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Fitting net photosynthetic light-response curves with *Microsoft Excel* – a critical look at the models

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Abstract

In this study, we presented the most commonly employed net photosynthetic light-response curves (P_N/I curves) fitted by the Solver function of *Microsoft Excel*. *Excel* is attractive not only due to its wide availability as a part of the *Microsoft Office* suite but also due to the increased level of familiarity of undergraduate students with this tool as opposed to other statistical packages. In this study, we explored the use of *Excel* as a didactic tool which was built upon a previously published paper presenting an *Excel* Solver tool for calculation of a net photosynthetic/chloroplastic CO_2-response curve. Using the *Excel* spreadsheets accompanying this paper, researchers and students can quickly and easily choose the best fitted P_N/I curve, selecting it by the minimal value of the sum of the squares of the errors. We also criticized the misuse of the asymptotic estimate of the maximum gross photosynthetic rate, the light saturation point estimated at a specific percentile of maximum net photosynthetic rate, and the quantum yield at zero photosynthetic photon flux density and we proposed the replacement of these variables by others more directly linked to plant ecophysiology.

Additional key words: curve fitting; iteration; nonlinear regression; P_N/I curve; Solver function.

Introduction

The net photosynthetic light-response curve (P_N/I curve) describes the net CO_2 assimilation by a plant leaf (P_N) as a function of an increase in the photosynthetic photon flux density (I) from the total absence of light to a high level of light, *e.g.* 2,000 μmol(photon) m^{-2} s^{-1}.

The curve presents several phases. At the beginning, from complete darkness to the light compensation point (I_{comp}), there is a rapid increase in P_N with I, due to the

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Abbreviations: ARE – average relative errors; C_c – chloroplast CO_2 concentration; C_i – intercellular CO_2 concentration; I – photosynthetic photon flux density; I_{comp} – light compensation point; I_{max} – light saturation point beyond which there is no significant change in P_N; I_{sat} – light saturation point; $I_{sat(n)}$ – light saturation point at a specific percentile (n) of P_{Nmax}; $I_{sat(85)}$ – light saturation point for $P_N + R_D$ equal to 85% of P_{Nmax}; $I_{sat(90)}$ – light saturation point for $P_N + R_D$ equal to 90% of P_{Nmax}; $I_{sat(95)}$ – light saturation point for $P_N + R_D$ equal to 95% of P_{Nmax}; $I_{(50)}$ – light saturation point for $P_N + R_D$ equal to 50% of P_{Nmax}; k – adjusting factor; P_g – gross photosynthetic rate; P_{gmax} – maximum gross photosynthetic rate; P_N – net photosynthetic rate; $P_{N(Imax)}$ – maximum net photosynthetic rate obtained at $I = I_{max}$; P_{Nmax} – maximum net photosynthetic rate; R_D – dark respiration; R^2 – coefficient of determination; SAE – sum of the absolute errors; SSE – sum of the squares of the errors; V_{max} – enzyme maximum velocity; β – adjusting factor; γ – adjusting factor; θ – convexity factor; ϕ – quantum yield; $\phi_{(I)}$ – quantum yield at a particular value of I; $\phi_{(I_{comp})}$ – quantum yield at $I = I_{comp}$; $\phi_{(I_{comp} - I_{200})}$ – quantum yield at the range between I_{comp} and I = 200 μmol(photon) m^{-2} s^{-1}; $\phi_{(I_0)}$ – quantum yield at I = 0 μmol(photon) m^{-2} s^{-1}; $\phi_{(I_0 - I_{comp})}$ – quantum yield at the range between I = 0 μmol(photon) m^{-2} s^{-1} and I_{comp}; ϕ_{max} – theoretical maximum quantum yield; χ^2 – Chi-square test.

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natural decrease in dark respiration (R_D), named the Kok effect (Kok 1949). The light compensation point is the value of I at which the CO_2 assimilated by photosynthesis is in balance with the CO_2 produced by light respiration and photorespiration, resulting in P_N equal to zero. Beyond this point, a supposed linear response of P_N to I can be seen until I reaches approximately 200 μmol (photon) m^{-2} s^{-1}. This linear portion is the range that many authors use to calculate the "maximum quantum yield" ($\phi_{(I_{comp} - I_{200})}$), which is the slope in that range, and beyond this range there is a region of nonlinear die-off before P_N reaches a semiplateau, where an increase in I does not provoke a proportional increase in P_N (Long and Hällgren 1993). The progressive curvature in the ratio $\delta P_N/\delta I$ in this region can be described by a convexity factor (θ) (Ögren 1993). Sometimes, after reaching the maximum value of P_N, a subsequent decrease in P_N with I, referred to as photoinhibition, can be observed (Ye 2007). Extensive literature is available describing the different phases of the curve and the effects of temperature, CO_2 concentration, pH, chemical inhibitors of photosynthesis, and other factors (Long and Hällgren 1993, Govindjee *et al*. 2005, Zeinalov 2005, Lambers *et al*. 2008).

Many mathematical models may be used to describe P_N/I curves. Various parameters and variables calculated from these models are used to describe the photosynthetic capacity, efficiency, and other aspects. Those variables include I_{comp}, the asymptotic estimate of the maximum gross photosynthetic rate (P_{gmax}), the light saturation point for $P_N + R_D$ equal to 50% of maximum net photosynthetic rate (P_{Nmax}) ($I_{(50)}$), the light saturation point for P_N equal to a percentile of the P_{Nmax} ($I_{sat(n)}$), the light saturation point (I_{sat}), the quantum yield at I = 0 μmol(photon) m^{-2} s^{-1} ($\phi_{(I_0)}$), the quantum yield obtained in the range between I_o and I_{comp} ($\phi_{(I_0 - I_{comp})}$), $\phi_{(I_{comp} - I_{200})}$, ϕ obtained at any value of I ($\phi_{(I)}$), θ, and R_D.

Certain models are modifications of the originals, but sometimes these modifications produce serious problems that are not handled properly or, at best, they produce meaningless information. Kaipiainen (2009) uses a modified Michaelis-Menten model (Eq. 2), assuming that $I_{(50)}$ is the value of I, when P_N is equal to 50% of P_{gmax}; however, that assumption is only true if the gross photosynthetic rate (P_g, which is equal to $P_N + R_D$) is equal to 50% of P_{gmax}. A nonrectangular hyperbola, in its original form (Eq. 6), was modified by Chen *et al*. (2008) to give the form of Eq. 7, which produces misleading information about the value of P_N in the absence of light (I = 0). In that case, when I = 0, it is expected that $P_N = -R_D$, but the modified model produces a value of $P_N = \frac{P_{gmax}}{2\theta} - R_D$. The original, exponential model (Eq. 8) has many different variations. One of these variations can be seen in the papers of Lootens *et al*. (2004) and Devacht *et al*. (2009), shown in Eq. 10, where it can be observed that $P_N = -R_D$, when $I = I_{comp}$, not when I = 0.

For the majority of P_N/I curves, P_{gmax} and $I_{sat(n)}$ have a mathematical definition but they do not often have the desired ecophysiological meaning, resulting in frequent misuse by researchers if not applied with caution. Presenting the definition of P_{gmax} as the light-saturated rate of CO_2 uptake is akin to defining it as "the point beyond which there is no significant change in P_N", which is not the case, because P_{gmax} is obtained when I is infinite. In other words, P_{gmax} is an abstraction, which forces the existence of $I_{sat(n)}$, which is by deduction also an abstraction. The same issues occur with the quantum yield (φ), which, given the variety of methods used for the calculation of this parameter, often serves more aptly as a source of doubt than as an explanatory variable.

Ye (2007) proposed a modified rectangular hyperbola model able to fit the photoinhibition stage and to estimate P_{gmax} and I_{sat}. The author also employs 4 different forms of φ: $\phi_{(I_0)}$, $\phi_{(I_{comp})}$, $\phi_{(I_0 - I_{comp})}$, and $\phi_{(I)}$. Although this model has several advantages in relation to the previous P_N/I curves, the values of P_{gmax} and I_{sat} produced are occasionally out of the expected range of ecophysiological meaning, thereby failing to resolve a key issue observed in other models calculating P_{gmax} and $I_{sat(n)}$.

Because the mathematical models describing the P_N/I curve are nonlinear, there are several problems that must be taken into account when fitting a regression curve. Researchers must have good statistical and mathematical knowledge and moderately advanced skill using statistical programs, which may not be available for students and at times researchers. *Microsoft Excel* can offer a good alternative in this case, not only in terms of fitting the regression curve but also affording the opportunity for the users to see and understand the use of each equation employed in the calculations (Brown 2001). Particularly in developing countries, an important consideration is that there are no additional expenses beyond the *Microsoft Office* package required to calculate P_N/I curves. There are many routines in *Microsoft Excel*, which were developed taking this philosophy into account. Examples include several exercises in ecology and evolution (Donovan and Welden 2002), resampling for the mean and the calculation of its confidence interval (Christie 2004), a tool for classical plant growth analysis (Hunt *et al*. 2002), and more appropriate for this work, a step-by-step tool to fit nonlinear regression (Brown 2001).

The net photosynthetic/chloroplastic CO_2 response curve (P_N/C_c curve) and the P_N/I curve are useful tools in plant physiology. Both curves assist researchers in understanding the effects of changes in one or more primary factors affecting photosynthesis. These models are also employed as a single leaf component of more complex models of entire plants or ecosystems (Harley and Baldocchi 1995, Lloyd *et al*. 1995).

Sharkey *et al*. (2007) developed an *Excel* routine to fit

net photosynthetic/intercellular CO_2 response (P_N/C_i) or P_N/C_c curve, but until now, no *Excel* routine has been developed to fit P_N/I curves. In this study, we presented the most common mathematical models for fitting P_N/I curves adjusted by the Solver function of *Microsoft Excel*. We proposed a new approach to find the maximum value of I (I_{max}) that saturates P_N, considering it as the point beyond which there is no significant change in P_N. For our applications, I_{max} and the maximum value of P_N obtained at $I = I_{max}$ ($P_{N(Imax)}$) are more appropriate than I_{sat} or $I_{sat(n)}$ and P_{gmax}, due to their realistic magnitudes that give them their intended ecophysiological meaning. Likewise, $\phi_{(I)}$ can provide much more information than all of the other calculations of ϕ.

Materials and methods

Primary data: Measurements were conducted on *Vochysia divergens* Pohl (Vochysiaceae) in a fragment of savanna ecosystem (Cerrado *stricto sensu*) located in Cuiabá, Mato Grosso, Brazil (15°43' S, 56°04' W).

A unique, disease free, mature leaf exposed to full sunlight was measured with a portable photosynthetic system (*LI-6400*, *LI-COR*, *Inc*., Logan, NE, USA) coupled with a standard red/blue LED broadleaf cuvette (*6400-02B*, *LI-COR, Inc*., Logan, NE, USA) and a CO_2 mixer (*6400-01*, *LI-COR, Inc*., Logan, NE, USA). The measurements were obtained with a block temperature of 28°C, 50 to 60% relative humidity, and 400 μmol(CO_2) mol(air)$^{-1}$ of CO_2 concentration inside the chamber.

The P_N/I curve was performed using the autoprogram function. In this case, we chose the sequence of desired light settings of 2,000; 1,500; 1,250; 1,000; 800, 500, 250, 100, 50, 25, and 0 μmol(photon) m^{-2} s^{-1}, a minimum wait time of 120 s, a maximum wait time of 200 s, and matching the infrared gas analyzers for 50 μmol(CO_2) mol(air)$^{-1}$ difference in the CO_2 concentration between the sample and the reference, which allowed them to be matched before every change in I. The measurement results are shown in Table 1.

Table 1. Original data obtained from the net photosynthetic light-response curves of *V. divergens*.

I [μmol(photon) m^{-2} s^{-1}]	P_N [μmol(CO_2) m^{-2} s^{-1}]
0	–1.55
25	0.06
50	1.60
100	4.10
250	8.74
500	12.00
800	13.60
1,000	14.30
1,250	14.80
1,500	15.00
2,000	15.50

Mathematical models for P_N/I curves: In this section, we presented the models that were the most frequently employed to fit P_N/I curves: the rectangular hyperbola Michaelis-Menten based models (Eqs. 1–3), the hyperbolic tangent based models (Eqs. 4,5), the nonrectangular hyperbola–based models (Eqs. 6,7), the exponential based models (Eqs. 8–10), and the Ye model (Eq. 11). All these mathematical models are well described in the literature (Baly 1935, Smith 1936, Webb *et al*. 1974, Jassby and Platt 1976, Prioul and Chartier 1977, Gallegos and Platt 1981, Prado *et al*. 1994, Prado and de Moraes 1997, Vervuren *et al*. 1999, Lootens *et al*. 2004, Ye 2007, Chen *et al*. 2008, Abe *et al*. 2009, Kaipiainen 2009).

We developed individual *Excel* routines for all of these models except for Eqs. 7 and 10 due to the previous comments about their performance.

$$P_N = \frac{\phi_{(I_o)} \times I \times P_{gmax}}{\phi_{(I_o)} \times I + P_{gmax}} - R_D \quad (1)$$

$$P_N = \frac{I \times P_{gmax}}{I + I_{(50)}} - R_D \quad (2)$$

$$P_N = \frac{\phi_{(I_o)} \times I \times P_{gmax}}{\sqrt{\phi_{(I_o)}^{\,2} \times I^2 + P_{gmax}^{\,2}}} - R_D \quad (3)$$

$$P_N = P_{gmax} \times \tanh\left(\frac{\phi_{(I_o)} \times I}{P_{gmax}}\right) - R_D \quad (4)$$

$$P_N = P_{gmax} \times \tanh\left(\frac{I}{I_{sat}}\right) - R_D \quad (5)$$

$$P_N = \frac{\phi_{(I_o)} \times I + P_{gmax} - \sqrt{\left(\phi_{(I_o)} \times I + P_{gmax}\right)^2 - 4\theta \times \phi_{(I_o)} \times I \times P_{gmax}}}{2\theta} - R_D \quad (6)$$

$$P_N = \frac{\phi_{(I_o)} \times I + \sqrt{\left(\phi_{(I_o)} \times I + P_{gmax}\right)^2 - 4\theta \times \phi_{(I_o)} \times I \times P_{gmax}}}{2\theta} - R_D \quad (7)$$

$$P_N = \left\{P_{gmax}\left[1 - \exp\left(\frac{-\phi_{(I_o)} \times I}{P_{gmax}}\right)\right]\right\} - R_D \quad (8)$$

$$P_N = \left\{P_{gmax}\ \left[1-\exp\left(-k\left(I-I_{comp}\right)\right)\right]\right\} - R_D \quad (9)$$

$$P_N = P_{gmax}\left\{1-\exp\left[-\phi_{(I_o)}\times\frac{\left(I-I_{comp}\right)}{P_{gmax}}\right]\right\} - R_D \quad (10)$$

$$P_N = \phi_{(I_o\_I_{comp})}\times\frac{1-\beta\times I}{1+\gamma\times I}\times\left(I-I_{comp}\right) \quad (11),$$

where: I – the photosynthetic photon flux density [μmol (photon) m^{-2} s^{-1}]; I_{comp} – the light compensation point [μmol(photon) m^{-2} s^{-1}]; I_{sat} – the light saturation point [μmol(photon) m^{-2} s^{-1}]; $I_{(50)}$ – the light saturation point at $P_N + R_D = 50\%$ of P_{gmax}; k – an adjusting factor [s m^2 μmol(photon)$^{-1}$]; P_{gmax} – the asymptotic estimate of the maximum gross photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; P_N – the net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; R_D – the dark respiration rate [μmol(CO_2) m^{-2} s^{-1}]; β – an adjusting factor (dimensionless); γ – an adjusting factor (dimensionless); θ – the convexity (dimensionless); $\phi_{(I_0)}$ – the quantum yield at $I = 0$ [μmol(CO_2) μmol (photon)$^{-1}$]; $\phi_{(I_0 - I_{comp})}$ – the quantum yield obtained at the range between I_o and I_{comp} [μmol(CO_2) μmol(photon)$^{-1}$].

Calculated variables: All variables and regression parameters used to analyze the P_N/I curves were calculated or taken from each model, respectively.

P_{gmax}, R_D, and I_{sat} were not any of the parameters in Eq. 11. Instead, they are calculated using Eqs. 12–14, respectively (Ye 2007).

$$P_{gmax} = \phi_{(I_o\_I_{comp})}\times\frac{1-\beta\times I_{sat}}{1+\gamma\times I_{sat}}\times\left(I_{sat}-I_{comp}\right)+R_D \quad (12)$$

$$R_D = \phi_{(I_o\_I_{comp})}\times I_{comp} \quad (13)$$

$$I_{sat} = \frac{\sqrt{\frac{(\beta+\gamma)\times\left(1+\gamma\times I_{comp}\right)}{\beta-1}}}{\gamma} \quad (14)$$

As $\phi_{(I_o)}$ is one of the parameters only in Eqs. 1, 3, 4, 6–8, and 10, for the other equations it was calculated as the derivative of these models at $I = 0$. For any value of I, the general $\phi_{(I)}$ is calculated as the derivative of the P_N/I curve with respect to I. The corresponding derivative equations for the mathematical models employed in this study to develop individual *Excel* routines (Eqs. 1–6,8,9, and 11) are presented in Eqs. 15–20, 21, 22, and 23, respectively.

$$\phi_{(I)} = \frac{\phi_{(I_o)}\times {P_{gmax}}^2}{\left(\phi_{(I_o)}\times I+P_{gmax}\right)^2} \quad (15)$$

$$\phi_{(I)} = \frac{I_{(50)}\times P_{gmax}}{\left(I+I_{sat(50)}\right)^2} \quad (16)$$

$$\phi_{(I)} = \frac{\phi_{(I_o)}\times {P_{gmax}}^3}{\left({\phi_{(I_o)}}^2\times I^2+{P_{gmax}}^2\right)^{3/2}} \quad (17)$$

$$\phi_{(I)} = \phi_{(I_o)}\times\operatorname{sech}^2\left(\frac{-\phi_{(I_o)}\times I}{P_{gmax}}\right) = \phi_{(I_o)}\times\left[\frac{1}{\cosh^2\left(\frac{-\phi_{(I_o)}\times I}{P_{gmax}}\right)}\right] \quad (18)$$

$$\phi_{(I)} = \frac{P_{gmax}}{I_{sat}}\times\operatorname{sech}^2\left(\frac{I}{I_{sat}}\right) = \frac{P_{gmax}}{I_{sat}}\times\left[\frac{1}{\cosh^2\left(\frac{I}{I_{sat}}\right)}\right] \quad (19)$$

$$\phi_{(I)} = \frac{\phi_{(I_o)}}{2\theta}\times\left[1-\frac{\phi_{(I_o)}\times I+P_{gmax}-2\theta\times P_{gmax}}{\sqrt{\left(\phi_{(I_o)}\times I+P_{gmax}\right)^2-4\theta\times\phi_{(I_o)}\times I\times P_{gmax}}}\right] \quad (20)$$

$$\phi_{(I)} = \phi_{(I_o)}\times\exp\left(\frac{-\phi_{(I_o)}\times I}{P_{gmax}}\right) \quad (21)$$

$$\phi_{(I)} = P_{gmax}\ \times k\times\exp\left[-k\left(I-I_{comp}\right)\right] \quad (22)$$

$$\phi_{(I)} = \phi_{(I_o\_I_{comp})}\times\frac{1-\beta\times\gamma\times I^2-2\beta\times I+(\gamma+\beta)I_{comp}}{\left(1+\gamma\times I\right)^2} \quad (23)$$

$\phi_{(I_{comp})}$ was calculated using Eqs. 15–23, replacing I with I_{comp}. $\phi_{(I_0 - I_{comp})}$ and $\phi_{(I_{comp} - I_{200})}$ were calculated as the slope of the linear regression of P_N for values of I between 0 and I_{comp} and between I_{comp} and 200 μmol (photon) m^{-2} s^{-1}, respectively.

I_{comp} is one of the parameters in Eqs. 9–11. For the other equations, it was calculated by isolating I in the mathematical model and setting P_N to zero. To develop individual *Excel* routines (Eqs. 1–6, and 8) for the mathematical models employed in this study, the corresponding equations are presented in Eqs. 24–29, and 30, respectively.

$$I_{comp} = \frac{P_{gmax}\times R_D}{\phi_{(I_o)}\times\left(P_{gmax}-R_D\right)} \quad (24)$$

$$I_{comp} = \frac{I_{(50)}-R_D}{P_{gmax}-R_D} \quad (25)$$

$$I_{comp} = \frac{R_D\times P_{gmax}}{\phi_{(I_o)}}\times\sqrt{\frac{1}{{P_{gmax}}^2-{R_D}^2}} \quad (26)$$

$$I_{comp} = \operatorname{arctanh}\left(\frac{R_D}{P_{gmax}}\right) \times \frac{P_{gmax}}{\phi_{(I_o)}} \quad (27)$$

$$I_{comp} = I_{sat} \times \operatorname{arctanh}\left(\frac{R_D}{P_{gmax}}\right) \quad (28)$$

$$I_{comp} = \frac{R_D \times \left(\phi_{(I_o)} \times R_D - P_{gmax}\right)}{\phi_{(I_o)} \times \left(R_D - P_{gmax}\right)} \quad (29)$$

$$I_{comp} = -\frac{P_{gmax}}{\phi_{(I_o)}} \times \left[\ln\left(1 - \frac{R_D}{P_{gmax}}\right)\right] \quad (30)$$

$I_{(50)}$ is one of the parameters of Eq. 2, but as mentioned before, this parameter does not correspond to the desired value of I, when P_N is 50% of P_{gmax}. In this study, we decided to calculate this variable by considering it to be the value of I, when P_N is 50% of P_{Nmax}, which means 0.5 ($P_{gmax} - R_D$). To develop individual *Excel* routines (Eqs. 1–6, 8, 9, and 11) for each of the mathematical models employed in this study, the corresponding general forms to calculate $I_{sat(n)}$ by inserting the desired percentile value (n) are presented in Eqs. 31–36, 37, 38, and 39, respectively.

$$I_{sat(n)} = \frac{P_{gmax} \times R_D\left(\frac{n}{100} - 1\right) - \frac{n}{100} \times {P_{gmax}}^2}{\phi_{(I_o)} \times \left[P_{gmax}\left(\frac{n}{100} - 1\right) + R_D\left(1 - \frac{n}{100}\right)\right]} \quad (31)$$

$$I_{sat(n)} = \frac{I_{(50)} \times \left(R_D \times \frac{n}{100} - P_{gmax} \times \frac{n}{100} - R_D\right)}{P_{gmax}\left(\frac{n}{100} - 1\right) + R_D\left(1 - \frac{n}{100}\right)} \quad (32)$$

$$I_{sat(n)} = \frac{P_{gmax} \times \left[\frac{n}{100} \times \left(P_{gmax} - R_D\right) + R_D\right]}{\phi_{(I_o)} \times \sqrt{{P_{gmax}}^2 - \left[\frac{n}{100} \times \left(P_{gmax} - R_D\right) + R_D\right]^2}} \quad (33)$$

$$I_{sat(n)} = \operatorname{arctanh}\left[\frac{\frac{n}{100} \times \left(P_{gmax} - R_D\right) + R_D}{P_{gmax}}\right] \times \frac{P_{gmax}}{\phi_{(I_o)}} \quad (34)$$

$$I_{sat(n)} = \operatorname{arctanh}\left[\frac{\frac{n}{100} \times \left(P_{gmax} - R_D\right) + R_D}{P_{gmax}}\right] \times I_{sat} \quad (35)$$

$$I_{sat(n)} = \frac{P_{gmax}\left[\frac{n}{100} \times P_{gmax} + R_D \times \left(1 - \frac{n}{100}\right)\right] - \theta\left[\frac{n}{100}\left(P_{gmax} - R_D\right) + R_D\right]^2}{\phi_{(I_o)}\left[P_{gmax}\left(1 - \frac{n}{100}\right) + R_D\left(\frac{n}{100} - 1\right)\right]} \quad (36)$$

$$I_{sat(n)} = \frac{P_{gmax}}{\phi_{(I_o)}} \times \left\{-\ln\left[1 - \frac{\frac{n}{100} \times \left(P_{gmax} - R_D\right) + R_D}{P_{gmax}}\right]\right\} \quad (37)$$

$$I_{sat(n)} = I_{comp} - \frac{\ln\left\{1 - \left[\frac{\frac{n}{100} \times \left(P_{gmax} - R_D\right)}{P_{gmax}}\right]\right\}}{k} \quad (38)$$

$$I_{sat(n)} = \frac{\left\{\left(\phi_{(I_o\_I_{comp})} \times \beta \times I_{comp}\right) - \left[\frac{n}{100} \times \left(P_{gmax} - R_D\right) \times \gamma\right] + \phi_{(I_o\_I_{comp})}\right\}}{2\phi_{(I_o\_I_{comp})} \times \beta} - \frac{\sqrt{\left\{\left[\left(\frac{n}{100} \times \left(P_{gmax} - R_D\right) \times \gamma\right) - \left(\phi_{(I_o\_I_{comp})} \times \beta \times I_{comp}\right) - \phi_{(I_o\_I_{comp})}\right]^2 - \left[4\phi_{(I_o\_I_{comp})} \times \beta \times \left(\phi_{(I_o\_I_{comp})} \times I_{comp} + \frac{n}{100} \times \left(P_{gmax} - R_D\right)\right)\right]\right\}}}{2\phi_{(I_o\_I_{comp})} \times \beta} \quad (39)$$

Although I_{sat} is a common variable in the literature, methodological problems in its calculation detract from its intended purpose. We developed a new method to calculate this variable in the *Excel* routine, which we called I_{max} to differentiate it from I_{sat}. I_{max} is defined as the point beyond which no significant change in P_N occurs. The equipment resolution is thereby taken into account. Given a low air flow rate [100 μmol(air) s^{-1}], the allowed difference between two infrared gas analyzers for the *LI-6400* is 0.4 μmol(CO_2) mol(air)$^{-1}$ (LI-COR 2004). Using a leaf chamber with 6 cm^2, therefore, one can calculate the maximum net photosynthetic rate that can be considered not significant multiplying the molar flow rate by the difference in the mol fraction of CO_2 and dividing it by the leaf area, which gives 0.067 μmol(CO_2) m^{-2} s^{-1}. If no significant change in P_N was found within a

50 μmol(photon) m^{-2} s^{-1} increment in I, we considered the previous value of I to be the exact value of I_{max}. At this point, the real light-saturated rate of net CO_2 uptake ($P_{N(Imax)}$) was also calculated.

The *Microsoft Excel* spreadsheet and the Solver function for fitting the models: A simple routine to minimize the sum of the squares of the errors (SSE), allowing the determination of function parameters using the Solver function of *Microsoft Excel 2010*, was developed according to Brown (2001). In this case, the method employed to fit the regression curves was the least squares estimation (Ratkowsky 1983, 1990, Seber and Wild 2003), although nowadays several error analysis methods have been used to determine the best fitting equation, such as the sum of the absolute errors (SAE), average relative errors (ARE), chi-square test (χ^2), *etc*. (Kumar and Sivanesan 2006).

Usually, the Solver function is not automatically installed in the *Excel* program; in this case, the users must enter in the "File" main menu, go to "Options", then "Add-Ins", and there they can find the "Solver Add-In". The users must choose it and then, pressing the key "Go" they are able to activate "Solver Add-In" in the proper box. Once properly activated, the "Solver" can be found in the "Data" main menu and, in this case, the users are able to start working with it.

The routine requires inputs of measurements of P_N and I as well as estimates of the sensitivity of the equipment used to obtain these measurements. Each of the potential fits is placed in a separate file, allowing users to choose which fits they would like to test.

The file contains two spreadsheets. The first is used to fit the regression, and it gives the goodness of fit (R^2) as its outputs, the SSE, and all of the ecophysiological and mathematical metrics. Two graphics are also generated, showing the relationship between P_N/I and the overlying fit and the relationship between ϕ and I. The second spreadsheet serves as a tutorial detailing how to use the Solver.

Because the fitting process is iterative, initial parameter estimates must be provided. The initial estimates and ranges used here were based on our own experience with the study of species and the literature values. However, there can be cases in which the maximum and minimum limits imposed for the parameter must be changed by the user in order to make it possible to fit the mathematical model.

The necessary primary parameters are P_{gmax}, $\phi_{(I_0)}$, and R_D, though certain fits require additional parameters. The initial estimates for P_{gmax} can be generated using values from Nobel (1991), who compiled data from various researchers showing that the maximal rate of the net CO_2 uptake for C_3 species ranges from 42 to 59 μmol(CO_2) m^{-2} s^{-1} and for C_4 species from 57 to 75 μmol(CO_2) m^{-2} s^{-1}. The initial estimates of R_D can be obtained as approximately 10% of the P_{Nmax}, as both parameters have a coupling relationship (Wertin and Teskey 2008).

The theoretical maximum value of the quantum yield is 0.1250 μmol(CO_2) μmol(photon)$^{-1}$ as 8 photons are required per one molecule of CO_2 fixed (Luo *et al*. 2000, Singsaas *et al*. 2001). However, the observed values range from 0.0266 to 0.0800 μmol(CO_2) μmol(photon)$^{-1}$, with the change due to leaf light absorption efficiency, photorespiration, cyclic photophosphorylation, and stress factors affecting gas exchange (Singsaas *et al*. 2001).

θ represents the ratio of the physical to the total resistances of CO_2 diffusion, describing the sharpness of the transition from light limitation to light saturation (Jones 1988). Its theoretical range is 0 to 1, but typical values of θ are within the range of 0.70 to 0.99 (Ögren 1993). The specific values of k in Eq. 9 range from 0.001 to 0.009 s m^2 μmol(photon)$^{-1}$ (Prado *et al*. 1994). The value of I_{comp} varies among species, but as the first approach, we used values from 5 to 150 μmol(photon) m^{-2} s^{-1} that include both C_3 and C_4 plants. Ye (2007) did not provide any information about the ranges of β and γ. We used the range between 0 and 1 for both variables. These values provide guidance to users in choosing the proper values for parameters, but it is fundamental to take into account the data measured. Looking at the P_N-I pairs plotted, it is easy to identify appropriate ranges for each parameter.

When executed, the Solver function completes the iterative process necessary to fit the chosen model with all of the variables of interest previously noted. With a minimal user input, a graph of the original data with a regressed P_N/I curve is produced. A graph of ϕ as a function of I is produced automatically.

To determine the best fit to the data, all of the model forms should be tested and compared *via* SSE. The best model presents the lowest SSE. We do not recommend the use of R^2 for model selection, as R^2 has no obvious meaning for nonlinear regression and it is not employed for this purpose (Ratkowsky 1983, 1990).

Estimating population means and confidence intervals for the regression parameters and the measured variables: The measurement of the original P_N-I data pairs is performed for each individual sample, so each leaf has its own P_N/I curve to be fitted. In this case, a single mathematical model is often (but not necessarily always) employed for all the replicates. It is also possible that intrinsic natural variations lead to differences between samples of the same population, which necessitates different mathematical models to be applied for specific cases. As previously mentioned, in each case, the method for choosing the best model is to identify the model showing the minimum SSE.

Once all the replicates were evaluated, it is necessary to characterize the population by obtaining the mean value and a confidence interval for each regression parameter or measured variable. As the aim of this manuscript was only to present the tools for researchers and students to fit the best P_N/I curve, we used only a single sample

and did not work with replicates. To deal with it, we recommend the method of resampling with replacement, using at least 1000 resamplings. It is easy to develop an *Excel* routine to perform this calculation for each variable, deriving the mean and confidence intervals following the step-by-step procedure detailed by Christie (2004).

Results and discussion

The aim of this work was to present the most common models used for P_N/I curves fitted by the Solver function of *Microsoft Excel*. The results are widely accessible and didactically useful, showing the computation of all of the regression parameters and calculated variables. Users can now compute both P_N/C_c curves according to the procedure explained by Sharkey *et al*. (2007), and P_N/I curves according to the procedure explained in this manuscript, using the same package, effectively presenting the biochemical and photochemical efficiency and capacity of the photosynthetic process.

In our opinion, there is no single definitive mathematical model to describe the P_N/I curve that should be employed in all situations. Several researchers have been developing mathematical models to fit P_N/I curves, and year after year, they found something, which was not taken into account at that moment, and then they presented a new mathematical model with that novelty. In our opinion, all of these improvements are welcome, but they must be considered very carefully because of their specificity, which renders very often any generalization impossible. From this perspective, the best P_N/I curve is the one among all available that fits best the original data.

In this study of *V. divergens*, the mathematical model, which fitted the best the original data of P_N/I curve, was the nonrectangular hyperbola (Eq. 6). This model was chosen because it had the lowest SSE (Fig. 1*F*). All of the regression parameters of the models employed are presented in Table 2.

Models based on the same principle are quite similar and in dependence on the original data they can provide solutions with high or low equality, and even precisely the same value. In this case, one could see that Eqs. 1 and 2, based on the Michaelis-Menten model (Fig. 1*A*,*B*), Eqs. 4 and 5, based on a hyperbolic tangent model (Fig. 1*D*,*E*), and Eqs. 8 and 9, based on an exponential model

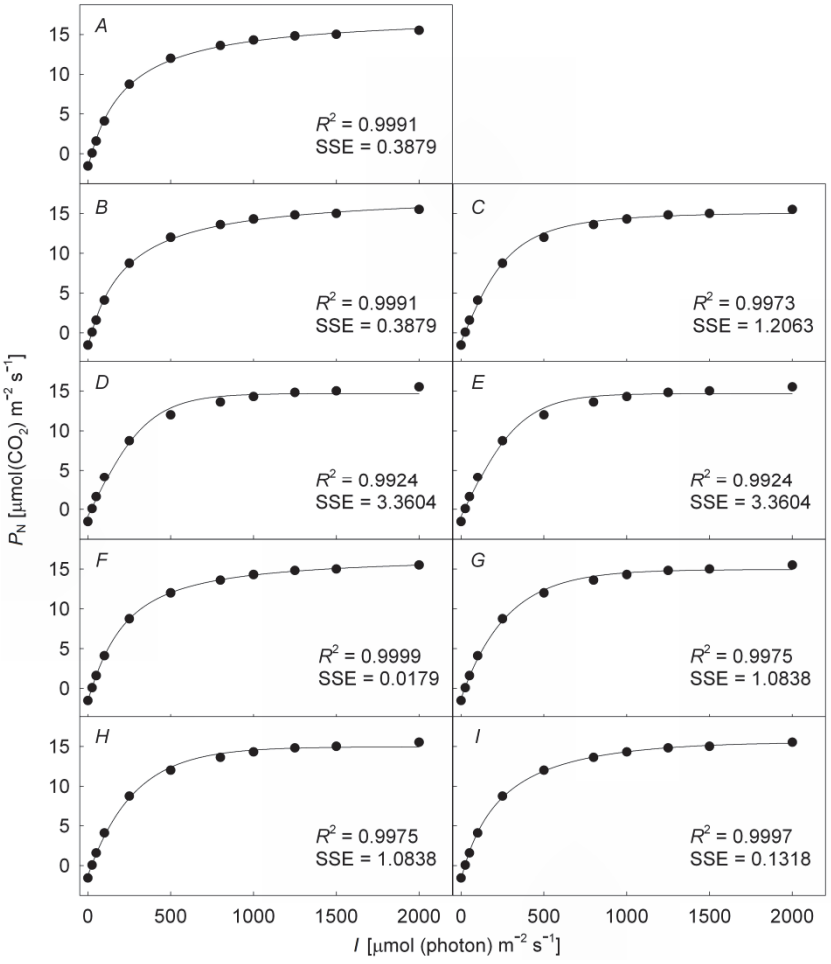

Fig. 1. Fitting P_N/I curves by applying different mathematical models for *V. divergens*. The models represented by the Eqs. 1–6, 8, 9, and 11 are represented by the regression lines in *A*–*F*, *G*, *H*, and *I*, respectively. P_N – net photosynthetic rate; I – photosynthetic photon flux density.

Table 2. The parameters of the regression models employed to fit the net photosynthetic light response curves of *V. divergens*. I_{comp} – light compensation point [μmol(photon) m^{-2} s^{-1}]; I_{sat} – light saturation point [μmol(photon) m^{-2} s^{-1}]; $I_{(50)}$ – light saturation point for $P_N + R_D$ equal to 50% of P_{Nmax} [μmol(photon) m^{-2} s^{-1}]; k – adjusting factor [s m^2 μmol(photon)$^{-1}$]; P_{gmax} – maximum gross photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; R_D – dark respiration [μmol(CO_2) m^{-2} s^{-1}]; β – adjusting factor (dimensionless); γ – adjusting factor (dimensionless); θ – convexity factor (dimensionless); $\phi_{(I_0)}$ – quantum yield at I = 0 μmol(photon) m^{-2} s^{-1} [μmol(CO_2) μmol(photon)$^{-1}$]; $\phi_{(I_0 - I_{comp})}$ – quantum yield at the range between I = 0 μmol(photon) m^{-2} s^{-1} and I_{comp} [μmol(CO_2) μmol(photon)$^{-1}$].

Parameters	Mathematical models								
	Eq. 1	Eq. 2	Eq. 3	Eq. 4	Eq. 5	Eq. 6	Eq. 8	Eq. 9	Eq. 11
P_{gmax}	19.5	19.5	16.4	15.5	15.5	18.4	16.2	15.6	
$\phi_{(I_o)}$	0.0899		0.0493	0.0433		0.0709	0.0597		
$\phi_{(I_0 - I_{comp})}$									0.0756
R_D	1.8	1.8	1.1	0.9	0.9	1.6	1.3	0.6	
I_{sat}					359.2				
$I_{(50)}$		216.4							
θ						0.4325			
K								0.0037	
I_{comp}								10.5	22.6
β									4.3E–5
γ									0.0039

Table 3. The variables calculated from the models. I_{comp} – light compensation point [μmol(photon) m^{-2} s^{-1}]; I_{max} – light saturation point beyond which there is no significant change in P_N [μmol(photon) m^{-2} s^{-1}]; I_{sat} – light saturation point [μmol(photon) m^{-2} s^{-1}]; $I_{sat(50)}$ – light saturation point for $P_N + R_D$ equal to 50% of P_{Nmax} [μmol(photon) m^{-2} s^{-1}]; $I_{sat(85)}$ – light saturation point for $P_N + R_D$ equal to 85% of P_{Nmax} [μmol(photon) m^{-2} s^{-1}]; $I_{sat(90)}$ – light saturation point for $A + R_D$ equal to 90% of P_{Nmax} [μmol(photon) m^{-2} s^{-1}]; $I_{sat(95)}$ light saturation point for $P_N + R_D$ equal to 95% of P_{Nmax} [μmol(photon) m^{-2} s^{-1}]; P_{gmax} – maximum gross photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; $P_{N(Imax)}$ – maximum net photosynthetic rate obtained at $I = I_{max}$ [μmol(CO_2) m^{-2} s^{-1}]; R_D – dark respiration [μmol(CO_2) m^{-2} s^{-1}]; $\phi_{(I_{comp})}$ – quantum yield at $I = I_{comp}$ [μmol(CO_2) μmol(photon)$^{-1}$]; $\phi_{(I_{comp} - I_{200})}$ – quantum yield at the range between I_{comp} and I = 200 μmol(photon) m^{-2} s^{-1} [μmol(CO_2) μmol(photon)$^{-1}$]; $\phi_{(I_0)}$ – quantum yield at I = 0 μmol(photon) m^{-2} s^{-1} [μmol(CO_2) μmol(photon)$^{-1}$]; $\phi_{(I_0 - I_{comp})}$ – quantum yield at the range between I = 0 μmol(photon) m^{-2} s^{-1} and I_{comp} [μmol(CO_2) μmol(photon)$^{-1}$]. *Photosynthetic active radiation values above the range employed to make the measurements. **Photosynthetic active radiation values above the maximum that reaches the Earth's surface.

Calculated variables	Mathematical models								
	Eq. 1	Eq. 2	Eq. 3	Eq. 4	Eq. 5	Eq. 6	Eq. 8	Eq. 9	Eq. 11
I_{comp}	22.5	22.5	22.7	20.2	20.2	23.4	22.0		
$I_{sat(50)}$	261.4	261.4	209.4	211.0	211.0	235.6	210.1	187.6	211.1
$I_{sat(85)}$	1,376.3	1,376.3	559.2	462.4	462.4	1,022.7	537.0	468.1	678.0
$I_{sat(90)}$	2,172.6*	2,172.6*	713.1	539.8	539.8	1,564.3	647.0	549.4	854.6
$I_{sat(95)}$	4,561.7**	4,561.7**	1,047.6	668.7	668.7	3,179.1**	835.2	665.9	1,148.4
I_{sat}									2,289.9*
I_{max}	1537.0	1,949.0	1,030.0	847.0	847.0	1,348.0	1,008.0	1,008.0	1,297.0
P_{gmax}									17.1
$P_{N(Imax)}$	15.2	15.7	14.5	14.4	14.4	14.9	14.5	14.5	14.9
R_D									1.7
$\phi_{(I_0)}$		0.0899			0.0433			0.0597	0.0824
$\phi_{(I_{comp})}$	0.0738	0.0738	0.0490	0.0431	0.0431	0.0638	0.0550	0.0574	0.0694
$\phi_{(I_0 - I_{comp})}$	0.0816	0.0816	0.0492	0.0432	0.0432	0.0674	0.0573	0.0586	0.0757
$\phi_{(I_{comp} - I_{200})}$	0.0410	0.0410	0.0416	0.0391	0.0391	0.0421	0.0400	0.0410	0.0409

(Fig. 1*G*,*H*) provided quite similar or even identical results; therefore, they had almost the same values for their regression parameters (Table 2), and the variables calculated from those models were approximately of the same magnitude (Table 3).

There is a difference between what is expected from the maximum theoretical velocity (V_{max}) for an enzyme and P_{gmax}. If we remember that V_{max} is a constant for a

given enzyme that is achieved, when all of the enzyme molecules have tightly bound their substrate, but that this rate of enzymatic activity is never actually achieved, then for all of the mathematical models, in which P_{gmax} is an asymptote reached at infinite I, there is no doubt that P_{gmax} has exactly the same connotation. In those cases, P_{gmax} can not be employed as an explanatory variable to define the maximum photosynthetic capacity of species, because it does not exist in real life. In this case, our proposition to employ $P_{N(I\max)}$ instead of P_{gmax} is justified by the fact that the primer is always found in the range of the measured data and it exactly represents the highest value of P_N to be achieved by that species in the experimental conditions to which it was subjected. This argument has to be taken into account, particularly because there is also an ecophysiological interest in knowing at which value of I the value of $P_{N(I\max)}$ can be achieved. Because for P_{gmax} the natural value of I is infinite by definition, several authors proposed the use of $I_{sat(n)}$, which is taken to be the corresponding value of light, when P_N reaches a percentile of P_{Nmax}, which leads to the same inadequacy. Independent of which fraction is taken into account, it is not reasonable to use $I_{sat(n)}$ as an ecophysiological indicator of the maximum light that saturates P_N, because it does not represent the desired concept.

The maximum instantaneous value of the solar irradiance perpendicularly incident on the Earth's atmosphere, which is known as the solar constant (S), is 1,366 ± 46 W m^{-2} (Jones *et al*. 2003, Nobel 2009). Because of atmospheric attenuation, on the clearest days, the maximum value of the solar irradiance that reaches the Earth's surface can be approximately 75% of S (Campbell and Norman 1998). Because the maximum value of I is approximately 50% of the fraction of S that reaches the Earth's surface (Stanhill and Fuchs 1977, Jacovides *et al*. 2003), the maximum value of I is 530 W m^{-2}. By converting this radiometric unit to a photometric unit and considering a wavelength of 550 nm to be representative of the photosynthetic active radiation (Campbell and Norman 1998, Jones *et al*. 2003), the maximum value of I is 2,435 μmol(photon) m^{-2} s^{-1} or slightly higher, due to the diffuse radiation that can enhance this value. Clearly, the values of I_{sat} above this maximum limit have no realistic ecophysiological meaning.

Several researchers adopt a value of I_{sat}, in which P_N is equal to 50% ($I_{sat(50)}$), 90% ($I_{sat(90)}$), or 95% ($I_{sat(95)}$) of the P_{gmax}, to overcome the fact that P_{gmax} is an asymptote, a value, in which I is infinite. When this method is used, not only P_{gmax} but also $I_{sat(n)}$ is a forced representation of the reality.

Kaipiainen (2009) uses a modified Michaelis-Menten model (Eq. 2) to estimate $I_{(50)}$, which does not have the same meaning as $I_{sat(50)}$, because, as we noted before, $I_{(50)}$ is the value of I, when $P_N + R_D = 0.5\ P_{gmax}$, and $I_{sat(50)}$ is the value of I, when $P_N = 0.5\ (P_{gmax} - R_D)$. However, Kaipiainen (2009) considers that despite the fraction of P_{gmax} that is taken, those different values of I obtained, when $P_N + R_D$ = 50, 90, or 95% of P_{gmax}, have "similar physiological meaning". We disagree with this statement, because if applying our approach (Eq. 32), $I_{sat(90)}$ and $I_{sat(95)}$ (Table 3) were 731 and 1,645% higher, respectively, than $I_{sat(50)}$ (Table 3). Obviously, these estimates cannot be considered to be similar in terms of plant ecophysiology. In addition, as one can see from Table 3, the estimates of $I_{sat(90)}$ derived from Eqs. 1 and 2 were out of the range employed to obtain the measurements, and the $I_{sat(95)}$ estimates derived from Eqs. 1, 2, and 6 were higher than the maximum theoretical value that can reach Earth's surface.

The modified rectangular hyperbola model (Eq. 11) proposed by Ye (2007) to solve this problem gave a reasonable value of P_{gmax} (Table 2). However, the magnitude of I_{sat} achieved (Table 3) using Eq. 14 was out of the range of the original data measured.

We suggest that $P_{N(I\max)}$ is a better variable to represent realistically the maximum net photosynthetic rate beyond which no significant increment can be achieved with an additional increment in I, and I_{max} is exactly that point at which $P_{N(I\max)}$ is found. These variables are much more appropriate for the representation of the photosynthetic capacity than any other variables proposed to date. Their interpretation is immediate and obvious, and their magnitude is always in the range, in which the measurements were performed.

Fig. 2 displays the results obtained for $\phi_{(I)}$ from each model; the results served as an important reminder to select the most appropriate model. For example, model estimates of $\phi_{(I)}$ that vary by 100% can be observed, especially at low I. The only way to be closer to the best approach of $\phi_{(I)}$ or any other variable taken from the mathematical model is by choosing the best fitted model. Because R^2 for nonlinear models cannot fulfill this expectation (Ratkowsky 1983, 1990), the best model can be achieved by choosing that with the minimal value of SSE. Fitting the best model provides the best approximation of reality; therefore, the search for the best model becomes crucial for interpreting all of the information, which the model can offer as correctly as possible.

Another important comment must be given with respect to quantum yield in Eqs. 1, 3, 4, 6–8, and 10 in which it appears as $\phi_{(I_0)}$, and in Eq. 11, in which it appears as $\phi_{(I_0 - I_{comp})}$. In all of these cases, $\phi_{(I_0)}$and $\phi_{(I_0 - I_{comp})}$ do not correspond with the original concept of this parameter, which tells us that the quantum yield (actually $\phi_{(I_{comp} - I_{200})}$, in our terminology) is the slope of the curve at its linear portion in the range of approx. $I = I_{comp}$ to I = 200 μmol(photon) m^{-2} s^{-1}, representing the "maximum quantum yield".

First of all, it is important to realize that $\phi_{(I_0)}$ is the derivative of the model at I equals to zero. In this case, it is obtained at darkness, when no photosynthesis is possible.

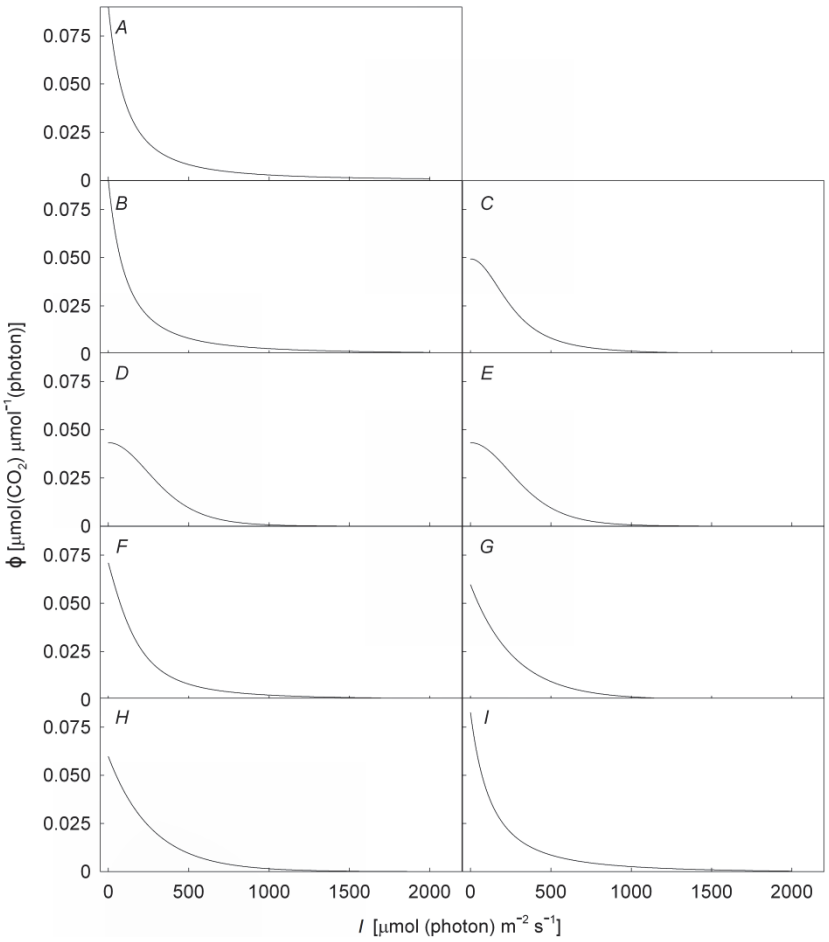

Fig. 2. The quantum yield as a function of light in *V. divergens*. The models represented by the derivative Eqs. 15–23 are represented by the curves in *A*–*I*, respectively. I – photosynthetic photon flux density; ϕ – quantum yield.

In this case, this variable cannot explain the "maximum quantum yield". Second, there is not a "linear portion" in that curvilinear model in its *stricto sensu*, and that shape is not due to a model that cannot correctly fit the data in that portion but arises, because the curvilinear shape is exactly how P_N changes with I in most cases. In fact, forcing any straight line in any portion of the curve could be unrealistic and arbitrary. In this case, it was obvious that $\phi_{(I_0)}$ is always the maximum value of the quantum yield, higher than any other point at the P_N/I curve. However, $\phi_{(I_0)}$ has no realistic meaning in terms of plant ecophysiology, because no positive net assimilation is possible in complete darkness.

The original definition of the quantum yield is "the ratio of the moles of product formed or substrate consumed to the moles of photons absorbed", the maximum quantum yield is definied as "the largest quantity of product formed or substrate consumed to the smallest number of photons absorbed" (Falkowski and Raven 2007). However, it is important to note that the relationship between the absorbed light and the O_2 evolution can be different from the relationship between the absorbed light and the CO_2 uptake or net CO_2 uptake. For ecophysiological purposes, the maximum ϕ appears to be better represented as the ratio of the net CO_2 evolution to the absorbed (or incident) light in the region of the P_N/I curve, where positive CO_2 assimilation begins. We prefer the approach adopted by Ye (2007), which describes the variation in $\phi_{(I)}$ with a progressive increase of I, which has an obvious decreasing tendency (Fig. 2). Additionally, we prefer to use the term "apparent quantum yield", when the light employed is not that absorbed by the leaf, but instead, the incident light, and no correction to eliminate the effect of photorespiration is performed as recommended to access the real "maximum quantum yield" (Long and Hällgren 1993, Singsaas *et al*. 2001).

In their critiques of different methods to calculate the quantum yield and the discrepancy between the published values, Singsaas *et al*. (2001) noted something that we have already mentioned about using O_2 evolution or CO_2 assimilation methods, which represent different moments in the process of photosynthesis. They also comment that to identify the correct maximum quantum yield, which they stated, must be the nearest to the theoretical value of 0.125, it is important to use the absorbed, not incident I values and to identify the linear portion of the P_N/I curve correctly, which can be accomplished by eliminating photorespiration at low O_2 keeping the intercellular CO_2 concentration (C_i) constant or correcting it mathematically. In our opinion, there are several questions that

must be considered in this context. As we stated in the previous paragraph, the most common shape of the P_N/I curve is curvilinear throughout its length, thus, no linear phase is clearly identifiable. However, if the shape is very close to linear, the method that Singsaas *et al*. (2001) employed to determine how much of the data could be included in that phase is inappropriate. As mentioned by Dubois *et al*. (2007), the upward progression in the coefficient of determination (R^2) with the withdrawal of observations is inherent to its definition, thus, this parameter cannot be used as a criterion to choose data to be included or excluded from the estimation. Depending on the focus, in terms of ecophysiological research, it appears to be much more reasonable to use one theoretical maximum quantum yield ($\phi_{max} = 0.125$) as the reference to identify how stress factors or specific treatments applied to the plant can affect quantum yield or any other P_N/I parameter or calculated variable.

For us, the approach of Ye (2007) to calculation of $\phi_{(I)}$ produces a result that is much more realistic and useful, because one can observe how the variable can change with I, and particularly for ecophysiological purposes, all of those points in the curve can be analyzed, when P_N is above the light compensation point, which means that a net CO_2 uptake is taking place. The variables $\phi_{(I_0)}$, $\phi_{(I_0 - I_{comp})}$, and $\phi_{(I_{comp})}$ all fail in representing the maximum value of the quantum yield, because they are defined below or at the light compensation point. $\phi_{(I_{comp} - I_{200})}$ is also problematic in fixing the point at which the calculation of the quantum yield must be performed, and depending on the species. Thus, this value may not be reasonable.

By applying the approach of Ye (2007), the values of quantum yield can be analyzed not only, when P_N is dependent on I, but also when P_N becomes progressively independent of I. This capability is very useful for evaluating differences in the photosynthetic efficiency between sun and shade leaves, for which, not always but frequently, no difference in ϕ can be observed in the initial portion of the P_N/I curve.

In conclusion, we consider that our *Excel* routines are an interesting alternative for users with any level of statistical knowledge to evaluate and quickly identify the best model of P_N/I curve that fits better their experimental data. We also consider that the variables, which better represent the light-saturated rate of CO_2 uptake, the light saturation point, and the quantum yield are $P_{N(I max)}$, I_{max}, and $\phi_{(I)}$, respectively.

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