Increasingly, photosynthetic electron transport rate is being calculated from chlorophyll fluorescence measurements. The fluorescence signal is a complex mixture of contributions from different depths within the mesophyll. One condition required for electron transport calculated from fluorescence to represent the rate accurately is that the ratio of photosynthetic capacity to light absorbed be constant throughout the leaf. In order to explore the fluorescence properties of leaves where this assumption is not true, a new approximation for \( \phi_{PSII} \) is used to generate \( F_m' \) and \( F_s \) values throughout the leaf. \( F_s \) is assumed to be proportional to the amount of light absorbed from the fluorescence measuring beam and constant, i.e. independent of the actinic irradiance or \( CO_2 \) concentration. This assumption is validated by measurements from Eucalyptus maculata, Flaveria bidentis and Triticum aestivum, with two different types of fluorometer, where irradiance or \( CO_2 \) response curves were measured with normal or inverted leaf orientations. The new approach enables fluorescence values to be generated at each layer in a multilayer model. Two applications using this approach are presented. First, the model is used to show that when quantum yield varies through a leaf, then fluorescence will lead to an incorrect estimate of electron transport rate. Secondly, since chlorophyll fluorescence is also used to calculate the \( CO_2 \) concentration at the sites of carboxylation within chloroplasts, \( C_c \), the model is also used to show that \( C_c \) may vary with depth. Significant variation in \( C_c \) through the mesophyll could lead to an apparent dependence of internal conductance on irradiance or \( CO_2 \).

**Keywords:** Chloroplast • Internal conductance • Leaf anatomy • Light profiles • Mesophyll conductance • Rubisco.

**Introduction**

Chlorophyll fluorescence is a powerful and versatile technique. The information gained from fluorescence depends on how the light is applied and how the fluorescence is captured. One of the most common applications is to use fluorescence signals to calculate the photosynthetic electron transport rate. This requires two fluorescence parameters to be measured, the first under actinic light and the second during a pulse of bright light that closes PSII. To calculate the electron transport rate, values for the fraction of incident light that is absorbed by the leaf, \( \alpha \), and the proportion of that light absorbed by PSII, \( \beta \), are needed. The two fluorescence parameters are used to calculate the photochemical efficiency of PSII, \( \phi_{PSII} \), which has been shown to be linearly related to quantum yields measured by gas exchange (Genty et al. 1989). By imaging fluorescence, it is possible to resolve photochemical efficiency spatially (Genty and Meyer 1995), even to the level of individual chloroplasts (Lawson et al. 2002). However, resolving the profile of photochemical efficiency through leaves has yet to be achieved. Fluorescence has also been used to measure the profile of light absorption through leaves (Takahashi et al. 1994, Koizumi et al. 1998, Vogelmann and Han, 2000, Vogelmann and Evans, 2002) and the distribution of chlorophyll through leaves (Vogelmann and Evans 2002, Evans and Vogelmann 2006).

At room temperature, chlorophyll fluorescence predominantly originates from PSII. In \( C_4 \) leaves, the contribution from PSI may be sufficiently great that it cannot be ignored (Siebke et al. 1997, Pfundel 1998, Kramer et al. 2004). At room temperature, chlorophyll fluorescence from a leaf has emission peaks at 686 and 740 nm. Fluorescence > 715 nm is usually measured, first to separate it from actinic light and, secondly, because chlorophyll absorbs strongly at 680 nm,
fluorescence <700 nm will mainly consist of signal from chloroplasts near the illuminated surface rather than properly representing the complete mesophyll. Leaf absorptance declines rapidly from about 0.92 at 680 nm to 0.52 at 710 nm and to 0.14 at 735 nm. Thus, at wavelengths > 715 nm, the fluorescence emerging from a leaf originates not only from near the surface but also from deep within the mesophyll. The actual profile of fluorescence emission through a leaf depends on the wavelength used to probe fluorescence. As this may differ from the spectral quality of the actinic light, there is potential for a mismatch to occur between the absorption profile of the actinic and chlorophyll fluorescence measuring lights.

The fluorescence that emerges from a leaf is the cumulative sum of the contributions from chloroplasts scattered throughout the mesophyll. It is known that the properties of chloroplasts differ, depending on their light environment. Chloroplasts near to the surface receiving light have greater Chl a/b ratios, and greater cytochrome f and Rubisco contents per unit chlorophyll (Terashima and Inoue 1985b). The diversity in chloroplast properties has the potential to result in a complex fluorescence signal that does not simply relate to whole-leaf photosynthesis. In fact, the strong linear relationships between photochemical efficiency and quantum yield can only occur if the ratio of photosynthetic capacity to light absorbed is the same for all chloroplasts in a leaf.

Another application of chlorophyll fluorescence is for estimating the CO₂ partial pressure at the sites of carboxylation within chloroplasts (Harley et al. 1992). This leads to the estimation of internal conductance which is defined as

\[ g_s = A / (C_i – C_c) \]

where \( A \) is the rate of CO₂ assimilation, and \( C_i \) and \( C_c \) are the CO₂ concentrations in the intercellular airspaces and at the sites of carboxylation, respectively. Internal conductance calculated from fluorescence was found to decline for a range of species as \( C_i \) increased (Flexas et al. 2007) or as irradiance decreased (Flexas et al. 2007, Hassiotou et al. 2008). Internal conductance calculated from a combination of carbon isotope discrimination and gas exchange also declined with increasing \( C_i \) in Nicotiana tabacum (Flexas et al. 2007). However, using the isotope discrimination method, internal conductance was found to be independent of both \( C_i \) and irradiance in Triticum aestivum (Tazoe et al. 2009). The difference between the true and observed internal conductance depends on the profiles of light absorption, photosynthetic capacity and internal conductance (Lloyd et al. 1992). Therefore, since the fluorescence and isotopic methods weight the sampling through the mesophyll differently, this could possibly explain the difference between these reports.

The objective of this study is to use a multilayer model of photosynthesis to examine the fluorescence properties that emerge for a whole leaf. This model developed from the approach of Terashima and Saeki (1985) and has been parameterized for spinach leaves (Evans and Vogelmann 2003) with profiles of light absorption and photosynthetic capacity, \( P_{max} \). However, to calculate whole-leaf photochemical efficiency in the absence of data at the chloroplast level, a new approximation is first introduced and validated. The impact of the interaction between the profiles of light absorption and \( P_{max} \) is demonstrated by measuring normal and inverted leaves.

**Results**

Despite the absence of data resolving fluorescence at the intraleaf level, it is possible to simulate the emergent properties of a leaf by making some reasonable assumptions. The first assumption is that as photochemical efficiency varies due to changing irradiance or CO₂ concentration, this is mainly due to changes in \( F_{m,j} \). In contrast, \( F_{s} \) stays fairly constant (Fig. 1). This is evident for a range of species, both C₃ and C₄. It is also independent of leaf orientation, i.e. whether light is applied to and fluorescence measured from the adaxial surface or whether the leaf is inverted during the measurement (Fig. 1A, C). When the CO₂ concentration is altered, the changes are also evident in \( F_{m,j} \) rather than \( F_{s,j} \) (Fig. 1E), although the changes are smaller because CO₂ varied the rate of electron transport less than irradiance. Consequently, if one calculates photochemical efficiency as \( \phi' = 1 – c/F_{m,j} \) where \( c \) is a constant for a given leaf and orientation, then \( \phi' \) is a good approximation to \( \phi_{PSII} \) (Fig. 1B, D, F).

The conventional use of \( \phi_{PSII} \) relies on the assumption that the ratio of \( P_{max} \) to light absorption is independent of depth from the leaf surface. This assumption breaks down if the color of light is changed or the leaf is inverted. Gas exchange was measured concurrently with fluorescence for two of the examples shown in Fig. 1. The rate of electron transport calculated from \( \phi_{PSII} (J_{s,j}) \) or \( \phi' (J_{s,j}) \) is compared with the rate of CO₂ assimilation (A) (Fig. 2). For Flaveria measured in the correct orientation, \( J_s \) was linearly related to \( A \). The slope of the regression was 6.3 mol e⁻ /mol CO₂⁻¹. The rate of electron transport calculated assuming a constant \( F_{s,j} \) followed \( J_s \) closely except for the two highest irradiances. In contrast, both \( J_{s,j} \) and \( J_{s,j} \) were overestimated for the inverted leaf. However, \( J_{s,j} \) was remarkably similar to \( J_i \) over the entire range of irradiance for the inverted leaf. For Triticum measured under varying CO₂ concentrations, \( J_{s,j} \) followed \( J_{s,j} \) but the agreement was not as close as that observed when irradiance varied. These examples demonstrate that, to a first approximation, it is reasonable to assume a constant \( F_{s,j} \) that is independent of \( \phi_{PSII} \). Consequently, Equations 5 and 6 in the Materials and Methods can be used to generate values for \( F_{s,j} \) and \( F_{m,j} \) for each layer in the model leaf.

The multilayer model based on spinach was used to explore the fluorescence properties of the leaf. The absorption

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profiles for blue and green light incident on a leaf are shown for both normal (adaxial) and inverted (abaxial) leaf orientations (Fig. 3). Due to the large extinction coefficient of the pigments in a leaf at around 450 nm, blue light is strongly absorbed near the surface. For a normally oriented leaf, 70% of blue light is absorbed in the first third of the mesophyll. Since green light is absorbed less strongly, it penetrates deeper into the mesophyll and only 42% of the green light is absorbed in the first third of the mesophyll. The observed profile of $P_{\text{max}}$ is similar to that for green light absorption except that it increases again slightly near the abaxial surface. For the simulations presented here, fluorescence parameters at each layer were calculated using the same wavelengths for both the actinic and fluorescence measuring light. The fluorescence values summed from each layer were used to calculate $\Phi_{\text{PSII}}$, and then the electron transport rate, $J_{\Phi}$. This rate was compared with the ‘true’ rate of electron transport, $J_{\text{M}}$, for different wavelengths of light and leaf orientations (Fig. 4). There was close agreement between $J_{\text{M}}$ and $J_{\Phi}$ when the leaf was measured under green light with
the correct orientation. This was expected because this condition almost satisfies the assumption that the ratio of $P_{\text{max}}$ to light absorbed is the same for each layer. However, for the other simulations, the two rates deviate progressively from each other as irradiance increases. When blue light applied to the adaxial surface, or green light to the abaxial surface, was modeled, the electron transport rate calculated from chlorophyll fluorescence overestimated the ‘true’ rate $J_M$ by 60% at high irradiance. The modeled response clearly resembled that observed for an inverted leaf (Fig. 2A), which again supports the assumption that $F_s = c I$ (Equation 5). The discrepancy between $J_M$ and $J_{\phi}$ was even greater when blue light was applied to the abaxial surface (Fig. 4).

Throughout this paper, it is assumed that the proportion of light absorbed by PSII, $\beta$, is 0.5, regardless of the color of the actinic or measuring light (which are the same in the
simulations presented here) and regardless of the depth in the leaf. It is conceivable that $\beta$ could vary with depth, although attempts to calculate this suggest that the difference between sun and shade chloroplasts is small (Evans 1988). The color of light, however, could be expected to alter $\beta$ as this has been put forward as one explanation of the response of quantum yield to wavelength (Evans 1987). The multilayer model could be used to investigate these assumptions, as any changes in $\beta$ would apply to both electron transport and fluorescence via Equation 1.

A sense of the underlying optical issue can be gained from the profiles of quantum yield through the leaf. When blue light applied to the adaxial surface is modeled, quantum yield is reduced close to the adaxial surface, but is relatively unchanged deep within the mesophyll (Fig. 5A). As irradiance increases, quantum yields are progressively reduced deeper into the mesophyll. However, even at high blue irradiance, quantum yield varies almost linearly with depth. In contrast, when green light applied to the adaxial surface is modeled, there is an almost uniform reduction in quantum yield that is virtually independent of depth (Fig. 5C). Consequently, the fluorescence signal, which is the sum of contributions from a range of depths, depends strongly on the relationship between the profiles of light absorption and $P_{\text{max}}$ through the mesophyll. Although the profile of internal conductance through a leaf is unknown, if one assumes that it scales with $P_{\text{max}}$, then the profile of the relative drawdown from $C_i$ to $C_c$ can be calculated (Fig. 5B, D). The profiles resemble that for quantum yield, but are not the same because they also reflect the profile of $P_{\text{max}}$. When blue light applied to the

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**Fig. 5** Modeled profiles of quantum yield (A, C) or relative drawdown, $C_i - C_c$ (B, D) through a leaf under blue (A, B) or green (C, D) light applied to the adaxial surface, using the profiles of light absorption and $P_{\text{max}}$ given in Fig. 3.
adaxial surface is modeled, relative $C_i - C_c$ is greatest near the adaxial surface and decreases almost to zero at the abaxial surface. However, when green light applied to the adaxial surface is modeled, relative $C_i - C_c$ is almost independent of depth. At the leaf level, variation in $C_i - C_c$ with depth would result in an apparent increase in internal conductance with increasing irradiance.

**Discussion**

It is remarkable that measurement of chlorophyll fluorescence from a leaf can give quantitatively accurate values of photochemical efficiency without any need for calibration. This occurs because $\phi_{PSII}$ is calculated from the ratio of two fluorescence parameters, which makes it independent of the absolute value for incident irradiance, leaf absorbance and photon distribution between PSII and PSI. Of course, to validate this claim, it is necessary to compare $\phi_{PSII}$ with an independent estimate, usually obtained from gas exchange. The comparison then needs accurate knowledge of the light parameters and either the CO₂ assimilation or oxygen evolution rates. At the time $\phi_{PSII}$ was proposed, a value for $\phi_{PSII}/\phi_{CO_2}$ of 15 was measured on maize leaves (Genty et al. 1989) which converts to 6.4 mol e⁻ (mol CO₂)⁻¹, assuming values for $\alpha$ and $\beta$ of 0.85 and 0.5, respectively. Similar values for $J/A$ have been reported for *Flaveria bidentis*, 6.5 (Siebke et al. 1997) and 6.7 (Dwyer et al. 2007), and the value obtained here [6.3 ± 0.2 mol e⁻ (mol CO₂)⁻¹, Fig. 2A] agrees with these values.

Concealed within the fluorescence measurement is the requirement that the ratio of $P_{max}$ to light absorbed is the same for all chloroplasts within the leaf. Because this condition appears to be met under many conditions, it tends to be forgotten. Alternatively, it is possible to correct for the error by calibrating $F_i$ against CO₂ assimilation measured under non-photorespiratory conditions. It is easy to demonstrate what happens when the ratio of $P_{max}$ to light absorbed varies by comparing measurements from a normal and inverted leaf (Fig. 2A), or when fluorescence is measured with a blue light and white light is used for the actinic source. Unraveling the basis for this from the deceptively simple fluorescence parameters is not trivial. The major hurdle is the scarcity of data on the profiles of light absorption and chlorophyll content inferred from multiple chlorophyll fluorescence images suggested that light absorption was influenced by both the extinction coefficient and path lengthening (Vogelmann and Evans 2002). This had previously been shown for *C. japonica* leaves that were paradermally sectioned (Terashima and Saeki 1983). However, some leaves are optically complex due to the presence of oil glands, such as in *Eucalyptus pauciflora* (Evans and Vogelmann 2006), or the presence of Kranz anatomy (Evans et al. 2007) or bundle sheath extensions (Karabourniotis et al. 2000). For these leaves, obtaining the profile of light absorption is more difficult.

The light absorption profiles used here were derived from spinach leaves (Vogelmann and Evans 2002, Evans and Vogelmann 2003). Blue and green light were analyzed as these represent the two extremes. Red or white light would be expected to be intermediate between them (Vogelmann and Han 2000, Vogelmann and Evans 2002). To model a leaf fully, the absorption profile of both the actinic and fluorescence measuring light need to be known. The wavelength of modulated light used to measure chlorophyll fluorescence differs between instruments.

**Profile of $P_{max}$**

Biochemical analysis of paradermal sections from palisade or spongy mesophyll demonstrated that chloroplasts acclimate to their local light environment (Terashima and Inoue 1985a, Terashima and Inoue 1985b). Coordinated changes to thylakoid components (cytochrome $f$, coupling factor), electron transport capacities and Rubisco content were found. Subsequently, detailed profiles of Rubisco through spinach leaves have been measured (Nishio et al. 1993) or inferred from $^{13}C$ labeling (Evans and Vogelmann 2003). Since the profiles for electron transport capacity, $J_{max}$ and Rubisco activity, $V_{max}$ are likely to be similar, the term $P_{max}$ has been used here rather than $J_{max}$ or $V_{max}$ as used in the $C3$ photosynthesis model (Farquhar et al. 1980, von Caemmerer 2000). A profile for $P_{max}$ was also derived for isobilateral *E. pauciflora* leaves (Evans and Vogelmann 2006). With only two profiles of $P_{max}$ available, there is a clear need for more work to be done. To some extent, the lack of data is a reflection of the difficulty in making the measurements. An alternative approach is to use chlorophyll fluorescence. Potentially this offers a simpler method with excellent spatial resolution.
At present we are limited by the ability to capture the weak fluorescence using realistic irradiances and exposure times of <1 s. However, improved sensitivity of CCD cameras should enable these measurements in the future such that \( P_{\text{max}} \) could be derived from irradiance response curves of \( \phi_{\text{PSII}} \). In this case, \( F_{i,j} \) would be used to measure \( I_j \) which would enable Equation 2 to be fitted to \( \phi_{\text{PSII}} \) collected at different irradiances.

**Implications for internal conductance**

At the leaf level, internal conductance is closely related to the surface area of chloroplasts exposed to intercellular airspace per unit leaf area (Evans et al. 1994). A rough assessment of how chloroplast surface area varied with depth was made for \( N.\ tabacum \) and revealed a 2-fold variation that peaked deep within the palisade layer and that was relatively similar to the profile of Rubisco through a spinach leaf (Evans 1998). An alternative to analyzing transverse sections is to use oblique paradermal sections. James et al. (1999) applied this method to \( Eucalyptus\ globulus \) leaves to compare the dorsiventral juvenile leaves with the isobilateral adult leaves. However, at present, there is no species for which profiles of both Rubisco and mesophyll surface area are known. Therefore, in order to model profiles of \( CO_2 \) drawdown, it was necessary to make the assumption that the ratio of Rubisco to exposed chloroplast surface area was constant and independent of depth. This assumption could be checked by measuring the profile of chloroplast thickness since chloroplast volume is closely correlated with Rubisco content at the leaf level. A constant ratio of Rubisco to chloroplast surface area should mean that chloroplast thickness is independent of depth, which could be assessed using confocal microscopy.

The assumption that exposed chloroplast surface area and Rubisco co-vari through the leaf means that the internal conductance for a layer is directly proportional to \( P_{\text{max}} \) for that layer. Consequently, the drawdown \( C_i - C_c \) will scale with \( J/P_{\text{max}}' \). The drawdown profiles differed greatly in green vs. blue light (Fig. 5B, D). When the profile of light absorption matched the profile of \( P_{\text{max}} \) as in green light, then \( C_i - C_c \) was nearly independent of depth. Under blue light where the two profiles differed considerably, the drawdown \( C_i - C_c \) decreased dramatically through the leaf. The value for internal conductance in this case would depend on the weighting placed on each layer. The calculation of internal conductance would be in error for the same reason that the calculated rate of electron transport was overestimated (Fig. 4). With the number of assumptions that have been made, it is not yet feasible to quantify the effect. However, the analysis indicates that internal conductance would appear to increase as irradiance increased in the blue light scenario. This is because as blue irradiance increases, it penetrates deeper into the mesophyll and additional chloroplasts are progressively engaged and contribute their internal conductance. In contrast, in green light, the balance between the contributions from various chloroplasts is independent of irradiance.

The current model is driven by profiles in \( P_{\text{max}} \) and absorbed irradiance. However, as \( CO_2 \) concentrations are reduced, at some point the limitation changes from electron transport to Rubisco. Since the drawdown \( C_i - C_c \) depends on the rate of \( CO_2 \) assimilation, an iterative solution would need to be used to solve the model. It is evident that under blue light, electron transport could be constrained by low \( CO_2 \) to a greater extent in the palisade compared with the spongy tissue. In turn, this would impact on the \( F_m' \) values which would alter \( \phi_{\text{PSII}} \), and hence the estimate of \( C_c \). Therefore, it is conceivable that internal conductance calculated from \( \phi_{\text{PSII}} \) could depend on \( C_c \). In this case, \( \phi_{\text{PSII}} \), would be influenced by the mismatch between the profiles of light absorption and \( P_{\text{max}}' \) and additionally by the further reduction in electron transport rate by low \( CO_2 \) concentrations. The finding that internal conductance calculated from \( \phi_{\text{PSII}} \) decreases as \( C_i \) increases (Flexas et al. 2007) is appealing because of the growing evidence supporting the role of cooipors in determining membrane permeability to \( CO_2 \) (Terashima et al. 2006, Uehlein et al. 2008). However, there is conflicting evidence on whether internal conductance decreases with increasing \( C_c \) when internal conductance is calculated from carbon isotope discrimination (Flexas et al. 2007, Tazoe et al. 2009). Considering that fluorescence signals correctly represent the complex sum of many sources only when the ratio of \( P_{\text{max}} \) to absorbed light is constant throughout the leaf, it seems possible that the variation in internal conductance calculated from \( \phi_{\text{PSII}} \) as either irradiance or \( CO_2 \) varies could be an artifact.

**Materials and Methods**

Chlorophyll fluorescence was measured with two PAM 101 fluorometers (H Walz, Effeltrich, Germany) positioned on opposite sides of a bifacial \( Eucalyptus\ maculata \) leaf (Ögren and Evans 1993). For the other species, combined fluorescence and gas exchange measurements were made with a 6400-40 LCF (LICOR, Lincoln, NE, USA). Both of these instruments use modulated red light to measure fluorescence. For the LCF, \( F_m' \) was calculated using the multiple intensity flash routine, and the actinic light was 90% red and 10% blue. With \( F.\ bidentis \), an NADP malic enzyme \( C_4 \) dicot, irradiance response curves were measured in 400 \( \mu \text{mol} \ \text{mol}^{-1} \text{ambient} \ CO_2 \) with the leaf in the normal orientation and then repeated with the leaf inverted, such that the actinic and fluorescence measuring lights were applied to and fluorescence measured from the abaxial surface. For \( T.\ aestivum \), the responses of fluorescence and gas exchange to \( C_i \) were measured by varying ambient \( CO_2 \) concentration, with an irradiance of 1,500 \( \mu \text{mol} \ \text{PAR quanta} \ m^{-2} s^{-1} \). In all cases,
the air contained 21% oxygen and leaf temperature was 23±1°C.

The multilayer model that has been parameterized for spinach (Evans and Vogelmann 2003) was used to calculate apparent photochemical efficiency. The model was also used to explore the profile of quantum yield and drawdown between Cᵢ and Cₑ. The model consists of 17 paradermal layers, each 40 μm thick, for which the fraction of light absorbed, aᵢ, and Pᵢ max for each layer is defined. The light absorbed by PSII in layer j, Iᵢ, is calculated as

$$Iᵢ = Iα β aᵢ$$  \hspace{1cm} (1)

where I is the incident irradiance, α is the leaf absorbance, β is the fraction of light absorbed by PSII and aᵢ is the fraction of light absorbed in layer j. The rate of electron transport for layer j is calculated as

$$Jᵢ = \Bigl\{ Iᵢ + Pᵢ max,ᵢ \Bigl[ (Iᵢ + Pᵢ max,ᵢ) - 4 \theta (Iᵢ + Pᵢ max,ᵢ) \Bigr]^{0.5} \Bigr\}^{θ}$$  \hspace{1cm} (2)

where θ is assumed to be 0.85. The modeled rate of electron transport for the leaf, Jₑ, is the sum of Jᵢ for all of the layers. The photochemical efficiency of layer j, \( \phi \text{PSII}_j \), is therefore

$$\phi \text{PSII}_j = \frac{Jᵢ}{Iᵢ}$$  \hspace{1cm} (3)

The fluorescence intensity depends on the irradiance absorbed by the chloroplast and the photochemical efficiency. At the leaf level, \( \phi \text{PSII} \) is calculated from the fluorescence under actinic light, \( Fᵥ \), and that under a saturating pulse, \( Fᵢ' \) (Genty et al. 1989)

$$\phi \text{PSII} = 1 - Fᵢ' / Fᵢ'$$  \hspace{1cm} (4)

To construct fluorescence emission from a leaf, one therefore needs to know both \( Fᵢ' \) and \( Fᵢ'' \) at each layer. As this information is lacking, we need to calculate them. This is possible from \( \phi \text{PSII} \) and the fraction of light absorbed at each layer, if we assume as an approximation that

$$Fᵢ'' = Fᵢ' (1 - \phi \text{PSII})$$  \hspace{1cm} (5)

where c is a constant. Then \( Fᵢ'' \) for layer j, \( Fᵢ''ᵢ \), can be calculated from Equations 1, 4 and 5 as

$$Fᵢ''ᵢ = Iᵢ c (1 - \phi \text{PSII}_j)$$  \hspace{1cm} (6)

The apparent photochemical efficiency for the leaf, \( \phi \text{PSII} \), can then be calculated using the sum of the values for both \( Fᵢ' \) and \( Fᵢ'' \)

$$\phi \text{PSII} = \frac{\sum Iᵢ c}{\sum (Iᵢ c / (1 - \phi \text{PSII}_j))}$$  \hspace{1cm} (7)

The rate of electron transport for a leaf, \( Jₑ \), is normally calculated as

$$Jₑ = \phi \text{PSII} \cdot I α β$$  \hspace{1cm} (8)

so we define the apparent rate of electron transport, \( Jₑp \), calculated from the apparent photochemical efficiency as

$$Jₑp = \phi \text{PSII} \cdot I α β$$  \hspace{1cm} (9)

In this paper, it is assumed that fluorescence is not absorbed within the leaf because fluorescence was measured at wavelengths >715 nm.

To calculate the relative drawdown \( Cᵢ - Cₑ \) for each layer, it was assumed that the surface area of chloroplasts exposed to intercellular airspace per unit leaf area for each layer was proportional to \( Pᵢ max \) for that layer which means that \( gᵢ \) is proportional to \( Pᵢ max \) and

$$(Cᵢ - Cₑᵢ) = Jᵢ / Pᵢ max,ᵢ$$  \hspace{1cm} (10)

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References


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