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The Kok effect in *Vicia faba* cannot be explained solely by changes in chloroplastic CO_2 concentration

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Summary

- The Kok effect – an abrupt decline in quantum yield (QY) of net CO_2 assimilation at low photosynthetic photon flux density (PPFD) – is widely used to estimate respiration in the light (R), which assumes the effect is caused by light suppression of R. A recent report suggested much of the Kok effect can be explained by declining chloroplastic CO_2 concentration (c_c) at low PPFD. Several predictions arise from the hypothesis that the Kok effect is caused by declining c_c, and we tested these predictions in *Vicia faba*.
- We measured CO_2 exchange at low PPFD, in 2% and 21% oxygen, in developing and mature leaves, which differed greatly in R in darkness.
- Our results contradicted each of the predictions based on the c_c effect: QY exceeded the theoretical maximum value for photosynthetic CO_2 uptake; QY was larger in 21% than 2% oxygen; and the change in QY at the Kok effect breakpoint was unaffected by oxygen.
- Our results strongly suggest the Kok effect arises largely from a progressive decline in R with PPFD that includes both oxygen-sensitive and -insensitive components. We suggest an improved Kok method that accounts for high c_c at low PPFD.

Introduction

Leaf respiration rate (nonphotorespiratory CO_2 release, R) is suppressed in the light, by up to 85% of the rate in the dark (R_{dark}; Hoefnagel *et al.*, 1998). This suppression is greater in mature leaves than in young leaves (Villar *et al.*, 1995), and when measured at high light than at low light (Atkin *et al.*, 2000). There is no consensus as to what causes this suppression or why it varies so widely (Krömer, 1995; Hoefnagel *et al.*, 1998; Buckley & Adams, 2011; Tcherkez *et al.*, 2017a,b). This uncertainty confounds reliable prediction of CO_2 exchange, as well as interpretation of processes related to CO_2 exchange, including photosynthesis and photorespiration, carbohydrate metabolism, anabolism and stable isotope discrimination (Krömer, 1995; Hoefnagel *et al.*, 1998; Noctor & Foyer, 1998; Ghashghaie *et al.*, 2003; Tcherkez & Hodges, 2008). It also reveals a major gap in our understanding of leaf respiration – an important and highly variable component of the carbon cycle that represents *c.* 5–20% of gross primary productivity and is widely predicted to be sensitive to climate change (Poorter *et al.*, 1990; Noguchi *et al.*, 1996; Ryan *et al.*, 1996; Waring *et al.*, 1998; Davey *et al.*, 2004; Atkin *et al.*, 2007).

Light suppression of R has long been suspected as the mechanism of the 'Kok effect' – an abrupt change in quantum yield (QY) of net CO_2 assimilation rate (A) that occurs at very low photosynthetic photon flux density (PPFD or i), often near the photosynthetic light compensation point (Fig. 1; Kok, 1948, 1949). If one extrapolates to $i = 0$ the A vs i relationship observed above the Kok effect breakpoint, the predicted value of R_{dark} is closer to zero than the observed value, which has often been interpreted as evidence for, and a measure of, the suppression of R by light (Villar *et al.*, 1994; Yin *et al.*, 2011; Heskel *et al.*, 2013). We distinguish in this study between the Kok effect (the change in QY itself) and the use of the Kok effect to infer R in the light (which we term the 'Kok method'). Although evidence from other methods supports the hypothesis that R is suppressed by light (Atkin *et al.*, 1997, 2000; Peisker & Apel, 2001; Tcherkez *et al.*, 2005, 2008), relatively few experiments have estimated changes in R across the narrow range of very low

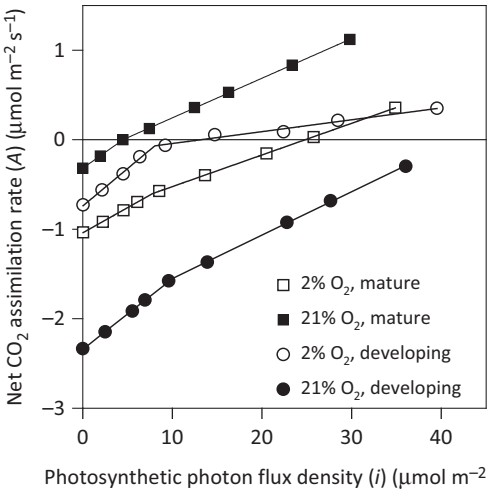

Fig. 1 Example responses of net CO_2 assimilation rate (A) to incident irradiance (i), demonstrating the Kok effect in *Vicia faba* in developing leaves (circles) and mature leaves (squares), measured at 21% oxygen (closed symbols) or 2% oxygen (open symbols). Lines shown are piecewise linear regressions. Each response curve is from a different leaf on a different individual.

PPFD at which the Kok effect is often observed (Atkin *et al.*, 1998).

Some evidence suggests the Kok effect involves changes in photosynthesis or photorespiration rather than R. For example, earlier studies found the effect to be absent at low oxygen (O_2) and in C_4 plants (Cornic & Jarvis, 1972; Ishii & Murata, 1978; Day *et al.*, 1985), which led to the hypothesis that the mechanism involves photorespiration. That idea was challenged by experiments reporting the Kok effect at saturating CO_2, a condition that suppresses photorespiration (Sharp *et al.*, 1984). An absence of the Kok effect in low O_2 might instead indicate a role for mitochondrial O_2 reduction (Healey & Myers, 1971) or chlororespiration (Peltier & Sarrey, 1988), either of which could be suppressed by low O_2. However, another study found R is not suppressed by 2% O_2 (Brooks & Farquhar, 1985), and a recent study of the Kok effect reported the effect at 2% O_2 (Yin *et al.*, 2011).

An alternative hypothesis for the mechanism of the Kok effect was proposed recently by Farquhar & Busch (2017, hereafter 'FB'). Those authors noted that chloroplastic CO_2 concentration (c_c) decreases as PPFD increases from darkness, as a consequence of diffusion (c_c must be above ambient to drive CO_2 diffusion into the leaf when $A < 0$ in darkness, and below ambient when $A > 0$ in the light). This drop in c_c below the light compensation point would be amplified by any increase in stomatal and/or mesophyll conductance (and hence in total conductance to CO_2, g_{tc}) with PPFD. Declining c_c would reduce QY as PPFD increases, and in a manner reminiscent of the Kok effect, with QY declining most rapidly below the light compensation point. This suggests that it may be premature to attribute the Kok effect to suppression of R.

Several predictions arise if one assumes R is constant and the Kok effect arises entirely from changes in c_c. Prediction no. 1 is that QY might approach but should not exceed the CO_2-saturated maximum QY for photosynthesis (*c.* 0.088; Ehleringer & Björkman, 1977; Fig. 2a,b). Prediction no. 2 is that QY should be greater in

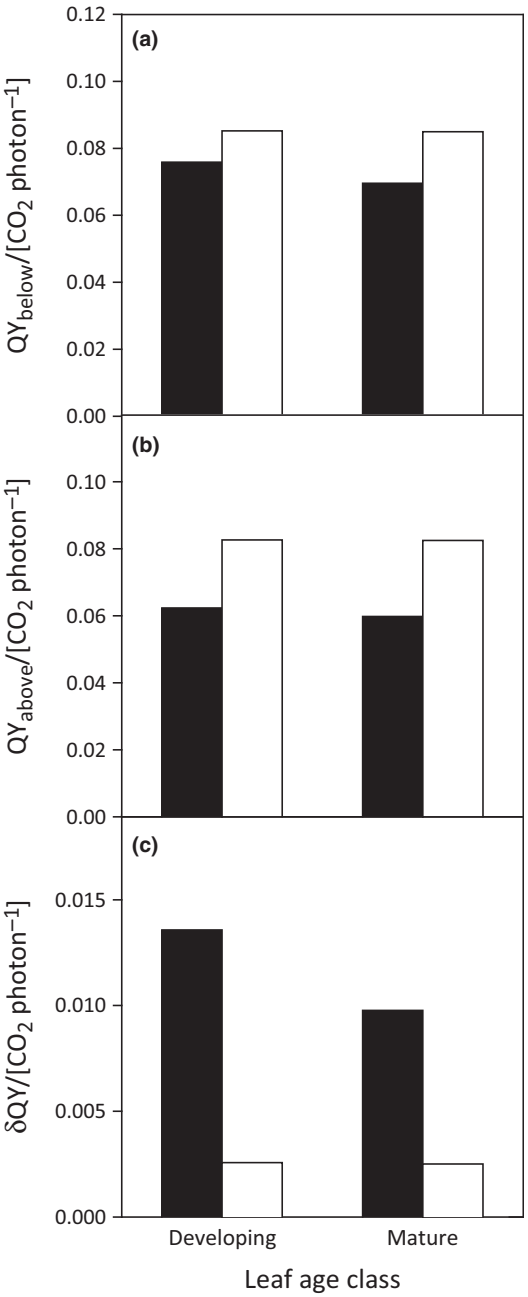

Fig. 2 Predicted effects of dark respiration and oxygen on features of the Kok effect, based on the hypothesis that changes in chloroplastic CO_2 concentration (c_c) with photosynthetic photon flux density (PPFD) cause the Kok effect, and using values of dark respiration rate (R_{dark}) observed in *Vicia faba* for developing and mature leaves in 21% (closed bars) and 2% (open bars) ambient oxygen concentrations (see Fig. 4). (a) Quantum yield below the Kok effect breakpoint (QY_{below}); (b) QY above the breakpoint (QY_{above}); (c) the decline in QY at the breakpoint (δQY).

2% O_2 than in 21% O_2 (Fig. 2a,b), because photorespiration reduces QY. Prediction no. 3 is that QY should be more sensitive to O_2 above the breakpoint than below it because c_c is lower above the breakpoint, so the decline in QY at the breakpoint (δQY) should be greater in 21% O_2 than in 2% O_2 (Fig. 2c).

Few published data are available to assess these predictions. Effects of O_2 on properties of the Kok effect are unknown, because

the effect was thought to be absent in low O_2 until the recent report by Yin *et al.* (2011). Our objective here was to test whether the phenomenology of the Kok effect in broad bean (*Vicia faba*) can be explained by the FB mechanism, by measuring the Kok effect in 21% and 2% O_2 and comparing results between developing and mature leaves, which differed greatly in R_{dark}.

Materials and Methods

Plant material

Individuals of broad bean (*Vicia faba* L.) were planted in 4-l pots containing commercial soil, perlite and vermiculite in the ratio 6 : 1 : 1 by volume, with 5 ml of dry slow-release fertilizer added. Plants were kept in a glasshouse at Sonoma State University (relative humidity, 30% : 65%; temperature, 23°C : 18°C (day : night)). Mature leaves *c.* 5–7 cm in length and 4–6 cm in width were sampled from the fourth, fifth or sixth node (counting from the stem apex) and were rejected if they showed any signs of senescence. Developing leaves were chosen from the first or second nodes, and were *c.* 15–25 mm in length and 10–20 mm in width.

Gas exchange measurement protocol

We measured A at nine levels of i: darkness and eight low i values ranging up to *c.* 30–40 μmol m^{-2} s^{-1}. i was measured directly at the leaf surface as described later. At each value of i, A was allowed to stabilize for 10 min, which preliminary trials found to be adequate to ensure stable gas exchange. Leaf to air water vapor mole fraction difference was kept at 15 ± 2 mmol mol^{-1} and leaf temperature was kept between 24.5 and 25.5°C, varying < 0.3°C within each experiment. c_a was kept at 600 ± 50 μmol mol^{-1}, varying < 20 μmol mol^{-1} within each experiment.

Gas exchange system

We measured gas exchange using an open-flow single-pass differential gas exchange system described previously (Buckley *et al.*, 2011), with several modifications. First, we used a 9.3 cm^2 circular leaf chamber made from high-density polyethylene with borosilicate glass windows above and below the leaf and neoprene foam gaskets. Air was circulated across both leaf surfaces independently through channels connected to a nickel-plated aluminum chamber containing a high-speed fan. Leaf temperature was measured with a fine-wire type T thermocouple that was kept in contact with the lower leaf surface. Second, we measured i in the leaf chamber with a GaAsP photodiode (G1118; Hamamatsu Inc., Hamamatsu, Japan) whose upper surface was 1.5 mm above the leaf surface. The photodiode was calibrated against a quantum sensor (LI-190; Li-Cor, Lincoln, NE, USA) under the light source used in this study: an LED fiber optic source (MSP-Series; DiCon LED, Richmond, CA, USA) operating in 'white' mode with a color temperature of 3000 K. Cellophane neutral density filters were placed on the chamber to modulate i. The light field varied < 7% across the chamber, as gauged by moving the photodiode. Third, we matched the gas analyzer before each measurement (allowing 5 min for reference gas to equilibrate in both cells), and corrected differential measurements retrospectively for any match drift between matching times. Fourth, the gas mix was buffered through a 2-l glass bottle to minimize fluctuations in reference gas composition. We zeroed the infrared gas analyzer for CO_2 using air stripped of CO_2 with Ascarite II and spanned it using a reference tank with CO_2 of known composition, and we zeroed the analyzer for H_2O using air stripped of water vapor using magnesium perchlorate and spanned it continuously in operation by passing the reference stream through a chilled mirror dewpoint hygrometer (Dew-10; General Eastern, Billerica, MA, USA).

Chamber leakage errors

Recent studies reported nonzero apparent CO_2 exchange with empty chambers, or with heat-killed leaves in the chambers of portable gas exchange systems (Jahnke, 2001; Flexas *et al.*, 2007). We performed empty chamber tests to test for this effect in our system, but found CO_2 exchange rate was very small (< 0.1 μmol m^{-2} s^{-1}) and independent of chamber pCO_2 between 100 and 1000 ppm.

Data analysis

To quantify the Kok effect, we fitted a segmented line to the relationship between A and i: $A = \min[QY_{below} \cdot i + b_{below}, QY_{above} \cdot i + b_{above}]$, where $\min[x,y]$ is the lesser of x and y, QY_{below} and QY_{above} are the slopes of A vs i below and above the Kok effect breakpoint, respectively, and b_{below} and b_{above} are the corresponding y-intercepts. We used Solver in Microsoft Excel to minimize the sum of squared errors between the segmented line and the data by adjusting QY_{below}, b_{below}, QY_{above} and b_{above}. Figure 1 shows examples of fitted responses.

We assessed differences in R_{dark}, QY_{below}, QY_{above}, δQY and i_{break} between treatments by analysis of variance on linear models with oxygen and leaf developmental stage as fixed independent categorical variables, followed by Tukey's HSD *post hoc* multiple comparison tests, using base R (functions LM(), ANOVA(), AOV() and TukeyHSD()) (R Core Team, 2013). All variables except QY_{above} were log-transformed before analysis to improve normality.

Simulations to generate predictions described in Table 1 and shown in Fig. 2.

To predict how the Kok effect should differ with R_{dark} and oxygen concentration if the effect arises only from changes in c_c in

Table 1 Predictions from the hypothesis that the Kok effect arises from effects of changes on chloroplastic CO_2 concentration on quantum yield (see also Fig. 2)

Prediction
(1) Quantum yield should not exceed the maximum value for photosynthetic CO_2 assimilation (*c.* 0.088)
(2) Quantum yield should be greater in 2% O_2 than in 21% O_2
(3) The drop in quantum yield at the breakpoint should be larger in 21% O_2 than in 2% O_2

relation to PPFD, we simulated A vs i using the same modeling assumptions as Farquhar & Busch (2017). Specifically, we modeled A as

$$A = \frac{\phi i}{4} \cdot \left(\frac{c_c - \Gamma_*}{c_c + 2\Gamma_*} \right) - R \qquad \text{Eqn 1}$$

where Γ_* is the photorespiratory CO_2 compensation point (= 40·(%oxygen/21), in units of ppm) and $\phi = \partial J/\partial i = (1 - f)/2$, where J is the potential electron transport rate and f is the fraction of photons absorbed by Photosystem II that do not contribute to photochemistry (0.3). Therefore, c_c and A also depend on g_{tc}, as

$$A = g_{tc}(c_a - c_c) \qquad \text{Eqn 2}$$

Combining Eqns 1 and 2 leads to a quadratic expression for c_c, whose larger root is applied to either equation to calculate A. Following Farquhar & Busch (2017), we assumed $g_{tc}/(\text{mol m}^{-2}\,\text{s}^{-1}) = 0.001 + ((1 - f)/3680)\cdot i/(\mu\text{mol m}^{-2}\,\text{s}^{-1})$, and assumed R was invariant with i, using mean values of R_{dark} observed in each treatment group for *V. faba*; these differences in R_{dark} were the only differences between developing and mature leaves in these simulations. We simulated A in this manner for 101 values of i between 0 and 100, and fitted segmented regressions to the results by minimizing the sum of SSbelow and SSabove (the sums of squared differences between the regression and the simulated values below and above the intersection of the two segments, respectively) while varying the slopes of both segments and the intercept of the upper segment using Solver in Microsoft Excel, with the intercept of the lower segment set at R_{dark}. The simulation spreadsheet is included as Supporting Information Methods S1.

Results

We observed the Kok effect in all developing leaves (18 of 18) and 61% of mature leaves (11 of 18) at 21% O_2, and in all developing leaves (10 of 10) and 86% of mature leaves (six of seven) at 2% O_2. Figure 1 shows an example from each treatment group.

The value of R_{dark} is greater in developing than mature leaves at 21% O_2

The value of R_{dark} averaged 2.51 ± 0.31 μmol $m^{-2}\,s^{-1}$ (mean ± standard error (SE)) in developing leaves vs 0.51 ± 0.06 μmol $m^{-2}\,s^{-1}$ in mature leaves at 21% O_2, and 1.18 ± 0.17 μmol $m^{-2}\,s^{-1}$ in developing leaves vs 0.66 ± 0.08 μmol $m^{-2}\,s^{-1}$ in mature leaves at 2% O_2 (Fig. 3). Thus, R_{dark} in mature leaves was not significantly affected by O_2 ($P < 0.05$, $n = 7$–18).

QY_{below} consistently exceeds the maximum QY for photosynthesis in developing leaves at 21% O_2

Contrary to Prediction no. 1 (Table 1), QY_{below} averaged 0.104 ± 0.009 CO_2 $photon^{-1}$ ($n = 18$) in developing leaves measured at 21% O_2 (Fig. 4a), exceeding the theoretical maximum QY for photosynthetic CO_2 assimilation of *c.* 0.088. Average QY in other treatments was below this maximum, ranging from 0.048 ± 0.004 (mature leaves at 21% O_2) to 0.069 ± 0.008 (developing leaves at 2% O_2; Fig. 4a,b).

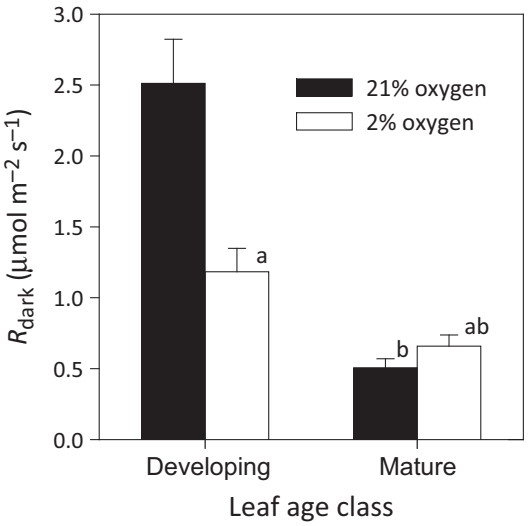

Fig. 3 Dark respiration rate (R_{dark}) in developing and mature leaves of *Vicia faba* measured in 21% and 2% ambient oxygen concentration. Means ± standard error. Bars with the same letter were not statistically different from one another (Tukey's HSD, $P > 0.05$).

QY is greater in 21% O_2 than in 2% O_2 in developing leaves

Contrary to Prediction no. 2, QY was significantly greater at 21% O_2 than at 2% O_2 in developing leaves, both below and above the breakpoint (Fig. 4a,b). QYs were statistically indistinguishable between 2% and 21% O_2 in mature leaves.

The drop in QY at the Kok effect breakpoint is unaffected by O_2

Contrary to Prediction no. 3, the drop in QY at the breakpoint was not significantly different between 21% and 2% O_2 ($P = 0.57$, df = 41; Fig. 4c).

Discussion

Our data contradict each of the three predictions (Table 1) from the hypothesis that changes in c_c at low PPFD explain the Kok effect, which strongly indicates that the Kok effect in *V. faba* is at least partly attributable to suppression of R by light. We present three lines of evidence for this conclusion:

- QY below the breakpoint substantially exceeded the theoretical maximum value for photosynthetic CO_2 assimilation of *c.* 0.088 in developing leaves at 21% O_2, averaging 0.104 ($n = 18$). Sharp *et al.* (1984) also presented three examples of QY_{below} exceeding this threshold in *Helianthus annuus*, ranging from 0.095 to 0.113. The simplest explanation for these observations is that R decreases as PPFD increases.

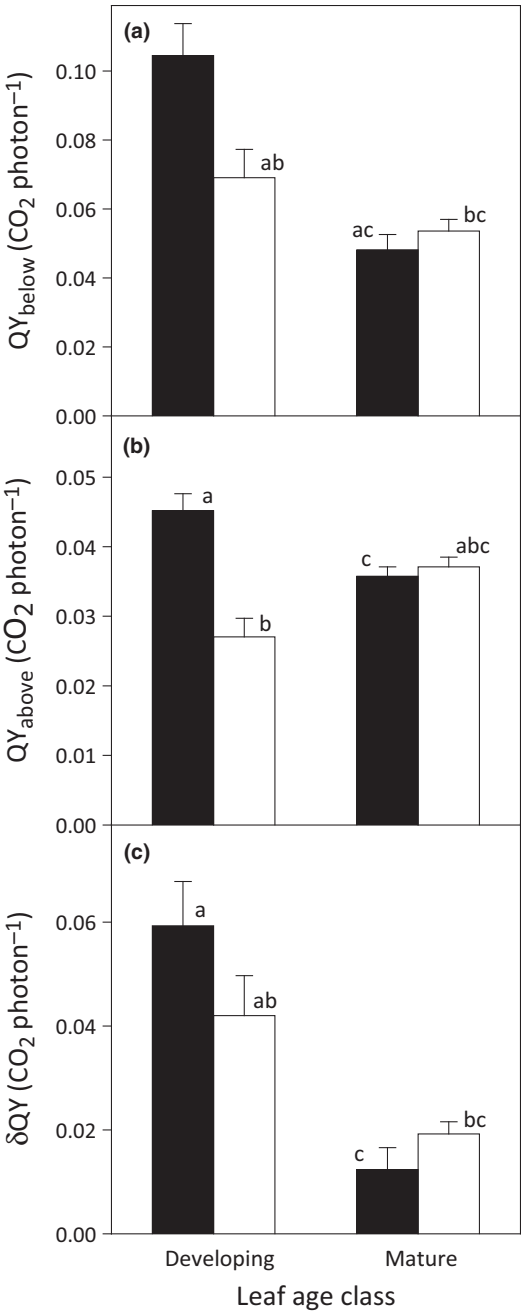

Fig. 4 Observed features of the Kok effect in *Vicia faba* in developing and mature leaves in 21% (closed bars) and 2% (open bars) ambient oxygen concentrations. (a) Quantum yield below the Kok effect breakpoint (QY_{below}); (b) QY above the breakpoint (QY_{above}); (c) the decline in QY at the breakpoint (δQY). Means ± standard error. Bars with the same letter were not statistically different from one another (Tukey's HSD, $P > 0.05$).

- QY was greater in 21% O_2 than in 2% O_2 in developing leaves, whereas a photosynthesis-mediated effect would predict the opposite. This implies that any effect of declining c_c on photosynthetic QY is substantially overridden by the decline in R with increasing PPFD, and it implies further that the suppression of R below the breakpoint is itself sensitive to oxygen.
- The drop in QY at the breakpoint (δQY) was unaffected by oxygen, whereas a photosynthesis-mediated Kok effect would predict δQY to be greater at 21% O_2 because QY is more sensitive to oxygen above the breakpoint than below it. This suggests that the decline in QY in the Kok effect also includes a component that is insensitive to oxygen.

QY could also exceed 0.088 if sources of ATP and NADPH other than the light reactions enhanced the rate of CO_2 fixation in very low PPFD. However, the most likely nonphotosynthetic sources of ATP and NADPH are themselves coupled to R (namely, the TCA cycle and the oxidative pentose phosphate pathway (OPPP), respectively), so a decline in their contribution to QY likely implies a decline in R as well. This is all the more likely given that QY below the breakpoint was enhanced by O_2, which could indicate the involvement of mitochondrial electron transport. Another possibility is that stomatal or mesophyll conductances to CO_2 might themselves respond to changes in c_c with PPFD and/or differences in pO_2 between treatments. Both g_s and g_m typically increase when c_c declines (Morison, 1998; Flexas *et al.*, 2012), which would act as a negative feedback on the decline in c_c resulting from positive responses of g_s and g_m to PPFD, thus muting, if anything, the effects noted by Farquhar & Busch (2017). Effects of pO_2 on g_s and g_m could, in theory, contradict Predictions nos 2 and 3 if they prevented or reduced the decline in c_c with increasing PPFD in 2% but not 21% O_2 – for example, if g_{tc} in darkness were dramatically larger, and dg_{tc}/di much smaller, in 2% O_2 than in 21% O_2 – but we are unaware of any evidence for such differences, and responses of g_s and g_m to pO_2 are insufficiently characterized to allow generalization. In any event, such effects would not contradict Prediction no. 1. Taken together, our data suggest that light suppression of R dominates the c_c effect in determining the phenomenology of the Kok effect in *V. faba*.

Our results also corroborate several previous conclusions. Sharp *et al.* (1984) measured the Kok effect as R_{dark} declined over several days in a single leaf, and reported that QY_{below} decreased in parallel with R_{dark} (cf Fig. 4a). We also verified Yin *et al.*'s (2011) finding of the Kok effect at 2% O_2. Our results, and those of Yin *et al.* (2011), stand apart from the 40-yr-old literature consensus, which has held that the effect is absent at low O_2 (Ishii & Murata, 1978; Sharp *et al.*, 1984; Kirschbaum & Farquhar, 1987). Our data expand the list of species in which the Kok effect has been observed in 2% O_2 to include *V. faba* in addition to rice, potato and maize (Yin *et al.*, 2011).

What causes suppression of *R* at low light?

It is unknown which of several nonphotorespiratory sources of CO_2 is or are involved in suppression of R at very low PPFD (Atkin *et al.*, 2000; Buckley & Adams, 2011; Tcherkez *et al.*, 2017a,b). Light is known to directly suppress the activity of several enzymes that regulate carbon flow through CO_2-releasing pathways (Hoefnagel *et al.*, 1998), including pyruvate dehydrogenase in the TCA cycle (Atkin *et al.*, 1998), pyruvate kinase in glycolysis (Xue *et al.*, 1996) and G6PDH in the OPPP (Buchanan, 1980). It is unknown whether these effects occur across similar PPFD ranges as the Kok effect, although Farr *et al.* (1994) found that the suppression of G6PDH by light in *Chlamydomonas reinhardtii* was strongest below a PPFD of *c.* 30 μmol m^{-2} s^{-1}, which is similar to the Kok effect breakpoint in many species (Sharp *et al.*, 1984; Kirschbaum

& Farquhar, 1987). Peltier & Sarrey (1988) found that a change in the QY of O_2 uptake persisted when mitochondrial function was suppressed by inhibitors. They concluded that the Kok effect did not involve mitochondria, but instead arose from suppression of chlororespiration by Photosystem I; it is unknown whether characterizing the Kok effect via CO_2 exchange rather than O_2 exchange would yield the same result.

Whatever the mechanism of the Kok effect, our results and the analysis by Farquhar & Busch (2017) suggest that it comprises at least three components. Two of these involve declining R with increasing PPFD: one that is sensitive to O_2 and another that is not. Only the latter component was present in mature leaves of *V. faba* in this study. The third component is the decline in photosynthetic QY caused by decreasing c_c at low PPFD, as noted by Farquhar & Busch (2017). Our results do not dispute the occurrence of the third component, but merely the hypothesis that it is solely responsible for the Kok effect. We join Farquhar & Busch (2017) in urging caution when inferring the numerical value of R in the light from the traditional Kok method. Accepting that R is substantially inhibited by light below the Kok effect breakpoint, careful measurement and analysis of CO_2 exchange in low light thus remains an important means for studying suppression of R by light.

We therefore propose an alternative method for estimating R_{light} from properties of the Kok effect. We show in Notes S1 that differentiating Eqn 1 and applying $dR/di = (R_{light} - R_{dark})/i_{break}$ (which assumes that R declines linearly with i below i_{break}) leads to the following approximate expression for R_{light}:

$$R_{light} \approx R_{dark} - i_{break}\left(QY_{below} - \frac{\phi}{4}\left(\frac{c_a + \frac{1}{2}\Gamma_*}{c_a + 2\Gamma_*}\right)\right) \qquad \text{Eqn 3}$$

The term involving c_a is an average of two values for the photosynthetic and photorespiratory component of QY below the breakpoint, computed using $c_c = c_a$ and $c_c \to \infty$, respectively, which represent lower and upper bounds on c_c below the light compensation point. Figure 5 shows how the estimate from Eqn 3 and that from the traditional Kok method vary in proportion to the 'true' R_{light} in a Monte Carlo sensitivity analysis in which we varied g_{min} (g_{tc} in darkness), dg_{tc}/di and i_{break} randomly in 944 simulations to represent a wide range of possible scenarios (additional details of these simulations are provided in Notes S1). The Kok method estimate invariably underestimated the magnitude of R_{light} (with a median ratio of estimated to true R_{light} of 0.80 and an interquartile range of 0.58 to 0.88), whereas Eqn 3 produced a more faithful estimate of R_{light}, with a median ratio of estimated to true R_{light} of 1.03 and an interquartile range of 0.88 to 1.12. We therefore tentatively suggest that Eqn 3 may be preferable to the traditional Kok method as an empirical tool to estimate R_{light} from CO_2 exchange measurements. We emphasize, however, that this (improved) approach is still approximate, and that the most rigorous approach is to explicitly measure and correct for the shifts in c_c at low PPFD noted by Farquhar & Busch (2017). Further improvement thus demands either a reliable, simple and field-robust method to estimate g_m and hence c_c, or other new knowledge sufficient to generalize the behavior of c_c below the Kok effect breakpoint.

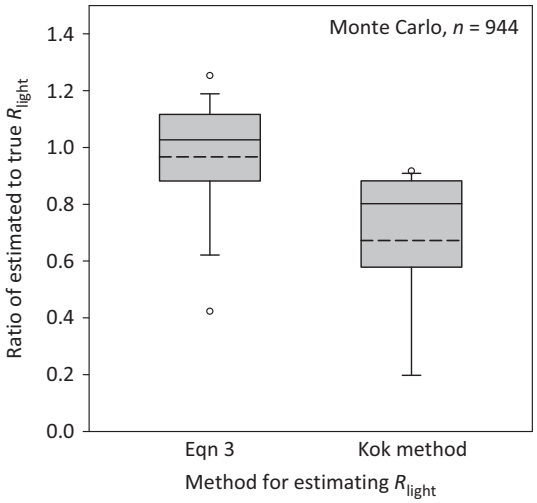

Fig. 5 Summary of Monte Carlo sensitivity analysis of estimates of light respiration rate (R_{light}) based on Eqn 3 and the traditional Kok method, in which total CO_2 conductance in darkness (g_{min}), sensitivity of total CO_2 conductance to light (dg_{tc}/di) and the photosynthetic photon flux density (PPFD) at which suppression of R by light stops (i_{break}) were simultaneously randomized in each of 944 simulations. The dashed lines indicate mean values; the solid midline, top and bottom of each box indicate the median, 75th and 25th percentiles, respectively, for the ratio of estimated to true R_{light}; the upper and lower whiskers indicate the 90th and 10th percentiles, respectively; and the upper and lower open symbols indicate the 95th and 5th percentiles, respectively.

Conclusion

Our results show that the properties of the Kok effect in *V. faba* in relation to R_{dark} and pO_2 cannot be explained by the effect of progressive decline in c_c with increasing PPFD on the QY of net photosynthesis. The Kok effect in *V. faba* persists in 2% O_2 and is largely caused by a progressive decline in R increasing PPFD that includes both oxygen-sensitive and -insensitive components. We suggest a modified version of the Kok method to estimate R in the light from CO_2 exchange.

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Author contributions

T.N.B. designed the research; T.N.B. and H.V. collected and analyzed the data; T.N.B., H.V. and M.A.A. interpreted the data and outlined the manuscript; T.N.B. drafted the manuscript; T.N.B., H.V. and M.A.A. edited the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Methods S1 EXCEL spreadsheet used to generate predictions for the effect of R_{dark} and oxygen on the Kok effect, assuming the effect is caused solely by changes in c_c.

Notes S1 Derivation of Eqn 3 and details of Monte Carlo simulation shown in Fig. 5.

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