

## Letters

## Reporting estimates of maximum potential electron transport rate

## Introduction

The  $C_3$  photosynthesis model of Farquhar, von Caemmerer & Berry (1980) is widely used to predict  $\text{CO}_2$  exchange and inform ecophysiological research. Users typically estimate the model's parameters by fitting it to response curves – a process fraught with potential errors and ambiguities (Long & Bernacchi, 2003; Dubois *et al.*, 2007; Flexas *et al.*, 2007; Rodeghiero *et al.*, 2007; Sharkey *et al.*, 2007; Kattge *et al.*, 2009; Miao *et al.*, 2009; Pons *et al.*, 2009; Gu *et al.*, 2010; Gilbert *et al.*, 2012; Tholen *et al.*, 2012). This letter concerns a widespread but simple ambiguity in photosynthetic parameter estimation that has largely eluded attention.

Maximum potential electron transport rate ( $J_{\max}$ ) is often estimated by fitting the photosynthesis model to  $\text{CO}_2$  response curves obtained at high photosynthetic photon flux ( $i$ ), then inferring the potential electron transport rate ( $J$ ) from the fitted model and reporting this estimate ( $J$  at high  $i$ , or  $J_{\text{high}}$ ) as  $J_{\max}$ . If  $J_{\text{high}}$  is subsequently applied to the model, an error will result if an asymptotic submodel for the response of  $J$  to  $i$  is employed – that is, a submodel in which  $J$  approaches  $J_{\max}$  but never reaches it (e.g. Fig. 1). The most common  $J$  versus  $i$  submodel is a nonrectangular hyperbola, in which  $J$  is calculated as the lesser root of a quadratic equation,  $\theta_j J^2 - (J_{\max} + \phi i) J + J_{\max} \phi i = 0$  (Farquhar & Wong, 1984), or

$$J = \frac{J_{\max} + \phi i - \sqrt{(J_{\max} + \phi i)^2 - 4\theta_j J_{\max} \phi i}}{2\theta_j}, \quad \text{Eqn 1(a)}$$

where  $\phi$  is the initial slope of  $J$  versus  $i$  and  $\theta_j$  is a dimensionless convexity parameter ( $\theta_j \leq 1$ ). Another, less widely used submodel for  $J$  versus  $i$ , given by Smith (1937), is

$$J = \frac{\phi i \cdot J_{\max}}{\sqrt{(\phi i)^2 + J_{\max}^2}}. \quad \text{Eqn 1(b)}$$

$J_{\text{high}}$  underestimates the values of  $J_{\max}$  appropriate to Eqns 1(a) and 1(b). To recover the appropriate values, Eqns 1(a) and 1(b) must be solved for  $J_{\max}$ . The resulting correction to  $J_{\text{high}}$  can be expressed as a percentage of the original value; that percentage correction depends on the value of  $i$  at which  $J_{\text{high}}$  was estimated ( $i_{\text{high}}$ ). For Eqn 1(a), the correction is

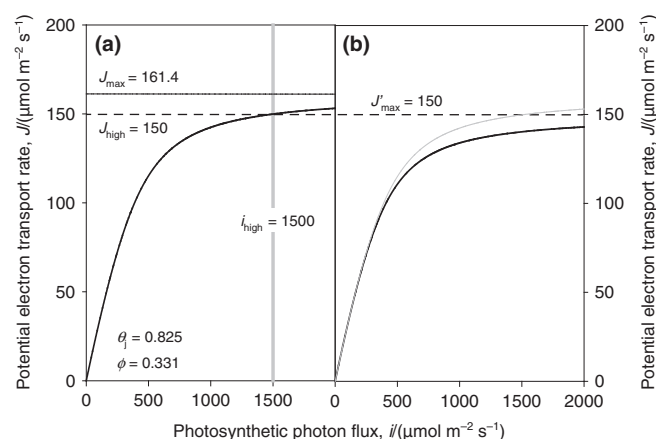
$$\% \text{ correction} = 100 \cdot \left( \frac{(1 - \theta_j) J_{\text{high}}}{\phi i_{\text{high}} - J_{\text{high}}} \right), \quad \text{Eqn 2(a)}$$

and for Eqn 1(b), the correction is

$$\% \text{ correction} = 100 \cdot \left( \frac{\phi i_{\text{high}}}{\sqrt{(\phi i_{\text{high}})^2 - J_{\text{high}}^2}} - 1 \right). \quad \text{Eqn 2(b)}$$

Because the correction increases with  $J_{\text{high}}$  for a given  $i_{\text{high}}$  (Eqn 2),  $J_{\text{high}}$  is also nonlinearly related to  $J_{\max}$ . To avoid this bias,  $i_{\text{high}}$  would need to be altered for each measurement so that it is always directly proportional to  $J_{\text{high}}$ ; however, this is impractical, so  $i_{\text{high}}$  is typically identical for all measurements in a given study.

Other approaches for estimating  $J_{\max}$  do not suffer from this problem. In some cases  $J_{\text{high}}$  is not reported as  $J_{\max}$ , but is instead used in conjunction with light-response data to estimate  $J_{\max}$  from Eqn 1 or a similar function (Niinemets *et al.*, 1999, 2009; Gu *et al.*, 2012). A related approach is to estimate light-saturated  $A$  ( $A_{\max}$ ) by fitting an asymptotic function to  $A$  versus  $i$  data, then estimating  $J_{\max}$  from  $A_{\max}$  (Bernacchi *et al.*, 2003). Another is to incorporate Eqn 1 directly into the photosynthesis model during the fitting process, which gives  $J_{\max}$  directly, bypassing  $J_{\text{high}}$  (Wulschleger,



**Fig. 1** Illustration of the error that results when the maximum value ( $J_{\max}$ ) of potential electron transport rate ( $J$ ) is estimated as the value of  $J$  measured at a high value ( $i_{\text{high}}$ ) of photosynthetic photon flux ( $i$ ), and the resulting estimate of  $J_{\max}$  ( $J_{\text{high}}$ ) is subsequently applied to an asymptotic model for  $J$ . (a) A typical response curve of  $J$  (solid curve) versus photosynthetic photon flux ( $i$ ) with  $J_{\max} = 161.4 \mu\text{mol m}^{-2} \text{s}^{-1}$  (solid horizontal line). The value of  $J$  is  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  at a high  $i$  ( $i_{\text{high}}$ ) of  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; this is  $J_{\text{high}}$  (dashed horizontal line). (b) If  $J_{\text{high}}$  is then used as the asymptote for  $J$  ( $J'_{\max}$ ; dashed horizontal line in b) in subsequent calculations of  $J$ , the resulting relationship (solid black curve in b) will differ from the true relationship (shown in a and with a grey line in b).

1993; Warren *et al.*, 2003; Ellsworth *et al.*, 2004; Crous *et al.*, 2008).

Our objective in this Letter is to draw attention to the distinction between  $J_{\text{high}}$  and  $J_{\text{max}}$ , to assess how commonly  $J_{\text{high}}$  is reported as  $J_{\text{max}}$  and the potential impact of this practice, and to recommend a change in reporting practice to prevent ambiguity in the meaning of  $J_{\text{max}}$ .

### How frequently is $J_{\text{high}}$ reported as $J_{\text{max}}$ ?

We re-analysed results from a sample of recent papers that reported new measurements of  $J_{\text{max}}$ . This sample was not meant to be exhaustive, but merely to provide enough information to assess how frequently  $J_{\text{high}}$  is reported as  $J_{\text{max}}$ , and to estimate how divergent the two values are across a typical data set. These papers were located by a full-text search using Google Scholar and the search terms 'Jmax', 'Farquhar', 'Caemmerer', 'Berry', 'electron transport' and 'cc' (the latter to maximize the proportion of papers reporting values based on chloroplastic  $\text{CO}_2$  concentration,  $c_c$ ) in the years 2004–2014. The search (performed on 21 May 2014 at 22:30 h GMT) yielded 71 nonduplicate peer-reviewed scientific journal articles that reported making novel measurements of  $J_{\text{max}}$  and also reported the associated values of  $i_{\text{high}}$  and  $J_{\text{max}}$  (a list of these papers with extracted results is included as Supporting Information Notes S1). In each of these papers, we recorded  $i_{\text{high}}$  (or its largest value if multiple values were reported) and the smallest and largest values of  $J_{\text{max}}$  reported in each paper.

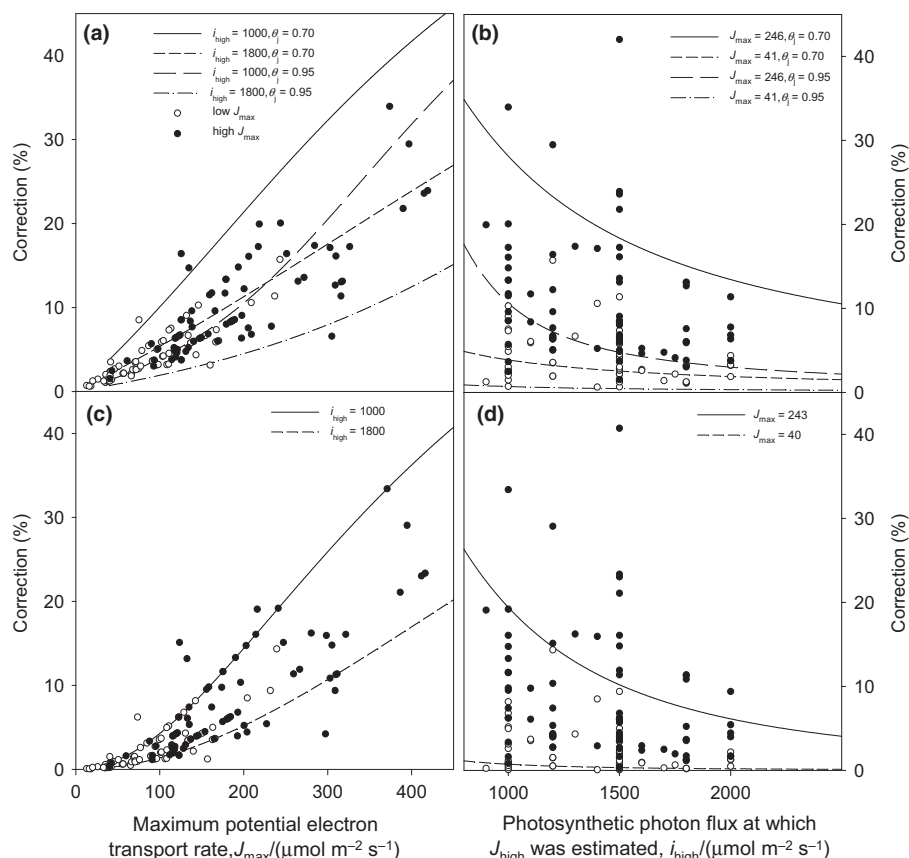
For 16 of these 71 papers (23%), it appeared that  $J_{\text{high}}$  was not reported as  $J_{\text{max}}$ . In 33 of the 71 papers (46%), the methods were not explained in sufficient detail to determine with certainty exactly how  $J_{\text{max}}$  was inferred from  $J_{\text{high}}$ . Many of these papers simply stated that they fitted the Farquhar *et al.* (1980) model to  $\text{CO}_2$  response curves, yet the original manuscript describing that model includes three different submodels for  $J$  versus  $i$ , including a nonasymptotic model (identical to Eqn 1a with  $\theta_j = 1.0$ ). Many papers cited other papers for the method, yet the cited papers either did not mention a  $J$  model or did not specify the parameter values used. A number of papers cited the proprietary PHOTOSYNTHESIS ASSISTANT software (Dundee Scientific, Dundee, UK) for the method, which apparently uses Eqn 2; however, those papers did not present Eqn 2 itself, nor the values of  $\phi$  used therein.

In another 22 papers (31%), it appeared likely that  $J_{\text{high}}$  was reported as  $J_{\text{max}}$ . These included numerous papers that cited the *A* versus  $c_i$  fitting spreadsheet provided by Sharkey *et al.* (2007) as the method used to estimate  $J_{\text{max}}$ . However, that paper took care to distinguish  $J$  from  $J_{\text{max}}$ , and the spreadsheet reported  $J$ , not  $J_{\text{max}}$ .

### How far is $J_{\text{high}}$ from the $J_{\text{max}}$ values appropriate to Eqns 1(a) and 1(b)?

To estimate the extent to which  $J_{\text{high}}$  underestimates the  $J_{\text{max}}$  values appropriate to Eqns 1(a) and 1(b), we used Eqns 2(a) and 2(b) to estimate the percentage corrections required to give the appropriate values for the smallest and largest values of  $J_{\text{max}}$  ( $J_{\text{high}}$ ) reported in

**Fig. 2** The percentage correction required to obtain the value of the maximum potential electron transport rate ( $J_{\text{max}}$ ) appropriate to asymptotic models from the value of  $J$  estimated at a photosynthetic photon flux ( $i$ ) of  $i_{\text{high}}$ , in relation to (a, c)  $J_{\text{max}}$ , and (b, d)  $i_{\text{high}}$ , with the percentage correction calculated using (a, b) Eqn 2(a), or (c, d) Eqn 2(b). The points are based on values taken from recent literature in which the potential electron transport rate at high  $i$  ( $J_{\text{high}}$ ) was reported as  $J_{\text{max}}$ ; closed circles, the largest value of  $J_{\text{max}}$  from each paper; open circles, the smallest value of  $J_{\text{max}}$  from each paper. The lines are simulated relationships using the values of  $J_{\text{high}}$ ,  $i_{\text{high}}$  and, for panels (a) and (b), the curvature parameter,  $\theta_j$ , as shown in the keys, and assuming an initial slope of  $J$  versus  $i$  ( $\phi$ ) of 0.331. The smaller and larger values of  $J_{\text{high}}$  and  $i_{\text{high}}$  used for these simulations represent the 25th and 75th percentiles of all values extracted from recent literature, as described in the main text. Panels (a, c) exclude one exceptional point for which  $J_{\text{max}} = 690 \mu\text{mol m}^{-2} \text{s}^{-1}$  and percentage correction = 42%. Panels (b, d) exclude two exceptional points for which  $i_{\text{high}}$  was  $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$  and percentage corrections were 3.1% and 6.6%, respectively.



each paper. These calculations required estimates for  $\phi$  and  $\theta_j$ ; we used  $\phi = 0.331$  (which represents a leaf absorptance of 0.86 (an average from 10 dicot species; Evans & Poorter, 2001) and assumes 23% of absorbed photons do not contribute to photochemistry; Farquhar *et al.*, 1980), and  $\theta_j = 0.825$  (the value reported by Bernacchi *et al.* (2003) at growth and measurement temperatures of 25°C in *Nicotiana tabacum* cv W38).

The percentage corrections calculated using Eqn 2(a) averaged 4.2% and 11.1% for the smallest and largest  $J_{\max}$  values in each paper, respectively, and ranged from 0.6% to 42.0% (Figs 2a,b, 3a). Using Eqn 2(b), the corrections averaged 2.4% and 9.2% for the smallest and largest  $J_{\max}$  values in each paper, respectively, and ranged from 0.1% to 40.7% (Figs 2c,d, 3b). The mean value of  $i_{\text{high}}$  was 1409  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . For reference, Fig. 2 also shows how  $J_{\max}$  and  $i_{\text{high}}$  affect the percentage correction for different values of the parameters in Eqns 1 and 2.

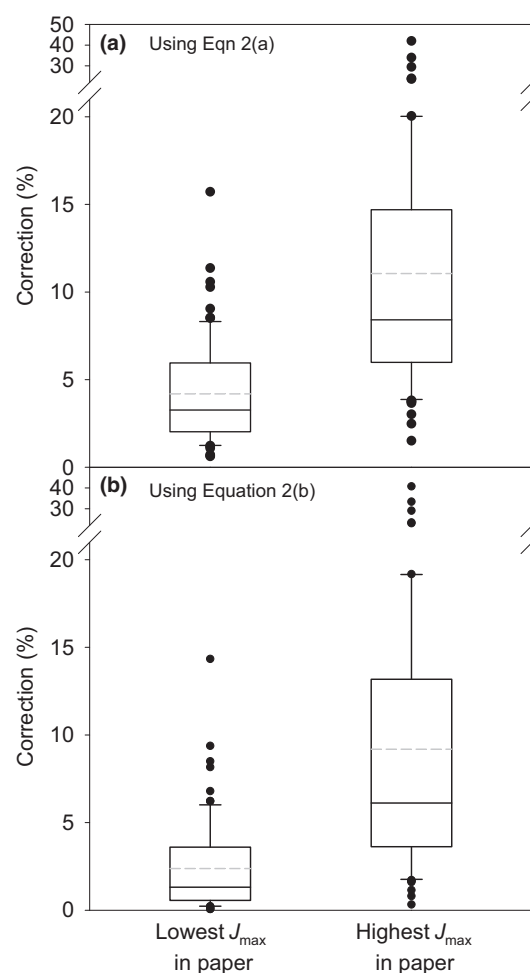
### Why $J_{\text{high}}$ should not be reported as $J_{\max}$

One could argue that it is fine to report  $J_{\text{high}}$  as  $J_{\max}$ , because  $J_{\text{high}}$  is 'truly saturated', and therefore should be labelled as  $J_{\max}$ . One could also argue that the problem is not with the practice of reporting  $J_{\text{high}}$  as  $J_{\max}$ , but with the use of asymptotic models for  $J$  versus  $i$  (e.g. Eqn 1), which are inaccurate and nonmechanistic. These arguments are misguided, for two reasons. The first reason is that published values of  $J_{\max}$  are in fact routinely applied to photosynthesis models using asymptotic models for  $J$  versus  $i$ , and  $J_{\text{high}}$  is not the correct parameter to apply to such models. Regardless of whether such models are ideal, accurate or mechanistic, the reality is that they are widely used, so reporting  $J_{\text{high}}$  as  $J_{\max}$  probably leads to errors in modelling. The second reason is that investigators rarely check whether  $J$  is truly light-saturated at the irradiance used in  $\text{CO}_2$  response curves – that would be impractical, and it might even be impossible, because  $\text{CO}_2$  exchange could be saturated at high irradiance and high  $\text{CO}_2$  for other reasons, such as triose phosphate utilisation limitation.

### Recommendations

One solution would be to demand that theoretical physiologists produce an alternative model that is not asymptotic. This is not a good solution because it remains unclear whether  $J$  is truly saturated at  $i_{\text{high}}$ , and also because any new model would certainly take many years to be fully adopted. Another solution would be for investigators to ensure that they always explicitly identify the equation and the associated parameter values that they used to infer  $J_{\max}$  from  $J$ . This would be helpful but it still carries the risk of ambiguity, because  $J_{\max}$  values can quickly become disconnected from the  $J$  versus  $i$  model used to estimate them – for example, when compiled into a meta-analysis or a trait database.

A better solution, which we recommend because it eliminates ambiguity and requires no effort, is simply to report  $J$  with an explicit annotation specifying the value of  $i$  at which it was



**Fig. 3** Distributions of calculated percentage corrections required to obtain the values of the maximum potential electron transport rate ( $J_{\max}$ ) appropriate to asymptotic models from the value of  $J$  estimated from photosynthetic  $\text{CO}_2$  response curves ( $J_{\text{high}}$ ), for 71 papers in the recent literature, computed using (a) Eqn 2(a) or (b) Eqn 2(b), as described in the main text. Boxes: bottom, 25th percentile; top, 75th percentile; horizontal solid line, median; horizontal dashed line, mean. Bars extending above and below boxes: 90th and 10th percentiles, respectively. Points, outliers beyond the 10th and 90th percentiles.

measured (e.g.  $J_{1500}$  for  $J$  measured at  $i = 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Some investigators already do this. We also recommend that modellers who wish to use  $J_{\max}$  values from the literature to simulate photosynthesis should retrospectively correct those values, where necessary, using Eqn 2(a) or 2(b) or an analogous expression based on their chosen submodel for  $J$  versus  $i$ .

### Acknowledgements

We thank the Editor, Richard Norby, and three anonymous reviewers for helpful comments on an earlier draft. T.N.B. was supported by the US National Science Foundation (Award no. 1146514) and by the Grains Research and Development Corporation (GRDC). This work was funded by the Spanish Ministry of Science and Innovation (research project AGL2012-34544).

Thomas N. Buckley<sup>1\*</sup> and Antonio Diaz-Espejo<sup>2</sup>

<sup>1</sup>IA Watson Grains Research Centre, Faculty of Agriculture and Environment, The University of Sydney, Narrabri, NSW 2390, Australia;

<sup>2</sup>Irrigation and Crop Ecophysiology Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC), Avenida Reina Mercedes 10, 41012 Sevilla, Spain

(\*Author for correspondence: tel +61 2 6799 2227; email t.buckley@sydney.edu.au)

## References

- Bernacchi C, Pimentel C, Long S. 2003. *In vivo* temperature response functions of parameters required to model RuBP-limited photosynthesis. *Plant, Cell & Environment* 26: 1419–1430.
- Crous KY, Walters MB, Ellsworth DS. 2008. Elevated CO<sub>2</sub> concentration affects leaf photosynthesis–nitrogen relationships in *Pinus taeda* over nine years in FACE. *Tree Physiology* 28: 607–614.
- Dubois J-JB, Fiscus EL, Booker FL, Flowers MD, Reid CD. 2007. Optimizing the statistical estimation of the parameters of the Farquhar–von Caemmerer–Berry model of photosynthesis. *New Phytologist* 176: 402–414.
- Ellsworth DS, Reich PB, Naumburg ES, Koch GW, Kubiske ME, Smith SD. 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO<sub>2</sub> across four free-air CO<sub>2</sub> enrichment experiments in forest, grassland and desert. *Global Change Biology* 10: 2121–2138.
- Evans JR, Poorter H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell & Environment* 24: 755–767.
- Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> species. *Planta* 149: 78–90.
- Farquhar GD, Wong SC. 1984. An empirical model of stomatal conductance. *Australian Journal of Plant Physiology* 11: 191–210.
- Flexas J, Diaz-Espejo A, Berry JA, Cifre J, Galmes J, Kaldenhoff R, Medrano H, Ribas-Carbo M. 2007. Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization. *Journal of Experimental Botany* 58: 1533–1543.
- Gilbert ME, Pou A, Zwieniecki MA, Holbrook NM. 2012. On measuring the response of mesophyll conductance to carbon dioxide with the variable *J* method. *Journal of Experimental Botany* 63: 413–425.
- Gu J, Yin X, Stomph T-J, Wang H, Struik PC. 2012. Physiological basis of genetic variation in leaf photosynthesis among rice (*Oryza sativa* L.) introgression lines under drought and well-watered conditions. *Journal of Experimental Botany* 63: 5137–5153.
- Gu L, Pallardy SG, Tu K, Law BE, Wullschlegel SD. 2010. Reliable estimation of biochemical parameters from C<sub>3</sub> leaf photosynthesis–intercellular carbon dioxide response curves. *Plant, Cell & Environment* 33: 1852–1874.
- Kattge J, Knorr W, Raddatz T, Wirth C. 2009. Quantifying photosynthetic capacity and its relationship to leaf nitrogen content for global-scale terrestrial biosphere models. *Global Change Biology* 15: 976–991.
- Long S, Bernacchi C. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany* 54: 2393–2401.
- Miao Z, Xu M, Lathrop RG, Wang Y. 2009. Comparison of the *A*–*C*<sub>c</sub> curve fitting methods in determining maximum ribulose 1,5-bisphosphate carboxylase/oxygenase carboxylation rate, potential light saturated electron transport rate and leaf dark respiration. *Plant, Cell & Environment* 32: 109–122.
- Niinemets Ü, Tenhunen J, Canta N, Chaves M, Faria T, Pereira J, Reynolds J. 1999. Interactive effects of nitrogen and phosphorus on the acclimation potential of foliage photosynthetic properties of cork oak, *Quercus suber*, to elevated atmospheric CO<sub>2</sub> concentrations. *Global Change Biology* 5: 455–470.
- Niinemets Ü, Wright IJ, Evans JR. 2009. Leaf mesophyll diffusion conductance in 35 Australian sclerophylls covering a broad range of foliage structural and physiological variation. *Journal of Experimental Botany* 60: 2433–2449.
- Pons TL, Flexas J, Von Caemmerer S, Evans JR, Genty B, Ribas-Carbo M, Bruognoli E. 2009. Estimating mesophyll conductance to CO<sub>2</sub>: methodology, potential errors, and recommendations. *Journal of Experimental Botany* 60: 2217–2234.
- Rodeghiero M, Niinemets Ü, Cescatti A. 2007. Major diffusion leaks of clamp-on leaf cuvettes still unaccounted: how erroneous are the estimates of Farquhar *et al.* model parameters? *Plant, Cell & Environment* 30: 1006–1022.
- Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL. 2007. Fitting photosynthetic carbon dioxide response curves for C<sub>3</sub> leaves. *Plant, Cell & Environment* 30: 1035–1040.
- Smith EL. 1937. The influence of light and carbon dioxide on photosynthesis. *The Journal of General Physiology* 20: 807–830.
- Tholen D, Ethier G, Genty B, Pepin S, Zhu X-G. 2012. Variable mesophyll conductance revisited: theoretical background and experimental implications. *Plant, Cell & Environment* 35: 2087–2103.
- Warren CR, Dreyer E, Adams MA. 2003. Photosynthesis–Rubisco relationships in foliage of *Pinus sylvestris* in response to nitrogen supply and the proposed role of Rubisco and amino acids as nitrogen stores. *Trees* 17: 359–366.
- Wullschlegel SD. 1993. Biochemical limitations to carbon assimilation in C<sub>3</sub> plants – a retrospective analysis of the *A/C*<sub>c</sub> curves from 109 species. *Journal of Experimental Botany* 44: 907–920.

## Supporting Information

Additional supporting information may be found in the online version of this article.

**Notes S1** List of papers examined in this study, with *J*<sub>max</sub> estimation method type, *i*<sub>high</sub>, and low and high *J*<sub>max</sub>.

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

**Key words:** electron transport, Farquhar model, maximum potential electron transport rate (*J*<sub>max</sub>), maximum velocity of carboxylation (*V*<sub>cmax</sub>), photosynthesis.