Editorial

Unravelling mechanisms and impacts of day respiration in plant leaves: an introduction to a Virtual Issue

Leaf respiration plays a key role in plant primary production due to its roles in determining rates of daily net carbon gain, nutrient acquisition, and growth. It is now well-accepted that leaf respiration differs considerably between darkness and light, not only in rate but also in its metabolic pathways and biological functions (Tcherkez *et al.*, 2012). As such, it is common practice to use different terms: light (day) and dark (night) respiration, with associated variables describing their rates, R_{light} (R_d) and R_{dark} (R_n). While leaf dark respiration has been documented for a long time, including its well-known relationship with growth, substrate supply and energy demand (O'Leary *et al.*, 2019), there is still considerable uncertainty about many aspects of day respiration including: (1) how to measure the rate R_{light}; (2) metabolic mechanisms underlying carbon dioxide (CO_2) release and oxygen (O_2) consumption; and (3) its impact on photosynthesis. This persistent uncertainty is highly problematic because it affects calculations of photosynthetic parameters, our understanding of nitrogen (N) assimilation and how it interacts with photorespiration, and calculations of carbon use efficiency or gross primary production at the canopy and/or ecosystem scale. This Virtual Issue compiles 33 articles that provide significant advances in addressing these challenges, propose new techniques to measure R_{light}, quantitatively assess the impact of R_{light} on calculated rates of carbon exchange in leaves, or enhance our understanding about day respiration through the documentation of diel patterns in R_{dark}. A specific review on day respiration can be found in Tcherkez *et al.* (2017b). Key aspects related to day respiration that are discussed in this Editorial are summarized in Fig. 1.

How to measure R_{light}?

In mathematical terms, day respiration participates in net photosynthetic CO_2 assimilation (A) according to:

$$A = v_c - \frac{v_o}{\xi} - R_{light} \qquad \text{Eqn 1}$$

where v_c and v_o are carboxylation and oxygenation velocities, respectively, and ξ is the stoichiometric coefficient between photorespiratory O_2 consumption and CO_2 release (very close to 2; Abadie *et al.*, 2016). At first glance, the role of R_{light} in influencing A seems very simple. However, what is not shown in Eqn 1 is the role that variations in R_{light} play in determining intracellular CO_2 concentrations (CO_2 mole fraction at carboxylation sites, c_c) – a parameter that is crucial in determining observed rates of net photosynthesis (A) through the dependence of v_c on c_c (Farquhar–von Caemmerer–Berry model). Moreover, R_{light} cannot be easily determined without the knowledge of c_c and thus of internal (mesophyll) conductance (g_m), or any other parameter that can solve the problem of having several variables convoluted in Eqn 1. Historically, two main techniques have been used to estimate R_{light}: the Laisk method and the Kok method (Atkin *et al.*, 2000). Both methods have advantages and disadvantages, which are reviewed in Tcherkez *et al.* (2017a,b). Of particular interest is the Kok method because it is relatively easy to implement and has therefore been used widely in surveys and rapid determinations of R_{light}. This method relies on exploitation of the Kok effect, that is, the abrupt change in slope in the A–light relationship in the region of very low light (0–40 µmol m^{-2} s^{-1} incident light). The decrease in slope as light intensity increases has long been interpreted as being a consequence of the inhibition of respiration by light reaching a maximum at the irradiance where the slope decreases. Hence the technique has been used to estimate R_{light} by linear extrapolation when taking into account changes in the intercellular concentration of CO_2 (Kirschbaum & Farquhar, 1987). The major problem with this method is that there is no definitive evidence that the Kok effect is due to the inhibition of respiration by light (Tcherkez *et al.*, 2017a). In other words, is R_{light} obtained with the Kok method actually representative of day respiration?

Four papers in this Virtual Issue make decisive advances on the question of whether R_{light} can be quantified using the Kok method. A useful starting point for understanding the discussion that follows is the relationship between A and absorbed irradiance (I_{abs}) explained further in Yin *et al.* (2020):

$$A = \frac{1 - \Gamma^*/c_c}{4(1 + 2\Gamma^*/c_c)} a\Phi \cdot I_{abs} - R_{light} \qquad \text{Eqn 2}$$
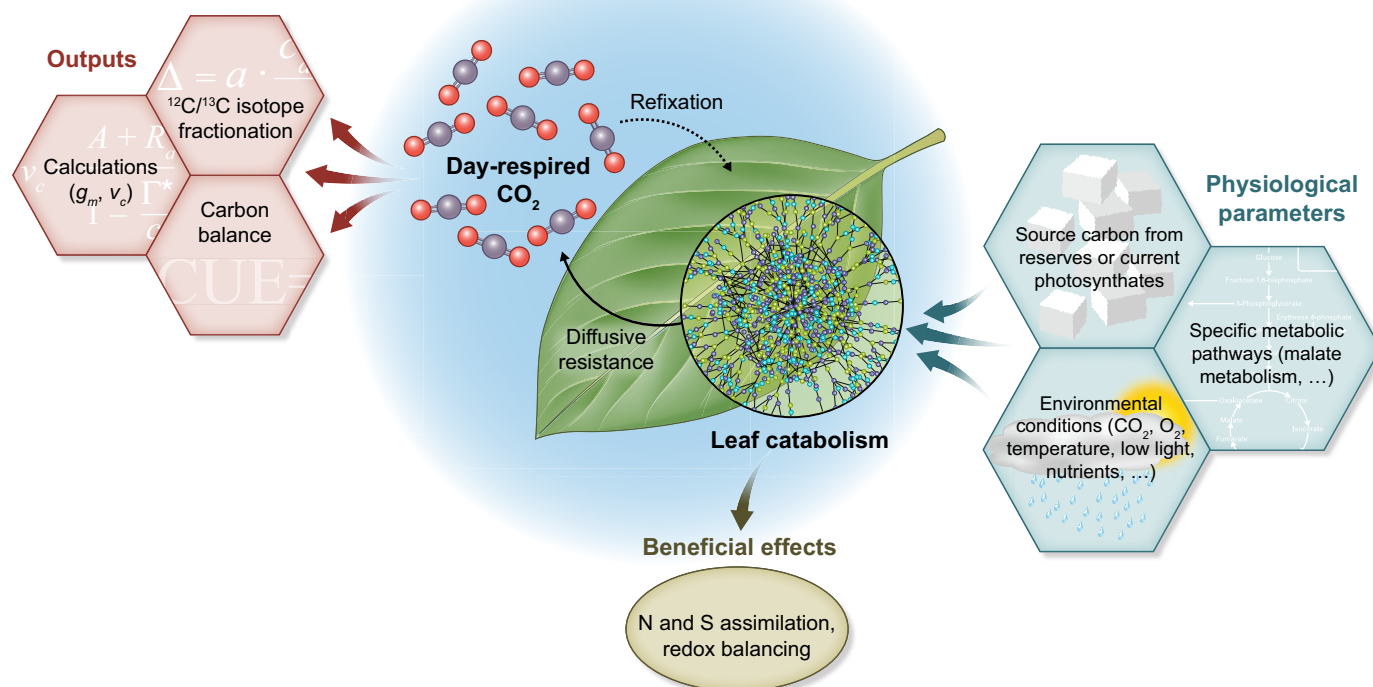
Fig. 1 Summary of questions tackled by papers in the Virtual Issue on day respiration and the Kok effect, differentiating physiological mechanisms influencing CO_2 release by day respiration (physiological parameters, right) and impacts of the day respiration rate for photosynthesis, gas exchange and variables derived therefrom (outputs, left).

where Γ^* is the CO_2 compensation point in the absence of day respiration, a is a parameter that encapsulates cyclic and pseudo-cyclic electron flows, Φ is the photochemical yield of photosystem II. Eqn 2 shows that an apparent change in slope in the A–I_{abs} relationship can come from either photosynthetic parameters (typically a, Φ or c_c/Γ^*) or a dependence of R_{light} with I_{abs}. Using a modelling exercise, Farquhar & Busch (2017) proposed that a change in c_c/Γ^* when irradiance varies in the low light region can effectively explain part, if not all, of the Kok effect. This assumption was experimentally tested by Buckley *et al.* (2017), who found that in broad bean (*Vicia faba*) leaves, there is a limited impact of both internal conductance and oxygen mole fraction (and thus Γ^*) on the Kok effect. This finding suggests that the effect of c_c/Γ^* is insufficient to explain the Kok effect. Moreover, using a semi-empirical determination of internal conductance to calculate c_c and an analysis of the light dependence of $a\Phi$, Yin *et al.* (2020) showed that in sunflower (*Helianthus annuus*) leaves, changes in c_c/Γ^* and $a\Phi$ were responsible for c. 25% and 11% of the change in slope in the Kok effect, respectively. Taken together, these results represent a major advance because they support the view that the Kok effect is, at least in part, a real respiratory phenomenon (i.e. R_{light} decreases with increasing irradiance). To gain insight on the metabolic basis of the Kok effect, Gauthier *et al.* (2020) carried out measurements on sunflower leaves labelled with ^{13}C-glucose. Using metabolic analyses, they showed that in the low irradiance region, there were light-dependent changes in metabolic fluxes, with a progressive increase in decarboxylating activities as

I_{abs} decreased. Interestingly, pathways other than the tricarboxylic acid pathway (TCAP) appeared to be involved, such as malate catabolism (by malic enzyme), perhaps indicating that the Kok effect is linked to metabolic changes associated with the onset of light-enhanced dark respiration. Taken as whole, we now have a fuller picture of the Kok effect, with it being a consequence of multiple factors, including the irradiance dependence of the CO_2 mole fraction at carboxylation sites (c_c), quantum yield ($a\Phi$) and R_{light}. That is, Kok-derived estimates are not entirely representative of R_{light}.

Given that the Kok effect is unlikely to be due solely to irradiance-dependent changes in respiratory metabolism, the question arises of what might be a better method for measuring R_{light}. Three new methods are proposed in this Virtual Issue. Gong *et al.* (2018) took advantage of the slow turnover of respiratory substrates to calculate R_{light} using an isotopic disequilibrium approach. That is, when the isotope composition of CO_2 of the atmosphere is changed abruptly, day respired-CO_2 has the isotope composition of 'old' substrates for some time and this represents an excellent opportunity to extract the value of R_{light}. One advantage of this technique is that it is independent of g_m, thus providing a way of estimating R_{light}, and in turn, assisting in calculating values of g_m and c_c.

Berghuijs *et al.* (2019) elaborated on the 2D model of photosynthetic cells described in Berghuijs *et al.* (2017) and used observed values of A to predict maximal carboxylation (V_{cmax}) and R_{light} by fitting. This technique assumes a certain geometry of the

cells to incorporate g_m implicitly (in practice, it is modelled with the diffusion coefficient of CO_2 in water, potentially corrected for porosity and viscosity) thereby allowing the calculation of R_{light}. This technique is interesting because it is more easily implementable than 3D-based models (lowering the demand for computing power). However, as always with geometric models, users must remain conscious that R_{light} estimates are highly sensitive to calculated electron transport (J) and the chosen value of Γ^*.

The method proposed by Moualeu-Ngangue *et al.* (2017) takes advantage of an A–c_i curve fitting where fluorescence and gas exchange measurements are made in parallel. Here, the input parameters are A, Φ, incident light and c_i, while R_{light} is a fitted parameter, as well as V_{cmax}, J and photosystem photon absorption parameters combined in a unique variable, denoted as τ. This study is very helpful because it clearly demonstrates that while estimating R_{light} is technically possible using photosynthetic response curves, there are many instances where fitted R_{light} values equal zero. In addition, R_{light} values are generally lower than that those found using fitting methods that assume a constant g_m. In other words, the persisting problem of mathematical interdependency between R_{light} and c_c (and thus g_m) mentioned above (Eqn 1) cannot be easily overcome: alternative techniques independent of J or c_c are required to estimate R_{light}, like the isotope disequilibrium technique proposed by Gong *et al.* (2018).

A further question that needs to be considered is what effect variations in R_{light} have on calculated photosynthetic parameters? This question is of importance because photosynthetic capacity is essential for photosynthetic modelling (when scaling up from leaves to whole ecosystems) and perhaps, calculated photosynthetic parameters are not very sensitive to R_{light} values. A comprehensive analysis of internal conductance showed that across plant lineages, g_m estimates are not very sensitive to the chosen value of R_{light} between 0.5 and 1.5 µmol m^{-2} s^{-1}, with variations in R_{light} leading to less than 10% changes in g_m (Xiong & Flexas, 2020). By contrast, estimating V_{cmax} appears to be very sensitive to the chosen R_{light} value, particularly when using the one-point A_{sat} method (De Kauwe *et al.*, 2016). Because of this, there is a much better agreement between V_{cmax} values calculated from A–c_i curves and A_{sat}, when observed values of R_{light} are used instead of assumed values (e.g. $R_{light} = 1.5\%$ of V_{cmax}) (De Kauwe *et al.*, 2016).

Day respiratory metabolism

A critical question is the origin of CO_2 produced by day respiration, not only because it may impact on the $^{12}C/^{13}C$ natural isotope composition but also because it reflects metabolic pathways mobilized by respiratory metabolism and the use of stored metabolites (reserves). In fact, it is important to remember that many pathways can generate CO_2 such as plastidial and mitochondrial pyruvate dehydrogenase, the TCAP, the γ-aminobutyrate (GABA) shunt, the malic enzyme or cytosolic pentose phosphates (reviewed in Tcherkez *et al.*, 2017b). Articles in this Virtual Issue provide very useful information on the origin of CO_2 generated by day respiration.

Gauthier *et al.* (2020) show that under low irradiance, the carbon flux through malic enzyme contributes to R_{light}. Under moderate irradiance, ^{13}C labelling and NMR analyses show that the TCAP makes a rather small contribution to day respiratory CO_2 efflux and is linked to N assimilation via glutamate synthesis (Abadie *et al.*, 2017), while the contribution of pyruvate dehydrogenase prevails (Lothier *et al.*, 2019), as found previously (Tcherkez *et al.*, 2008). The relatively low contribution of the TCAP to CO_2 evolution is not only linked to the capture of TCAP intermediates to feed N assimilation but also related to the down-regulation of TCAP enzyme activity in the light (Tcherkez *et al.*, 2012). This effect is nicely illustrated in Ahmad Rashid *et al.* (2020) where metabolomics were used to show the opposite behavior of citrate plus aconitate (which is more abundant at night) and other TCAP intermediates (more abundant in the light). Also, in mitochondrial mutants affected in Complex I activity, rates of day respiration are higher, due to pyruvate dehydrogenase activity being higher while anaplerotic bicarbonate fixation by phospho*enol*pyruvate carboxylase (PEPc) is lower (Lothier *et al.*, 2019). This raises the question of the influence of PEPc on observed rates of R_{light}. That is, R_{light} can be viewed as a net efflux resulting from CO_2 production by catabolism, refixation (accounted for in calculations reviewed above) and PEPc activity. Direct assessment of PEPc fixation by labelling shows that the flux is moderate under ordinary conditions but can reach 1 µmol m^{-2} s^{-1} or more at high photorespiration (Abadie & Tcherkez, 2019). Conversely, the bicarbonate leaf pool could reform CO_2 by acid-base equilibration and this could contribute to CO_2 efflux. Feeding cut poplar (*Populus deltoides*) leaves with ^{13}C-bicarbonate shows that the contribution of bicarbonate to R_{light} is very small, only up to 20% when bicarbonate is fed with concentrations as high as 11 mM (Stutz & Hanson, 2019).

Processes in which respiration plays a role were summarized and hierarchized in 'core principles' by O'Leary *et al.* (2019). In brief, these principles relate to: (1) metabolic functions (ATP and NADH energy generation, carbon skeleton synthesis, redox balancing); (2) metabolic flexibility (alternative routes); (3) regulation with supply (sugars, intracellular ADP regeneration) and demand (such as ATP utilization); (4) effects of plant life cycle (such as carbon investment in growth at different developmental stages); and (5) its roles in acclimation to environmental parameters such as temperature, or tolerance to salinity (Munns *et al.*, 2020). These five principles are relevant to day respiration (as they are to dark respiration). Here, special emphasis can be given to aspect (1) because in leaves, nutrient assimilation takes place in the light and thus the generation of carbon skeletons needed by N and S assimilation is essential (electron utilization for N and S assimilation is further discussed in Tcherkez & Limami, 2019). Mitochondrial metabolism is also important for redox homeostasis since excess redox power and photorespiratory-derived NADH can be consumed by the mitochondria via NADH re-oxidation through the respiratory electron chain (via the alternative and cytochrome pathways). In Dahal *et al.* (2015) and Dahal & Vanlerberghe (2017), R_{light} was estimated using the Kok method during progressive drought in either wild-type plants or alternative oxidase (AOX) knock-down mutants or over-expressors. Interestingly, R_{light} was found to decrease under drought but this decline is not observed in over-expressors. In addition, there was a negative relationship between electron

pressure on PSII (represented by $1 - q_p$ where q_p is photochemical quenching) and R_{light}. While the link between these results and the regulation of catabolism (decarboxylations) remains to be elucidated, these studies suggest that there is a beneficial effect of the electron flow via AOX to sustain PSII function, i.e. mitigation of electron pressure in the chloroplast. Similarly, at high irradiance, the engagement of the AOX (measured with $^{16}O/^{18}O$ respiratory discrimination in the dark) seems to be beneficial for PSII photochemistry across four species (Florez-Sarasa *et al.*, 2016).

How does day respiration respond to environmental constraints?

There is relatively little practical data on how day respiration varies with environmental conditions. In the recent past, the relationship between dark or day respiration, temperature and CO_2 has been examined (e.g. Ayub *et al.*, 2014) and surveys across different biomes have been carried out (e.g. Heskel *et al.*, 2016). However, the response of R_{light} to temperature, growth CO_2 mole fraction and nutrients is not very well known, and furthermore, it is possible that the response to such environmental parameters varies when different techniques are used to measure R_{light}.

A number of studies on R_{dark} are included in this Virtual Issue since they provide information on how R_{light} may respond to environmental parameters. For instance, V_{cmax} calculated from A_{sat} at different temperatures requires the knowledge of R_{light}; in cases were R_{light} estimates are not available, one can use R_{dark} as a surrogate. Possible uncertainties in doing so are discussed in Atkin *et al.* (2015). The response of photosynthesis and R_{dark} to key parameters involved in climate change (temperature and CO_2) is thoroughly reviewed in Dusenge *et al.* (2019). The impact of temperature was also explored in Aspinwall *et al.* (2016) where daily average air temperature and prevailing leaf temperature were found to have contrasting effects on R_{dark}. In addition, Aspinwall *et al.* (2016) provides the surprising result that the $R_{dark} : A_{sat}$ ratio (at prevailing leaf temperature in each growth treatment) was negatively related to total nonstructural carbohydrates, suggesting that carbon partitioning to respiration changed as A increased. It is worth noting that in the short-term (i.e. minutes to hours), it has also been shown with isotopic labelling that the carbon allocation to TCAP intermediates in illuminated leaves is not proportional to the rate of assimilation, with allocation to the TCAP being lower under conditions that increase assimilation rates (i.e. conditions that lower photorespiration) (Abadie *et al.*, 2018).

This Virtual Issue also comprises highly valuable articles that directly address day respiration and include measurements of R_{light} under different environmental conditions. Way *et al.* (2019) looked at the response of day respiration to temperature in forest red gum (*Eucalyptus tereticornis*) both in the short-term (via rapid changes in leaf measurement temperature) and in the long-term (via a comparison of plants grown at different temperatures). Due to acclimation, there was little effect of growth temperature on R_{light} (and R_{dark}) taking place at the prevailing temperature of each environment, regardless of whether the Laisk or the Kok method was used. There was also little effect of growth temperature on R_{light} calculated from A–c_i curve fitting (Kumarathunge *et al.*, 2019). In

contrast to earlier studies pointing to leaf respiration being less temperature sensitive in the light than in darkness (Atkin *et al.*, 2005, and references therein), recent work reported in this Virtual Issue found that R_{light} was more sensitive than R_{dark} to measured temperature, with higher Q_{10} values. Interestingly, R_{light} obtained with the Laisk method was sometimes negative (net CO_2 fixation), perhaps showing the prevalence of PEPc fixation (mentioned above) over catabolic decarboxylation at low temperature (Way *et al.*, 2019). This quite complicated effect of temperature is important to keep in mind, particularly when attempting to predict how rates of nonphotorespiratory CO_2 release in the light respond to short- and long-term changes in temperature. When the temperature varies, R_{light} is the result of both changes in the degree of inhibition of respiration by light (i.e. the extent to which R_{light} differs from R_{dark}) and thermal acclimation, and thus both phenomena may affect computations of plant, ecosystem or global CO_2 net exchange (Heskel *et al.*, 2013; Vanderwel *et al.*, 2015). The effect of neglecting the inhibition of respiration by light has been examined precisely using whole canopy CO_2 exchange in forest gum trees by Drake *et al.* (2016). They found that while autotrophic respiration (R_a) is visibly lower (on average by 21%) when light inhibition is accounted for, gross primary production (GPP) and the $R_a : GPP$ ratio are only impacted by a few percent (and the dependence on temperature is also minimally affected).

In addition to temperature, nutrient availability is also a fundamental parameter that dictates respiration rate, and this not only includes N but also phosphorus (P), sulphur (S) or potassium (Tcherkez, 2017). Using a survey across many species of different life forms grown under contrasted N/P conditions, Crous *et al.* (2017) demonstrated the relationship between R_{light} (measured with the Kok method and expressed per leaf surface area) and N and P elemental content (per leaf surface area). It is worth noting that R_{dark} has also been found to be related to N and P content (per leaf surface area) in a survey of tropical trees, also included in this Virtual Issue (Rowland *et al.*, 2017). The relationship between R_{light} and N and P seems to be specific to these nutrients since there is no correlation between R_{light} and leaf mass per surface area (Crous *et al.*, 2017), unlike R_{dark} (Rowland *et al.*, 2017). Interestingly, the dataset of Crous *et al.* (2017) did not reveal a close relationship between R_{light} and R_{dark}, with $R_{light} < R_{dark}$ in 84% of cases and $R_{light} > R_{dark}$ in 16% of cases. When the dataset is restricted to measurements where $R_{light} < R_{dark}$, the average $R_{light} : R_{dark}$ ratio is about 0.64 (i.e. 36% inhibition) which falls within the range of values commonly observed. Furthermore, the rather unusual situation where $R_{light} > R_{dark}$ is associated with significantly larger A_{max} (per unit of mass), lower N elemental content (per leaf surface area) and concerns more herbs than woody species. Collectively, these observations suggest a possible link between nitrogen use efficiency (and/or C : N ratio) and day respiration – a topic that requires further work in the future. In addition, Clemente-Moreno *et al.* (2020) present an interesting correlation analysis between mitochondrial electron chain capacity via the cytochrome pathway (assayed in the dark using $^{16}O/^{18}O$ fractionation) and some metabolites (quantified in leaves sampled in the light), using Antarctic pearlwort (*Colobathus quitensis*) and a temperate counterpart of the same family, rainbow pink (*Dianthus chinensis*). That

study suggests a possible relationship between mitochondrial electron transfer capacity and sulphur (S) assimilation, which is in turn beneficial to antioxidant metabolism (glutathione synthesis) and thus growth at low temperature.

Conclusion: day respiration is an essential parameter of leaf photosynthesis

Taken as a whole, papers of this Virtual Issue show the critical importance of day respiration for leaf photosynthesis, since it interacts with quantum yield, correlates to N and S elemental content and/or assimilation, and participates in tolerance to oxidative stress. Of course, measuring R_{light} remains challenging and most methods have their drawbacks. Importantly, the papers presented in this Virtual Issue have helped in identifying and addressing such problems. Moreover, this Virtual Issue combines papers that deal with modelling, biochemistry and physiological experiments. Such a combination reflects the fact that: (1) coupling different approaches (trans-disciplinarity) is critical to the provision of significant advances; and (2) classical physiology such as gas exchange remains essential and must not be overlooked despite the increasing role played by next-generation omics in plant science.

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