from the Kok effect region would cause an overestimation of ϕ while including points from the transition region would cause an underestimation.

The linear region of each light-response curve varied considerably among the light-response curves we measured. Thus, the range of PPFD valid for ϕ measurements was determined by evaluating each light-response curve individually. Data from two representative species are presented in Fig. 3. The light-response of *H. tuberosa*

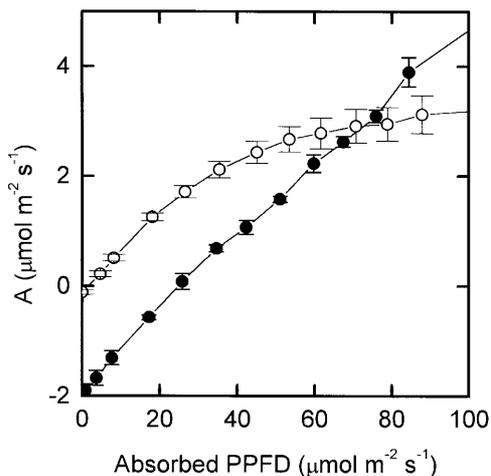


Fig. 3 Light-response of photosynthesis for sun and shade grown plants measured with gas-exchange. *Parthenocissus quinquefolia* (L.) Planchon. (○) grew in a forest understory and *Helianthus tuberosus* L. (●) grew in a nearby open meadow. Data points are the mean \pm 1 SE of four observations

grown in full sunlight was apparently linear over a wider range of PPFD than was the *Parthenocissus quinquefolia* grown in the understory. The nonlinearity of the light-response is shown by the linear regressions where data points were sequentially added to the regression analysis (Fig. 4). Since the Kok effect (Sharp et al. 1984) was evident at the lowest two PPFD levels in the *H. tuberosus* data (Fig. 3), these points were excluded from the analysis in Fig. 4. Quantum yield was calculated from each regression line fit to different numbers of points starting at low PPFD. The nonlinearity in the *P. quinquefolia* data is evident by the sharp drop in calculated quantum yield numbers (Fig. 4a). This was also the case with the *H. tuberosus* data although the effect is not as severe. An abrupt drop occurred in the curve in both cases as irradiance levels approached the transition to light-saturated photosynthesis.

We expected the light-response of shaded leaves to be nonlinear even at quite low light. The light response of *P. quinquefolia* was nonlinear even below $20 \mu\text{mol m}^{-2} \text{s}^{-1}$, which was typical of the shade plants we measured (results not shown). Consequently, there was a very strong dependence of the quantum yield value on the range of data used in the regression. In the example shown, the correlation coefficient (r^2) remained above 0.95 even when the non-linearity of the light response caused the estimated quantum yield to drop by more than 30%. Thus the truly linear region of the light-response curve was often not present, or occurred in a narrow range of light intensities. This fact was not evident from the r^2 of the regression.

The curvature of the sun-grown *H. tuberosus* response was not as obvious, but still resulted in a 20% reduction in calculated quantum yield values over the apparently light-limited range of photosynthesis. This was not evident from the correlation coefficient (Fig. 4b), but result-

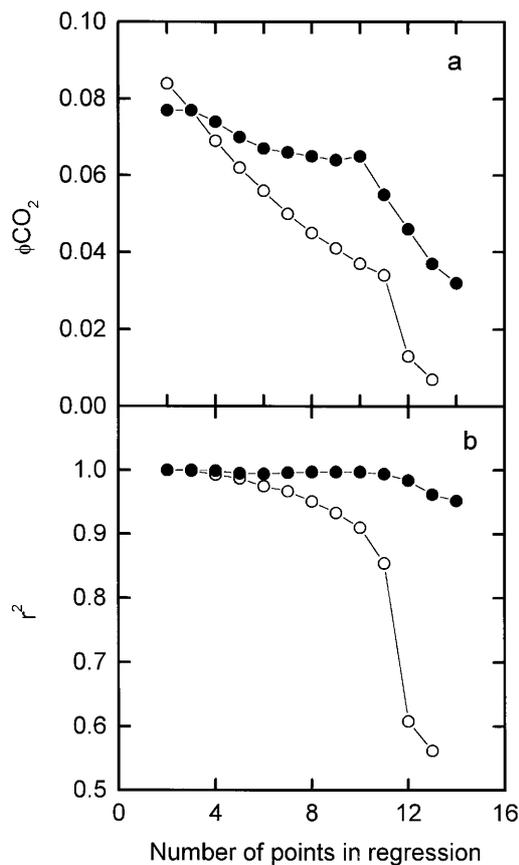


Fig. 4 The effect of nonlinearity in the light-response curve on **a** regression-derived ϕCO_2 and **b** r^2 . The averaged light-response data from Fig. 3 were fit using successive linear regressions on subsets of the data ranging from 3 to 14 points. Regressions were re-calculated as data points were sequentially added to the regression starting with the lowest photosynthetic photon flux density (PPFD). Because the Kok effect was present in the *H. tuberosus* data (●, see Methods), the first two points were left off the regression analysis. Symbols are as in Fig. 3

ed in a truly linear region of photosynthesis that only extended to $40 \mu\text{mol m}^{-2} \text{s}^{-1}$. In this case, the curvature did not result from the light saturation of photosynthesis but rather is a result of changes in C_i . This behavior in light response curves was called an “apparent Kok effect” by Kirschbaum and Farquhar (1987). This occurs when photosynthesis responds to light independently of stomatal conductance during gas-exchange measurements. Increases in CO_2 assimilation without an accommodating change in stomatal conductance result in decreased C_i . Because stomatal conductance did not change substantially during our measurements, C_i increased as photosynthesis declined. This was the case with most of the field measurements we made (data not shown).

To demonstrate the consequences of the apparent Kok effect on ϕ measurements, we made measurements under three conditions (Fig. 5). In the first experiment, we measured leaves under 21% O_2 while holding the CO_2 entering the leaf cuvette (C_e) constant at $600 \mu\text{mol mol}^{-1}$ (closed circles). In the second experiment we measured

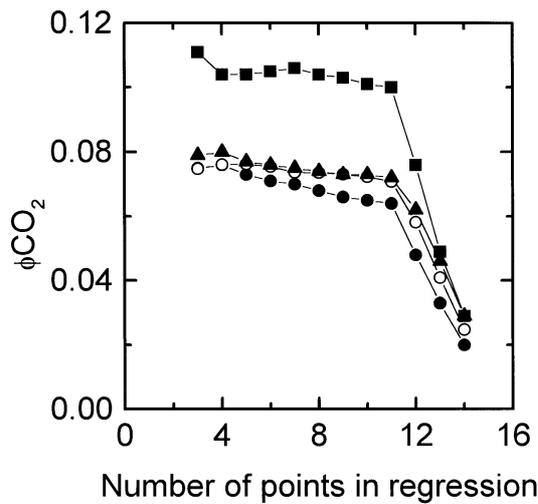


Fig. 5 The effect of measurement conditions on the linearity of quantum yield measurements. The data are fit from linear regressions as in Fig. 4 to light-response data collected from *Phaseolus vulgaris* L. leaves while holding (1) the CO_2 mole fraction entering the leaf cuvette (C_e) at $600 \mu\text{mol mol}^{-1}$ (●), (2) C_e at $600 \mu\text{mol mol}^{-1}$ in a 2% O_2 atmosphere (■), (3) the leaf-airspace CO_2 mol fraction (C_i) at $400 \mu\text{mol mol}^{-1}$ (○), and (4) C_e held at $600 \mu\text{mol mol}^{-1}$ corrected to a C_i of $400 \mu\text{mol mol}^{-1}$ as described in the appendix (▲)

leaves under 2% O_2 and held C_e constant at $600 \mu\text{mol mol}^{-1}$ (closed squares). In the third experiment, we measured under 21% O_2 but adjusted C_e at each measurement point so C_i remained between 387 and $417 \mu\text{mol mol}^{-1}$ (open circles). When C_e was held constant at atmospheric O_2 , quantum yield varied with the number of points measured by 15% (0.076–0.065), while C_i varied between 468 and $568 \mu\text{mol mol}^{-1}$. This compares with a drop of 3% at low O_2 , and 7% with a constant C_i . To further investigate the quantitative effects of changing C_i , we corrected the measurements made at constant C_e to a constant C_i of $400 \mu\text{mol mol}^{-1}$ (triangles; see Appendix 1 for calculations). The ϕ values from these corrected calculations were indistinguishable from the data collected at a constant C_i , and the reduction in ϕ dropped to 9%. Thus, by eliminating photorespiration at low O_2 , keeping it constant by adjusting C_i , or correcting for changes in photorespiration using the mathematical relationships developed for photorespiration, we increased the linear region of the light-response. This supports our observation that the photosynthetic light response can be affected by C_i when measurements are made at atmospheric O_2 levels yielding erroneously low quantum yield calculations. Since most of our field measurements were made under 21% O_2 and constant C_e , this helps explain the nonlinear response of the *H. tuberosa* leaves (Fig. 3).

Technical problems

Aside from the biological problems resulting from the growth or measurement conditions of plants, there are

also a number of technical problems that can affect ϕ . Among these, errors in light and CO_2 measurement will have the greatest impact on ϕ . Irradiance measurements inside the gas-exchange chamber can be problematic as many light sources using a single bulb can provide highly nonuniform irradiance inside the chamber. This problem can be avoided by using a LED light source with many small diodes distributed across the surface which provide even lighting across the chamber surface (Tennessen et al. 1995). Irradiance measurements can also be problematic if differences in the spectra between the artificial light source and sunlight are not taken into account during calibration of the measurement device. Neither of these was a problem in the present study as we used a factory-calibrated LED light source. Any calibration problems affecting CO_2 assimilation measurements will also impact ϕ calculations. Most notable among these are the flow meter and infra-red gas analyzer (IRGA) calibrations within the measurement system. Since these problems result from the measurement equipment and the user's knowledge, extensive discussion of these is beyond the scope of this manuscript.

Calculating ϕ from photosynthesis measurements

From our analyses, it appears that substantial underestimation of ϕ can result from the nonlinear portion of the light-response curve appearing at a lower than expected PPFD. This problem can affect both measures of maximum and apparent ϕ , and may be the major reason for the wide variation in published ϕ values (Figs. 1, 2). This condition is more easily diagnosed when measurements are made at close PPFD intervals and the data are analyzed with a multiple regression approach. In addition to this, measurements of apparent ϕ could be reduced by as much as 15% by subtle changes in the slope of the linear portion of the light-response curve, caused in large part by changes in photorespiration as the ratio of CO_2 to O_2 changes in the chloroplast. Much of this anomaly can be eliminated by reducing or keeping constant the rate of photorespiration during the measurement period. If ϕ_{max} is of interest, eliminating photorespiration by making measurements at low O_2 or high CO_2 partial pressures is preferable. Since most ecophysiological studies are concerned with ϕ under growth conditions, these measurements are not applicable. Instead, measurement can be made while adjusting conditions to keep C_i constant, or lacking that type of control, by correcting the measurements using the equations developed by Sharkey (1988). These techniques, in addition to proper instrument and light-source calibration, will improve the consistency of ϕ measurements so they can be compared across ecophysiological studies.

Conclusions

We have found considerable variation among estimates of ϕ when it was measured in the context of larger photo-

synthesis studies. Although it was not usually possible to determine in any individual case what caused low photosynthetic efficiency, methodological errors can cause substantial underestimation of ϕ . Erroneously low values can be caused by nonlinearity of the photosynthetic light response. The nonlinearity can result from the leaf becoming partially light-saturated at relatively low light even in leaves of plants grown in full sunlight. This condition did not vary in any predictable manner and had to be evaluated on a case-by-case basis using many data points collected between 0 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. Changes in photorespiration can also cause nonlinearity in the light-response of photosynthesis in near-ambient O_2 and CO_2 concentrations. Although we did not find evidence of photodamage or photoprotection during our measurements, these could have affected some of the previously published studies. Because of the ease by which ϕ can be underestimated, we reject the hypothesis that the intrinsic efficiency of photosynthesis varies substantially among plant species or growth conditions unless plants experience stressful environments. Thus, our analysis supports the contention of Long et al. (1993) and Björkman and Demmig (1987) that intrinsic quantum yield varies little from the mean values of 0.092 for ϕCO_2 and 0.108 for ϕO_2 in unstressed plants. Furthermore, we conclude that operational ϕ varies little from our mean value of 0.081.

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Appendix

The correction factor for photosynthesis measurements is based on the mechanistic model of photorespiration described by Sharkey (1988). The analysis is based upon the determination of the ratio of oxygenation to carboxylation of RuBP, Φ , where:

$$\Phi = 2\Gamma^*/C \quad (1)$$

where C is the CO_2 partial pressure at the site of carboxylation and Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration as defined by Farquhar et al. (1980). Γ^* can be estimated based on the leaf temperature from the empirical relationship determined by Brooks and Farquhar (1985):

$$\Gamma^* = 42.7 + 1.68(T - 25) + 0.0012(T - 25)^2 \quad (2)$$

$$\Gamma^* = \Gamma^* \cdot P \quad (2a)$$

where P is atmospheric pressure.

The rate of oxygenation is calculated as:

$$v_o = (A + R_d) / (\Phi - 0.5)$$

where A is net CO_2 assimilation rate, and R_d is respiration in the dark, determined by extrapolating the linear portion of the light-response curve to zero.

Once the rate of oxygenation is known, the two major effects of photorespiration, the emission of CO_2 and the loss of RuBP regeneration capacity, can be subtracted from the photosynthesis data. Because one molecule of CO_2 is released for every two oxygenen-

ation events, the CO_2 efflux is half of v_o . The loss of RuBP regeneration capacity is equal to v_o . Therefore we subtracted $1.5 \times v_o$ at each point. Next we calculated v_o for a C_i of 400 $\mu\text{mol mol}^{-1}$ and added $1.5 \times v_o(400)$ back to each data point. Because this model is based on CO_2 partial pressure at the site of carboxylation, C_c , we used a mesophyll conductance, g_m , of 0.2 $\text{mol m}^{-2} \text{s}^{-1} \text{bar}^{-1}$ to calculate C_c from C_i and A . This value was chosen based on the average values of g_m for plants with light-saturated photosynthesis rates of approximately 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$, as determined from different species (summarized in Evans and Loreto 2000). When we applied this correction to the light-response data from *P. vulgaris* plants, ϕ was increased by 5–12% in the light-limited region of the curve, and the linearity of the light response was substantially improved resulting in ϕ calculations nearly identical to those measured at a C_i of 400 (Fig. 5).

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