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A portable reflectance-absorptance-transmittance meter for photosynthetic work on vascular plant leaves

R.J. RITCHIE[*,+] and J.W. RUNCIE[**,***]

Tropical Environmental Plant Biology Unit, Faculty of Technology and Environment, Prince of Songkla University – Phuket, 83120 Thailand[*]
School of Biological Sciences, University of Sydney, NSW 2006, Australia[**]
Aquation Pty Ltd, Umina Beach NSW, Australia[***]

Abstract

PAM (pulse amplitude modulation) fluorometers can be used to estimate the electron transport rate (ETR) [μmol(e^-) m^{-2} s^{-1}] from photosynthetic yield determinations, provided the absorptance (Abt_λ) of the photoorganism is known. The standard assumed value used for absorptance is 0.84 (leaf absorptance factor, Abt_F). We described a reflectance-absorptance-transmittance (RAT) meter for routine experimental measurements of the actual absorptance of leaves. The RAT uses a red-green-blue (RGB) LED diode light source to measure absorptances at wavelengths suitable for use with PAM fluorometers and infrared gas analysers. Results using the RAT were compared to Abt_λ spectra using a Taylor integrating sphere on bird's nest fern (*Asplenium nidus*), banana, *Doryanthes excelsa*, *Kalanchoe daigremontiana*, and sugarcane. Parallel venation had no significant effect upon Abt_{465} in banana, *Doryanthes*, a *Dendrobium* orchid, pineapple, and sugarcane, but there was a slight difference in the case of the fern *A. nidus*. The average Abt_{465} (≈ 0.96) and Abt_{625} (≈ 0.89) were ≈14% and 6% higher than the standard value (Abt_F = 0.84). The PAR-range $Abt_{400–700}$ was only ≈ 5% higher than the standard value (≈ 0.88) based on averaged absorptance from the blue, green, and red light data and from where the RGB-diode was used as a 'white' light source. In some species, absorptances at blue and red wavelengths are quite different (*e.g.* water lily). Reflectance measurements of leaves using the RAT would also be useful for remote sensing studies.

Additional key words: absorptance; electron transport rate; integrating sphere; leaf absorptance factor; PAM fluorometry; reflectance.

Introduction

Absorptance (Abt_λ) is defined as the amount of irradiance (I) absorbed by a translucent object at a specified wavelength (λ). We have designed a reflectance-absorptance-transmittance (RAT) meter to experimentally measure the absorptance of plants at blue (465 nm), green (525 nm), or red (625 nm) wavelengths. The RAT was designed to be a simple portable device for making relevant absorptance readings of leaves more generally useable than the cumbersome and often unavailable integrating sphere equipment for measuring absorptances. The design of the RAT device is based upon Schultz (1996). The RAT uses a RGB LED diode light source so that absorptances can be measured separately at blue (465 nm), green (525 nm), or red (625 nm) wavelengths or in a "white" light source with all three colour diodes activated. Absorptances can thus be measured for both the

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[+]Corresponding author; phone: +66 76 276 108, fax: +66 76 276 102, e-mail: raymond.r@phuket.psu.ac.th
Abbreviations: A_{PSII} – allocation factor of photons to PSII between PSII and PSI; Abt_λ – absorptance at a given wavelength or wavelength range, Abt_F – default leaf absorptance factor; CL – confidence limit; I – irradiance [μmol(photon) m^{-2} s^{-1}] PPFD; ETR – absolute electron transport rate; rETR – relative electron transport rate; PAM fluorometry – pulse amplitude modulation fluorometry; P_g – gross photosynthesis; R – reflectance; RAT – reflectance-absorptance-transmittance; T – transmittance.
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blue (465 nm) and red (625 nm) wavelengths, commonly used as light sources in PAM fluorometers and infrared gas analysers (IRGAs). Where the absorptance of white light is required (for some types of PAM fluorometers and many IRGAs), the absorptance in RBG diode "white" light can be measured or the absorptance estimated from absorptances measured in blue, green, and red wavelengths. In this study, comparisons were made to absorptance scans on material using a Taylor integrating sphere attached to a spectrophotometer. We showed that the RAT device is convenient for estimating absorptance in the laboratory and field.

Fluorescent techniques, such as those used by PAM and photosynthetic efficiency apparatus (PEA) fluorometers, are very useful for estimating photosynthesis of plants, offering the great advantages of speed of measurement and hence very large amounts of data can be collected in short periods of time. Adequate measurements of irradiance are essential for any serious photosynthetic work; however, PAM and PEA methods make particular demands on adequate measurements of irradiance because both measured and derived data are expressed in terms of photons. Essentially PAM and PEA fluorometers estimate photosynthesis in the same way: a beam of light is projected onto a photosynthetic material and chlorophyll fluorescence is measured. The proportion of photons absorbed by PSII and actually used for electron transport is calculated as the quantum yield of photochemical efficiency of PSII, which also goes by several different names including simply yield and the photochemical efficiency of PSII (Y, Y_{PSII}, or Φ_{PSII}; Genty *et al.* 1989, Krause and Weis 1991, Schreiber *et al.* 1995). In oxygenic photoorganisms, it is assumed that about one half of the photons absorbed are absorbed by PSII and one half by PSI and so the allocation factor for photons absorbed by PSII compared to total photons is 0.5 ($A_{PSII} = 0.5$) (Melis 1989). The proportion of photons from the light source that are absorbed by the photoorganism is termed the absorptance, the wavelength of which needs to be specified (Abt_λ). PAM fluorometers estimate photosynthesis as the ETR, [µmol(e^-) m^{-2} s^{-1}]. This is calculated as the product of photochemical efficiency of PSII (Y_{PSII}), an allocation factor to PSII compared to total photons absorbed by both photosystems (0.5), absorptance (Abt_λ), and irradiance (I) [µmol(photon) m^{-2} s^{-1}]: $ETR = Y_{PSII} \times 0.5 \times Abt_\lambda \times I$. Hence, knowing the amount of light absorbed by the photosynthetic surface (Abt_λ) is essential for calculating the photosynthetic rate in terms of ETR using a fluorescence-based estimate of photosynthesis. In the case of gas-exchange techniques (O_2 electrode and IRGA methods) and ^{14}C fixation, while it is useful to know how much incident light is absorbed by the plant, this information is not essential for the calculation of the photosynthetic rate. Nevertheless, absorptance measurements are needed to calculate actual, rather than apparent, photosynthetic efficiency where photosynthesis is measured as an oxygen or carbon flux (Cheng *et al.* 2000). Experimentally determined absorptances are useful for any kind of photosynthetic study.

Absorptance (Abt_λ) is usually expressed as a percentage Abt_λ [%] and should not be confused with absorbance (A or Abs), which is derived from transmittance and is based upon the Beer-Lambert law. Absorptance is usually expressed as a percentage. The standard default value (Abt_F) of 0.84 derived from a fluorescence study by Björkman and Demmig (1987) is an overall mean absorptance calculated from a large number of leaves of vascular plants (n = 44) determined using an Ulbricht-type integrating sphere attached to a spectrophotometer. Absorptances were measured at 25 nm intervals and the overall mean absorptance for the PAR range (400–700 nm) was calculated (Björkman and Demmig 1987). It is therefore a mean value for 400–700 nm (PAR) light. Their results are closely comparable to those made earlier by McCree (1972), and Abt_F = 0.84 is the default value incorporated into the software of *WALZ*® PAM machines. A default absorptance value of $Abt_F = 0.84$ might be valid to use as a standard value for devices using a white light source with a colour temperature of 6,000 K but it is not valid for devices using a blue or red light source. Estimates of ETR based on $Abt_F = 0.84$ are most properly designated relative ETR (rETR) to reflect the fact that they are based on an experimentally measured fluorescence yield (Y_{PSII}) and irradiance but not on an experimentally determined absorptance value (Abt_λ). Both studies noted considerable variation among species. An actual experimental measurement of absorptance in a particular experimental system is better than relying on a default standard value.

The usual method for measuring the amount of light absorbed by a plant is using a Taylor or Ulbricht integrating sphere (Gates *et al.* 1965, McCree 1972, Ehleringer *et al.* 1976, Ehleringer 1981, Lee and Graham 1986, Björkman and Demmig 1987, Lee *et al.* 1990, Schultz 1996, Delfine *et al.* 1999, Knapp and Carter 1998, Cheng *et al.* 2000, Carter and Knapp 2001,Valladares *et al.* 2002, Bauerle *et al.* 2004, Runcie and Durako 2004, Merzlyak *et al.* 2008, Gorton *et al.* 2010, Stemke and Santiago 2011, Davis and Hangarter 2012). Integrating spheres are usually attached to a benchtop spectrophotometer or spectroradiometer. Portable spectroradiometers equipped with an integrating sphere are more practical for field experiments. Schultz (1996) in his study of absorptance properties of grapevine leaves (*Vitis vinifera* L.) used a simple Ulbricht sphere of his own design that was useable in the field fitted with a *Li-Cor* quantum light detector (PPFD 400–700 nm).

Some broad generalisations can be made about the absorptance properties of vascular plants based upon reflectance-absorptance-transmittance scans using integrating spheres attached to spectrophotometers or spectroradiometers (Gates *et al.* 1965, McCree 1972, Gausman and Allen 1973, Lee and Graham 1986, Björkman and Demmig 1987, Lee *et al.* 1990, Knapp and Carter 1998, Carter and Knapp 2001, Merzlyak e*t al.* 2008, Gorton *et al.* 2010). Most plants have a broad flat region of high absorptance

from about 400 to 500 nm ($Abt_{400–500} \approx 0.95$); another region of high absorptance is in the red region near the *in vivo* absorption chlorophyll *a* peak at 680 nm ($Abt_{625–690} \approx 0.9 – 0.95$). In the green part of the spectrum (≈ 550 nm), absorptances typically are about 20% lower than in blue and red light ($Abt_{550} \approx 0.6–0.75$). In the far red (>700 nm) Abt_λ rapidly falls to near zero. Some authors, such as McCree (1972), Gausman and Allen (1973), and Björkman and Demmig (1987), felt it justifiable to calculate mean Abt_λ for each of a range of wavelengths based on many species of plants. Overall mean absorptance for the entire PAR range ($Abt_{400–700}$) is generally about 0.84–0.9 (Gates *et al.* 1965, McCree 1972, Björkman and Demmig 1987, Lee *et al.* 1990, Schultz 1996, Evans and Poorter 2001, Valladares *et al.* 2002). Surprisingly, there appears to be little if any systematic difference in $Abs_{400–700}$ based on rainforest sun and extreme shade plants [sun plants: 0.886 ± 0.023 (n = 12); shade plants: 0.902 ± 0.037 (n = 13)] (Lee and Graham 1986), but Valladares *et al.* (2002), concentrating on rainforest shade plants, found an average $Abt_{400–700}$ of about 0.868 ± 0.00825 (n = 24). The mean PAR light absorptance value ($Abt_{400–700}$) is generally lower than the absorptances based on blue or red light because the calculation includes low absorptance readings in the green part of the spectrum.

If there was little variation in absorptance between plants, as might be inferred from above, there would be little point in measuring absorptance routinely: a correctly chosen default value would suffice. Absorptance data on hand (*see* above) shows that with due caution a default value of about 0.95 for blue light and ≈ 0.9 for red light sources would be adequate for most purposes. These mean blue and red light absorptance values are considerably different to the $Abt_F = 0.84$ value currently in common use.

There are two groups of vascular plants where absorptances of mature leaves are very different from those found in typical vascular plants. Xerophytic plants have a very wide range of PAR light absorptance ($Abt_{400–700}$) ranging from 0.29 to 0.92 (Ehleringer *et al.* 1976, Ehleringer 1981, Stemke and Santiago 2011). In most, but not all cases, the very low absorptances are due to hirsute vestiture on the leaves or stems. A second group of vascular plants with unusual, generally low absorptances, are seagrasses (Cummings and Zimmerman 2003, Runcie and Durako 2004, Enríquez 2005, Durako 2007, Ralph *et al.* 2007). Absorptance values range from as low as 0.30 to about 0.80. Enríquez (2005) noted that not only were absorptances of *Thalassia testudinum* lower than terrestrial angiosperms but they were also highly variable depending on the maturity of the leaves and the collection site ($Abt_{400–700} \approx 0.4$ to 0.67; $Abt_{680} \approx 0.30$ to 0.79). The low absorptances of seagrasses are attributed to their photosynthetic epidermal cells and lack of palisade mesophyll. Absorptances found in freshwater aquatic angiosperms and in macroalgae also cover a wide range down to as low as 0.25 (Frost-Christensen and Sand-Jensen 1992, 1995). Many leaves have substantial amounts of anthocyanins in the epidermal cells, giving leaves a wide variety of colours. Many leaves are variegated, such as *Coleus* species (Burger and Edwards 1996), or change in anthocyanin content with season (Merzlyak *et al.* 2008). The main effects of anthocyanins are to change the absorptance properties of leaves in the green part of the spectrum. Measurements on variegated *Coleus* leaves show that presence or absence of anthocyanins has little effect on absorptance in the photosynthetically critical blue and red parts of the spectrum (Burger and Edwards 1996). This might appear to be a rather surprising result but the seeming anomaly arises mainly from the very different spectral sensitivities of human eyesight compared to the light absorption properties of vascular plants.

The literature above clearly demonstrates that experimentally determined absorptances on mature leaves of most vascular plants at blue or red wavelengths is a factor of 1.06–1.15 higher than the default absorptance value (Abt_F or $Abt_{680} = 0.84$) and so use of the default absorptance underestimates ETR by approximately 10%. Absorptance information on physiologically interesting developing leaves, xerophytic desert plants, and aquatic macrophytes show widely ranging absorptances. Using a default absorptance value on such plants is highly misleading. Appropriate Abt_λ values also need to be selected based on the use of a blue or red LED diode or a 6,000 K quartz-halogen actinic light-source based PAM machine. An absorptance factor (Abt_λ) based upon the mean absorptance of 400–700 nm PPFD light is not appropriate if you are using a blue or red-LED diode based PAM machine. Findings by Björkman and Demmig (1987), Schultz (1996) and Bauerle *et al.* (2004) that immature leaves can have blue and red absorptances as low as 0.25 is another good reason why using experimentally measured absorptance values is preferable to using default values.

Little information is available on absorptances of nonvascular, terrestrial plants and other photosynthetic organisms, such as algae, lichens, corals, *etc.* Only one published report on absorptances was found for bryophytes: this report (Conde-Álvarez *et al.* 2002) is for a specialised aquatic liverwort and so is unlikely to be representative. Information on absorptance properties of lichens is also very limited and may also be nonrepresentative (Anthony *et al.* 2002, Gauslaa and Ustvedt 2003, Solhaug *et al.* 2003, Solhaug *et al.* 2010, Ritchie 2014). One particular problem in lichens is that they often have nonphotosynthetic, blue absorbing pigments such as parietin in their thalli, which would be expected to result in quite different absorptance properties in blue and red light. Some lichens absorb large and very difficult to quantify amounts of light by pigments in the fungal thallus rather than the algal symbionts (Solhaug *et al.* 2010), others such as *Dirinaria picta* do not (Ritchie 2014). The UV-blue absorbing lichen pigment, parietin, would be expected to interfere strongly with ETR measurements made with a blue LED diode system but not where a red

LED diode was used. Other pigments such as anthocyanins might be less important because they do not strongly absorb blue or red light (Burger and Edwards 1996, Merzlyak *et al.* 2008).

Few data on absorptances of macrophytic algae or corals are available. Beach *et al.* (2006) measured the absorptances in representatives of the major classes of macroscopic algae. Absorptances of green algae were qualitatively similar to terrestrial vascular plants over the PAR range (400–700 nm). Green algae were strongly absorbing in the blue and red parts of the spectrum. Brown and red algae were able to absorb green, yellow, and orange light better than green algae. Mean absorptances in the PAR range varied from 62 to 90% depending on the thickness of the thallus, the degree of calcification, and the pigment composition of the alga: reflectances varied from a low of 7% to as much as 23%. Absorptances of corals are difficult to estimate and some published values might be misleading (Beer *et al.* 1998, Enríquez *et al.* 2005, Stambler and Dubinsky 2005, Rodríguez-Román *et al.* 2006, Hennige *et al.* 2009). Most PAM-based estimates of photosynthesis in macrophytic algae and corals are actually rETR estimates based on the default overall absorptance (Abt_F) of 0.84 and so could be seriously erroneous.

It is important to note that the absorptances even of mature leaves of some plant species are very much lower than the standard default value ($Abt_F = 0.84$) (Ehleringer *et al.* 1976, Ehleringer 1981, Björkman and Demmig 1987, Frost-Christensen and Sand-Jensen 1992, 1995; Burger and Edwards 1996, Schultz 1996, Cummings and Zimmerman 2003, Bauerle *et al.* 2004, Runcie and Durako 2004, Enríquez 2005, Durako 2007, Ralph *et al.* 2007, Merzlyak *et al.* 2008, Stemke and Santiago 2011). In photosynthetic work, particularly when dealing with productivity issues, it is important to have absolute measurements of photosynthesis such as ETR rather than relative rates (rETR). For example, if the actual absorptance is 0.25, then use of the default value of 0.84 will result in an overestimation of photosynthesis by a factor of 3.4.

The advantage of having a portable RAT device is that actual absorptance measurements can be used to estimate ETR and quantum efficiencies instead of relying on a standard default absorptance value. Since it is known that absorptance properties of young leaves are substantially different to mature leaves, actual absorptance measurements are needed for studies of photosynthesis over the course of leaf development. The method is nondestructive and so the absorptance of individual leaves can be measured with the RAT device before being used for PAM measurements, enabling the time course of establishment of photosynthetic capacity to be followed.

Materials and methods

Plants used in testing the RAT: The vascular plants selected were: bird's nest fern (*Asplenium nidus* L.), banana (*Musa x paradisiacal* L.), gymea lily (*Doryanthes excelsa* (Correa)), *Kalanchoe daigremontiana* (Raym.-Hamet & H. Perrier), oil palm (*Elaeis guineensis* Jacq.), orchid, *Dendrobium spp*. (D. cv. Viravuth Pink), pineapple (*Ananas comosus* L.), rice (*Oryza sativa* L.), river red gum [*Eucalyptus camaldulenis* (Dehnh)], sugarcane (*Saccharum* spp.), and water lily (*Nymphaea caerulea* Saligny). Plants were collected from the gardens of the campuses of Prince of Songkla University-Phuket (Thailand) and the University of Sydney (NSW, Australia) and from the School of Biological Sciences greenhouses at the University of Sydney. Bird's nest fern, banana, rice, river red gum, water lily, and oil palm are C_3 species, pineapple and the *Dendrobium* orchid are CAM species, *Doryanthes* is a presumptive CAM or weak CAM species, *Kalanchoe* is an obligate CAM species, and sugarcane is a C_4 species. Leaves were cut into convenient pieces for conducting the measurements and placed in Petri dishes with moistened filter paper, but excessive delays (> 15 min) in making measurements after collection were avoided. The RAT is small and light (835 g) and has its own internal batteries so it can be used by hand in the field where repeated measurements on the same leaves *in situ* are of interest.

The RAT machine: The RAT machine was conceived by Raymond J. Ritchie and built by John Runcie **(***Aquation Pty Ltd.*, V 1.0**,** www.aquation.com.au**)**. The design of the RAT machine is shown in Fig. 1 and is similar to the device made by Schultz (1996). The prototype version used a single blue diode (465 nm) to measure transmission and reflectance and to calculate absorptances appropriate for PAM experiments using a PAM machine fitted with a blue diode (Ritchie 2013, Ritchie and Runcie 2013): the machine and software were later upgraded to use a RGB diode (*SML-LX1610RGBW/A*, *Lumex Corp*., Palatine, IL 60087, USA) to allow reflectance, absorptance, and transmission to be measured at three different wavelengths (465, 525, and 625 nm) and in combination as 'white' light. The machine measures reflectance and transmittance and then calculates absorptance by subtraction.

Absorptance measurements using the RAT: The RGB-LED diode light source allows blue, green, and red light absorptance to be estimated separately and hence average absorptance over the entire 400–700 nm range ($Abt_{400–700}$) can be estimated for systems using a quartz halogen light source by taking the average absorptance over the PAR range. If the voltages supplied to the red, green, and blue channels of the RGB-LED diode are suitably adjusted, an approximate white light can be obtained and $Abt_{400–700}$ estimated in 'white' light directly.

The RAT measures transmittance through a specimen to obtain T [%] and also measures reflectance (R, %) using

a detector diode set at 45º to the light beam in an arrangement based upon Schultz (1996). Absorptance is calculated as Abt [%] = 100 – T [%] – R [%] (Runcie and Durako 2004). The machine is calibrated using a black card and a white standard surface for each colour channel and when the RGB-LED diode was used as a 'white' light source. Using the RGB-LED diode installed as standard equipment, if blue, green, and red channels were adjusted to 50, 100, and 75% of the maximum voltage rating of the three channels, the resultant light approximated sunlight (6,000 K). So-called 'white light' LED diodes are commercially available, however, they only appear white to the human eye: spectrally their light does not resemble sunlight or incandescent light sources. White LEDs generally comprise a blue LED with a phosphor that re-emits light across the visible spectrum and so the spectrum of white LEDs have a distinct peak in the blue, and a less intense much broader peak extending from the blue into the red parts of the PAR spectrum (Cope and Bugbee 2013).

The machine was calibrated as described in the instruction manual using a black and white (0 and 100% reflectance, respectively) polyester card standards (waterproof paper) for the particular light source being used (red, green, blue, or a RGB combination of sources giving "white" light). Calibration steps involved firstly measuring 100% transmittance with no sample, 0% reflectance with the black card, and 100% reflectance with the white card. The software then calculates reflectance %, transmission %, and absorptance % of a sample placed in the light path. The RAT needs to be recalibrated for each coloured light source.

Absorptance spectra using an integrating sphere: Absorptance spectra of plant leaves were also measured using a Taylor integrating sphere attachment (*ISR-240A*) on a *Shimadzu UV-2550* UV-visible spectrophotometer (*Shimadzu*, Kyoto, Japan) at the University of Sydney, NSW, Australia. The Taylor sphere configuration used in the present study did not allow simultaneous measurements of R_λ and T_λ; absorptance was calculated as Abt_λ [%] = 100 – R_λ [%] – T_λ [%]. In the case of the Ulbricht sphere configuration used by Schultz (1996), McCree (1972), and Björkman and Demmig (1987) absorptance is measured directly because the specimen is placed inside the integrating sphere. In our study, transmittance was measured by placing a specimen in the "IN" sample holder and the light path passed through the specimen into the Taylor sphere. After recording the T_λ scan, the specimen was moved to the reflectance "REFL" sample port and scanned again to get the reflectance readings. Slight differences in orientation of the sample during the transmittance and reflectance measurement steps are a potential source of error. Nonphotosynthetic absorptance was estimated by zeroing and base-lining the spectrophotometer on 750 nm (McCree 1972, Cummings and Zimmerman 2003). The Ulbricht sphere configuration measures absorptance directly and does not provide transmittance and reflectance data as separate data.

Statistics: Unless otherwise stated all values quoted are means ± 95% confidence limits (CL) with the number of plant types (n_1) and the total number of data points (n_2), quoted in brackets (n_1, n_2). Simultaneous access to a Taylor sphere and the RAT machine was limited due to time and logistics. Where comparisons are made between Taylor sphere and RAT measurements it is between plants collected in Australia and not between RAT measurements made on Thai material with material collected in Australia.

Results

Transmittance (T), reflectance (R) and absorptance (Abt) in blue light: T, R, and calculated Abt values, using the 465 nm blue-diode light setting, on a variety of vascular plants including one fern [bird's nest fern: *Asplenium nidus* (L.)] are shown in Table 1. Similar T, R, and Abt values obtained using the red light source diode (625 nm) are shown in Table 2. All measurements were based on at least 16 determinations. Since the angle at which reflectance is measured forms a longitudinal axis (Fig. 1), for leaves with obvious parallel venation it was necessary to measure R, A, and T on leaves with veins parallel and at right angles to this axis. Those vascular plants, which exhibited parallel venation, were all measured in a longitudinal and in a latitudinal direction and the means and variances tested for significant differences using *t*-tests. Only the bird's nest fern showed a significant difference in light absorption characteristics in a longitudinal and latitudinal direction: reflectance (R_{465}) was extremely low in the latitudinal direction and absorptance was very high ($Abt_{465} \approx 98$ %). In the case of the angiosperms with parallel venation, no significant differences were found and so overall mean R_{465}, Abt_{465}, and T_{465} values were calculated. Like many eucalypts, the leaves of the river red gum (*Eucalyptus camaldulensis*) are pendulous and their adaxial and abaxial surfaces are almost identical in appearance: RAT properties of abaxial and adaxial surface were not significantly different and so overall means ± 95% CL were calculated for blue and red light. The mean R_{465}, Abt_{465}, and T_{465} values for the leaves of the 11 different species of vascular plant under blue light were not greatly different from one another and so it was meaningful to calculate overall mean values based on 12 plants (adult and seedling leaves of one species) and a total of 388 observations: R_{465} = 3.03 ± 0.825 %, T_{465} = 1.05 ± 0.94 %, and Abt_{465} = 95.9 ± 1.12 %. RAT measurements made using 625 nm light for the same species as listed in Table 1 are compiled together in Table 2. There appear to be some general differences in the

Table 1. Spectral characteristics of selected plants at 465 nm (blue diode). R_{465}, T_{465}, and Abt_{465} – reflectance, transmittance, and absorptance at 465 nm, respectively.

Species	R_{465} [%]	T_{465} [%]	Abt_{465} [%]
Bird's nest fern (*Asplenium nidus*)			
Latitudinal	1.34 ± 0.09 (n = 20)	0.01 ± 0.02 (n = 20)	98.7 ± 0.09 (n = 20)
Longitudinal	1.20 ± 0.03 (n = 20)	0.85 ± 0.20 (n = 20)	97.9 ± 0.25 (n = 20)
Overall mean	1.27 ± 0.05 (n = 40)	0.43 ± 0.20(n = 40)	98.3 ± 0.13 (n = 40)
Banana (*Musa × paradisiaca*) Latitudinal + longitudinal	3.91 ± 0.08 (n = 32)	0.41 ± 0.034 (n = 32)	95.7 ± 0.07 (n = 32)
Gymea lily *(Doryanthes excelsa)* Latitudinal + longitudinal	4.62 ± 0.21 (n = 56)	0.24 ± 0.039 (n = 56)	95.3 ± 0.21 (n = 56)
Kalanchoe daigremontiana	3.72 ± 0.44 (n = 16)	0.98 ± 0.092 (n = 16)	95.3 ± 0.41 (n = 16)
Oil palm (*Elaeis guineensis*) seedling	3.82 ± 0.41 (n = 20)	2.69 ± 0.35 (n = 20)	93.5 ± 0.49 (n = 20)
Oil palm (*E. guineensis*) mature leaves Latitudinal + longitudinal	2.32 ± 0.42 (n = 32)	0.52 ± 0.043 (n = 32)	97.2 ± 0.43 (n = 32)
Orchid (*Dendrobium* spp.) Latitudinal + longitudinal	3.07 ± 0.17 (n = 32)	0.22 ± 0.090 (n = 32)	96.7 ± 0.25 (n = 32)
Pineapple (*Ananas comosus*) Latitudinal + longitudinal	1.89 ± 0.13 (n = 32)	0.18 ± 0.016 (n = 32)	97.9 ± 0.14 (n = 32)
Rice (*Oryza sativa*) Latitudinal + longitudinal	0.85 ± 0.34 (n = 24)	6.90 ± 0.98 (n = 24)	92.4 ± 1.3 (n = 24)
River red gum *(Eucalyptus camaldulensis)* Adaxial + abaxial	3.82 ± 0.51 (n = 32)	0.32 ± 0.019 (n = 32)	95.8 ± 0.50 (n = 32)
Sugarcane (*Saccharum* spp.) Latitudinal + longitudinal	5.50 ± 0.19 (n = 32)	2.18 ± 0.22 (n = 32)	92.3 ± 0.17 (n = 32)
Water lily (*Nymphaea caerulea*)	1.30± 0.17 (n = 40)	0.53± 0.14 (n = 40)	98.2 ± 0.19 (n = 40)
Overall mean for all leaves tested (12 plants, n_{total} = 388)	3.0 ± 0.83	1.05 ± 0.94	95.9 ± 1.12

Table 2. Spectral characteristics of selected plants at 625 nm (red diode). R_{625}, T_{625}, and Abt_{625} – reflectance, transmittance, and absorptance at 625 nm, respectively.

Species	R_{625} [%]	T_{625} [%]	Abt_{625} [%]
Bird's nest fern (*Asplenium nidus*)			
Latitudinal	4.62 ± 0.76 (n = 16)	6.11 ± 0.43 (n = 16)	89.3 ± 1.1 (n = 16)
Longitudinal	2.27 ± 0.69 (n = 16)	5.89 ± 0.47 (n = 16)	92.0 ± 0.85 (n = 16)
Overall mean	3.45 ± 0.49 (n = 32)	6.00 ± 0.31 (n = 32)	90.7 ± 0.65 (n = 32)
Banana (*Musa × paradisiaca*)	0.80 ± 0.24 (n = 16)	2.72 ± 0.29 (n = 16)	96.5 ± 0.41 (n = 16)
Gymea lily *(Doryanthes excelsa)*	7.62 ± 0.71 (n = 32)	0.791 ± 0.19 (n = 32)	90.7 ± 2.0 (n = 32)
Kalanchoe daigremontiana	5.76 ± 0.69 (n = 16)	4.40 ± 0.28 (n = 16)	89.9 ± 0.65 (n = 16)
Oil palm (*Elaeis guineensis*) seedling	4.59 ± 1.50 (n = 16)	10.91 ± 0.99 (n = 16)	84.5 ± 2.1 (n = 16)
Oil palm (*E. guineensis*) mature leaves	2.16 ± 0.85 (n = 16)	2.33 ± 0.55 (n = 16)	95.6 ± 1.0 (n = 16)
Orchid (*Dendrobium spp*)	7.37 ± 1.09 (n = 16)	3.39 ± 0.62 (n = 16)	89.3 ± 1.4 (n = 16)
Pineapple (*Ananas comosus*)	4.72 ± 1.90 (n = 16)	1.28 ± 0.35 (n = 16)	94.0 ± 2.1 (n = 16)
Rice (*Oryza sativa*)	7.46 ± 0.97 (n = 24)	17.4 ± 0.91 (n = 24)	75.2 ± 1.7 (n = 24)
River red gum *(Eucalyptus camaldulensis)*	9.61 ± 1.07 (n = 32)	1.9 ± 0.14 (n = 32)	88.8 ± 1.2 (n = 32)
Sugarcane (*Saccharum spp*)	7.01 ± 1.03 (n = 16)	5.30 ± 0.44 (n = 16)	87.7 ± 1.2 (n = 16)
Water lily (*Nymphaea caerulea*)	12.4 ± 2.0 (n = 16)	4.71 ± 0.46 (n = 16)	82.9 ± 1.8 (n = 16)
Overall mean for all leaves tested	6.16 ± 1.84 (12 plants, n_{total} = 224)	3.74 ± 1.58 (12 plants, n_{total} = 208)	90.1 ± 2.1 (12 plants, n_{total} = 224)

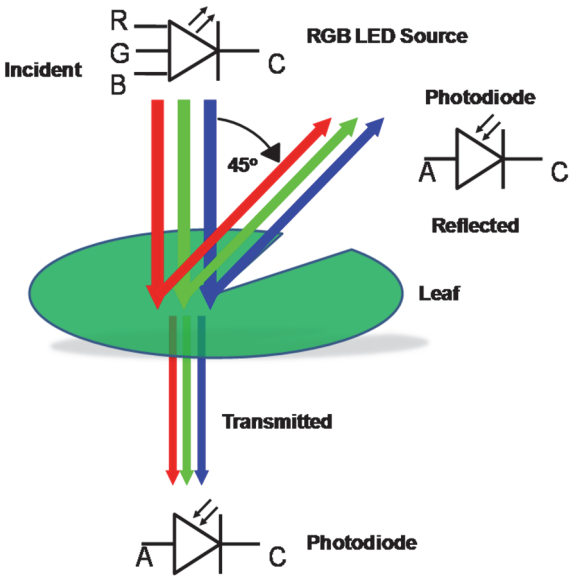

Fig. 1. Basic arrangement of RGB-LED diode light source and transmittance and reflection detecting photodiodes of the RGB-RAT machine. Blue (465 nm), green (525 nm), and red (625 nm) light can be selected using the RAT software. Adapted from Schultz (1996). The working distance is about 2.5 cm above the stage on which the transmittance detector diode is housed.

absorptance properties of vascular plants under blue and red light: reflectances are generally higher in red light and transmission through the leaves is much higher in red light than in blue light. Absorptance is generally about 7% lower in red light than in blue light. Variability of Abt_{625} among species and within the same species between different leaves also appears to be higher than in blue light. The overall mean values for R, Abt, and T at 625 nm based on 12 plants and 248 observations were: R_{625} = 6.28 ± 1.69 %, $T_{625\,nm}$ = 5.06 ± 2.71 %, and Abt_{625} = 88.6 ± 3.17 %.

RAT absorptance measurements in blue, green, and red light: RAT absorptance measurements made using blue 465 nm light (Table 1), green light (525 nm), and red light (625 nm) (Table 2) for the vascular plants used in the present study are compiled in Table 3. The overall mean absorptances for the blue, green, and red light were calculated to give an estimate of absorptance in the PAR range ($Abt_{400–700}$). Absorptances in 'white' light provided by the RGB-LED diode are also shown and in nearly all cases are very similar to those calculated by taking the mean absorptance from the red, green, and blue light sources measured separately. *Kalanchoe* is exceptional: the mean absorptance from the blue, green, and red measurements was 89.3 ± 0.441 % (n = 48) but the value found for the 'white' light source was quite different [$Abt_{400–700}$ = 76.5 ± 1.2 % (n = 16)]. Taking the overall means of all the plants used in the present study, the absorptance in the PAR range (400–700 nm) was estimated to be about 86 to 89% based upon both the means of the red, green, and blue light determinations of absorptance and the 'white' light (Table 3). However, some plants we found that the overall mean absorptances based on separate RGB measurements were significantly different to estimates using 'white' light, *Kalanchoe* and banana being the most conspicuous examples.

Reflectance measurements of vegetation are also important in remote sensing applications. RAT reflectance measurements from Tables 1 and 2 and those made in green light (525 nm) and in composite 'white' light from the RGB-LED diode light source have been compiled in Table 4. The overall mean reflectances for the blue, green, and red light have been calculated to give an estimate of reflectance in the PAR range ($Abt_{400–700}$). In nearly all cases, the calculated mean from the blue, red, and green measurements of reflectance were very similar to those made using the 'white' light source. Banana leaves have a very low average reflectance ($R_{400–700}$ < 3%) and the two methods of estimation of reflectance over the PAR range give different but nevertheless both very low results. The estimates of reflectance of oil palm seedling leaves in 'white light' and based on averaging the reflectance in blue, green, and red light were also very different. Taking the overall means of all the plants used in the present study, the reflectance of leaves in the PAR range was estimated to be about 6% based upon the means of the red, green, and blue light determinations of absorptance and upon using the RGB-LED diode as a 'white' light source. The highest overall average reflectances were found in green light (R_{525} = 9.24 ± 1.63 %), followed by red light (R_{625} = 6.28 ± 1.69 %), and were lowest for blue light (R_{465} = 3.03 ± 0.825 %).

RAT measurements using a Taylor integrating sphere: Our T, Abt, and T scans on banana, *Doryanthes*, and sugarcane from 350 to 750 nm using the Taylor integrating sphere attachment on the *Shimadzu* spectrophotometer are shown in Fig. 2. All the Abt_λ *vs*. λ curves are similar to the mean curve plotted by McCree (McCree 1972) and are closely comparable to scans obtained by other researchers using integrating spheres on a variety of land plants (Ehleringer *et al.* 1976, Ehleringer 1981, Lee and Graham 1986, Björkman and Demmig 1987, Knapp and Carter 1998, Carter and Knapp 2001, Merzlyak *et al.* 2008, Gorton *et al.* 2010). Measurements at 465, 524, and 625 nm using the RGB-RAT are shown for comparison. There is good agreement in the calculated Abt_{465}, Abt_{525} and Abt_{625} based on the Taylor sphere and the RAT machine although the RAT tends to slightly overestimate the absorptances. However, each colour of the RGB diode used in the RAT machine has a bandwidth of about 50 nm, whereas the absorptances calculated from an integrating sphere have bandwidths of only 0.5 or a few nm. More detailed studies would require a large data set of integrating sphere data averaged over wide bandwidths

Table 3. Estimates of absorptance in "white light" PAR 400–700 nm.

Species	Blue light 465 nm (Table 1)	Green light 525 nm	Red light 625 nm (Table 2)	Overall mean	White light (RGB-diode)
Bird's nest fern (*Asplenium nidus*)	98.3 ± 0.126 (n = 40)	79.0 ± 0.933 (n = 16)	90.7 ± 0.649 (n = 32)	89.3 ± 0.126 (n = 98)	87.8 ± 0.645 (n = 16)
Banana (*Musa* × *paradisiaca*)	95.7 ± 0.072 (n = 32)	93.0 ± 0.234 (n = 16)	96.5 ± 0.414 (n = 16)	95.1 ± 0.160 (n = 64)	91.3 ± 0.716 (n = 16)
Gymea lily *(Doryanthes excelsa)*	95.3 ± 0.206 (n = 56)	84.9 ± 3.88 (n = 16)	90.7 ± 2.03 (n = 32)	90.3 ± 1.44 (n = 104)	87.1 ± 3.25 (n = 16)
Kalanchoe daigremontiana	95.3 ± 0.406 (n = 16)	82.6 ±1.08 (n = 16)	89.9 ± 0.645 (n = 16)	89.3 ±0.441 (n = 48)	76.5 ±1.20 (n = 16)
Oil palm (*Elaeis guineensis*) seedling	93.5 ± 0.490 (n = 20)	69.3 ± 2.32 (n = 16)	84.5 ± 2.11 (n = 16)	82.4 ± 1.06 (n = 52)	83.6 ± 1.51 (n = 16)
Oil palm (*E. guineensis*) mature leaves	97.2 ± 0.428 (n = 32)	87.8 ± 0.803 (n = 16)	95.6 ± 0.999 (n = 16)	93.5 ± 0.948 (n = 64)	92.8 ± 0.523 (n = 16)
Orchid (*Dendrobium spp*)	96.7 ± 0.253 (n = 32)	87.1 ± 1.97 (n = 16)	89.3 ± 1.35 (n = 16)	91.0 ± 0.801 (n = 64)	91.2 ± 0.855 (n = 16)
Pineapple (*Ananas comosus*)	97.9 ± 0.136 (n = 32)	85.2 ± 2.16 (n = 16)	94.0 ± 2.1 (n = 16)	92.4 ± 1.01 (n = 64)	89.0 ± 1.41 (n = 16)
Rice (*Oryza sativa*)	92.4 ± 1.34 (n = 24)	69.3 ± 1.67 (n = 24)	75.2 ± 1.67 (n = 24)	81.2 ± 1.71 (n = 72)	79.0 ± 2.47 (n = 24)
River red gum *(Eucalyptus camaldulensis)*	95.8 ± 0.498 (n = 32)	-	88.8 ± 1.17 (n = 32)	-	-
Sugarcane (*Saccharum spp*)	92.3 ± 0.17 (n = 32)	79.5 ± 0.81 (n = 16)	87.7 ± 1.2 (n = 16)	86.5 ± 0.47 (n = 64)	86.0 ± 0.73 (n = 16)
Water lily (*Nymphaea caerulea*)	98.2 ± 0.19 (n = 40)	77.9 ± 1.2 (n = 16)	82.9 ± 1.8 (n = 16)	86.3 ± 0.72 (n = 72)	86.0 ± 1.2 (n = 16)
Overall mean	95.9 ± 1.1 (12 plants, n_{total}= 388)	80.9 ± 4.4 (11 plants, n_{total}= 184)	88.6 ± 3.2 (12 plants, n_{total}= 248)	89.0 ± 2.4 (11plants, n_{total}= 786)	86.1 ± 3.0 (11 plants, n_{total} = 184)

and RAT data on the same material collected at the same time. Any systematic difference is likely to be only a few percent. The results of McCree (1972) are in general agreement with our results shown in Tables 1, 2, 3 and Fig. 2 and the calculated mean absorptances for the PAR range also agree with those of Björkman and Demmig (1987).

Discussion

The most detailed information available on the absorptance of plants at various wavelengths is the data of McCree (1972) who measured absorptances of 22 commonly grown crop species at 25 nm intervals from 350 to 750 nm (McCree 1972). Measurements were made by McCree (1972) on a laboratory constructed Ulbricht integrating sphere attached to a spectroradiometer. The absorptance data for the range of cereal, oil seed, field and vegetable crops were consistent, despite differences in leaf morphology. The most important wavelengths for the purposes of the present study are 430 nm (the Chl *a* peak *in vivo*), 465 nm (the blue peak for the RGB-LED diode used in the present study). Other important wavelengths are 625, 650, 670, and 680 nm. 625 nm is the wavelength of the red channel of the RGB-LED diode used in the RAT machine, 650 or 670 nm are the wavelengths of the red LED diodes of many types of PAM machines and 680 nm is the red *in vivo* peak for Chl *a*. Interpolation of absorptances by linear regression were made to estimate absorptances at 430 and 465 nm (absorptance data for 425, 450, and 475 nm, n = 66), and at 670 and 680 nm using the data from McCree (1972) one wavelength interval below and above the desired wavelengths (n = 44). The estimates of absorptances at critical wavelengths were: Abt_{430} = 92.7 ± 0.76 %, Abt_{465} = 92.6 ± 1.03 %, Abt_{625} = 87.6 ± 1.22 %, and Abt_{650} = 90.5 ± 0.905 % (calculated directly from the data of McCree 1972), Abt_{670} = 92.4 ± 1.3 % and Abt_{680} = 90.4 ± 1.60 %. Where interpolation was used to calculate the absorptance values, correlations in all cases were r>0.99. The overall mean Abt_{465} and Abt_{625} values found in the present study based on a different set of selected vascular plants (Table 1: Abt_{465} = 95.9 ± 1.12 %, Table 2: Abt_{625} = 88.6 ± 3.17 %) differs by only 3.6% and 1.1% from the mean values calculated from McCree (1972).

Table 4. Estimates of reflectance (R_λ) in the PAR range (400–700 nm).

Species	Blue light 465 nm (Table 1)	Green light 525 nm	Red light 625 nm (Table 2)	Overall mean of blue, green & red	White light (RGB-diode)
Bird's nest fern (*Asplenium nidus*)	1.27 ± 0.045 (n = 40)	9.53 ± 0.77 (n = 32)	3.45 ± 0.49 (n = 32)	4.75 ± 0.30 (n = 108)	5.23 ± 0.53 (n = 32)
Banana (*Musa* × *paradisiaca*)	3.91 ± 0.078 (n = 32)	3.20 ± 0.15 (n = 16)	0.80 ± 0.24 (n = 16)	2.64 ± 0.098 (n = 64)	0.818 ± 0.12 (n = 16)
Gymea lily *(Doryanthes excelsa)*	4.62 ± 0.21 (n = 56)	13.08 ± 3.3 (n = 16)	7.62 ± 0.71 (n = 32)	7.83 ± 0.91 (n = 104)	11.2 ± 2.7 (n = 16)
Kalanchoe daigremontiana	3.72 ± 0.44 (n = 16)	9.9 ± 1.0 (n = 16)	5.76 ± 0.69 (n = 16)	6.46 ± 0.41 (n = 48)	5.67 ± 0.81 (n = 16)
Oil palm (*Elaeis guineensis*) seedling	3.82 ± 0.41 (n = 20)	9.92 ± 1.4 (n = 16)	4.59 ± 1.5 (n = 16)	6.11 ± 0.69 (n = 52)	3.51 ± 0.84 (n = 16)
Oil palm (*E. guineensis*) mature leaves	2.32 ± 0.42 (n = 32)	4.82 ± 0.39 (n = 16)	2.16 ± 0.85 (n = 16)	3.10 ± 0.34 (n = 64)	2.80 ± 0.41 (n = 16)
Orchid (*Dendrobium spp*)	3.07 ± 0.17 (n = 32)	9.26 ± 1.6 (n = 16)	7.37 ± 1.1 (n = 16)	6.57 ± 0.66 (n = 64)	6.71 ± 0.88 (n = 16)
Pineapple (*Ananas comosus*)	1.89 ± 0.13 (n = 32)	10.4 ± 1.4 (n = 16)	4.72 ± 1.90 (n = 16)	5.67 ± 0.79 (n = 64)	7.43 ± 1.03 (n = 16)
Rice (*Oryza sativa*)	0.846 ± 0.34 (n = 24)	7.99 ± 1.1 (n = 24)	7.46 ± 0.97 (n = 24)	5.43 ± 0.90 (n = 72)	4.66 ± 0.75 (n = 24)
River red gum *(Eucalyptus camaldulensis*	3.82 ± 0.51 (n = 32)	-	9.61 ± 1.1 (n = 32)	-	-
Sugarcane (*Saccharum spp*)	5.50 ± 0.19 (n = 32)	10.8 ± 0.65 (n = 16)	7.01 ± 1.03 (n = 16)	7.77 ± 0.41 (n = 64)	7.52 ± 0.50 (n = 16)
Water lily (*Nymphaea caerulea*)	1.30 ± 0.17 (n = 40)	13.08 ± 1.2 (n = 16)	12.4 ± 2.0 (n = 16)	8.93 ± 0.79 (n = 72)	8.59 ± 0.83 (n = 16)
Overall mean for all leaves tested	3.03 ± 0.83 (12 plants, n_{total} = 388)	9.24 ± 1.6 (11 plants, n_{total} = 200)	6.28 ± 1.7 (12 plants, n_{total} = 248)	5.98 ± 1.10 (11 plants, n_{total} = 776)	5.68 ± 1.54 (11 plants, n_{total} = 200)

The overall mean Abt_{465} found in the present study on 12 different plants was 95.9 ± 1.12 % (n_{plants} = 12, n_{total} = 388) and is similar to the Abt_{465} value which can be calculated by interpolation from the study of McCree (1972), *i.e.* Abt_{465} = 92.6 ± 1.03 % (n = 22) on a largely different set of vascular plants. The only plant species included in both the study by McCree (1972) and the present study was rice (*Oryza sativa* L.). The mean absorptance at 625 nm found in the present study (Table 2: Abt_{625} = 88.6 ± 3.17 %, n_{plants} = 12, n_{total} = 248) is also in good agreement with the mean value calculable from the data of McCree (1972), *i.e.* 87.6 ± 1.22 % (n = 22). Overall mean Abt_λ values *vs*. wavelength do have some value but are limited by the range of plants studied. Only two species included in McCree's study were C_4 (corn and sorghum), there were no CAM species and no characteristically tropical crops, such as banana, sugarcane, pineapple, rubber, or oil palm. Björkman and Demmig (1987) performed their study in northern Australia and included tropical plants and mangroves in their study. A mean absorptance value of $Abt_{650} \approx 90 \pm 1.4$ % can be calculated from the data of Björkman and Demmig (1987). This is not significantly different from a value that can be calculated from McCree (1972) (Abt_{650} = 90.5 ± 0.905 %, n = 22) and is not significantly different from that found in the present study for 625 nm (Abt_{625}= 88.6 ± 3.17 %). Gausman and Allen (1973) measured absorptances on 30 vascular plants but only at 550 and 650 nm in the 400–700 nm range. Their study included many of the same species used by McCree (1972) but also included banana, sugarcane, cotton, and three tree species (river red gum, *Eucalyptus camaldulensis* Dehnh; a fig species, *Ficus elastica* Roxb. and privet, *Ligustrum lucidum* Ait.). No submergent aquatic angiosperms were included in the studies of McCree (1972), Gausman and Allen (1973) or Björkman and Demmig (1987). Overall, the absorptances of tree species are poorly documented (Gausman and Allen 1973, Lee and Graham 1986, Björkman and Demmig 1987, Lee *et al*. 1990, Knapp and Carter 1998, Carter and Knapp 2001, Bauerle *et al*. 2004, Merzlyak *et al*. 2008). This is somewhat surprising considering the importance of the optical properties of tree vegetation in remote sensing studies (Jones and Vaughan 2010).

Although mature leaves generally have blue and red absorptances of about 90 to 95%, it was demonstrated early on by Björkman and Demmig (1987) in *Hedera canariensis*, by Schultz (1996) in *Vitis vinifera*, grapevine, and Bauerle *et al*. (2004) in red maple (*Acer rubrum* L.) that the absorptance properties of immature leaves are much lower than for mature leaves. In boreal deciduous

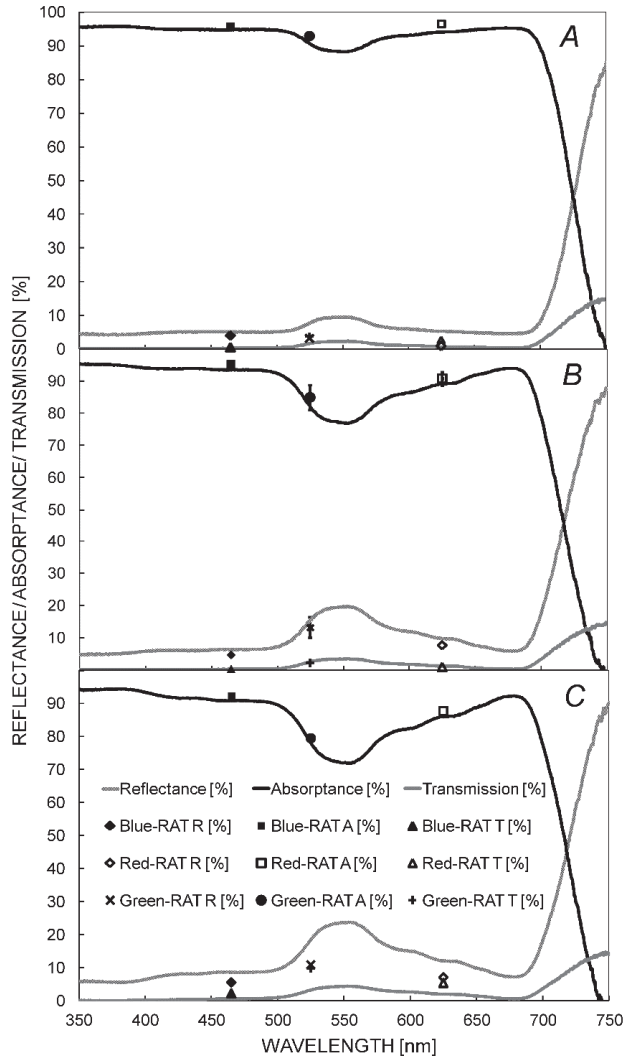

Fig. 2. Comparison of reflectance, absorptance, and transmittance measurements made using the RAT (465, 525, and 625 nm) and Taylor sphere scans of leaves of banana (*Musa* × *paradisiacal L.*) (*A*), Gymea lily (*Doryanthes excelsa*) leaves (*B*) and sugarcane (*Saccharum* spp.) (*C*) The RAT reflectance (R), absorptance (Abt) and transmittance (T) values at 465 nm and 625 nm are taken from Tables 1 and 2. For banana R_{525} = 3.2 ± 0.15, Abt_{525} = 93 ± 0.23 %, and T_{525} = 3.78 ± 0.13 % (n = 16). For *Doryanthes* R_{525} = 13.1 ± 3.3 %, Abt_{525} = 84.9 ± 3.9 % and T_{525} = 2.02 ± 0.70 % (n = 16). For sugarcane, at 525 nm R = 10.8 ± 0.65 %, Abt = 79.5 ± 0.81 %, and T = 9.71 ± 0.28 % (n = 16). Banana, *Doryanthes*, and sugarcane leaves have parallel venation but it was shown that longitudinally and latitudinally arranged leaves did not give significantly different R_λ, Abt_λ, or T_λ values. Data are mean values ± 95% CL of the combined data (Tables 1, 2, 3). Some of the error bars do not show because they are less than ± 1%.

forests, anthocyanin content of leaves varies with the season and not simply their age (Merzlyak *et al.* 2008). The RAT machine offers the opportunity to conveniently do routine absorptance measurements in different seasons and on leaves at different stages of development (the example of oil palm adult and juvenile leaves in the present study). This would help in better estimating productivity of crops and forests.

The RAT gives plausible values for reflectance (R_λ) and transmittance (T_λ) and hence calculated absorptance (Abt_λ) of plant leaves included in the study at both blue (465 nm) and red (625 nm) wavelengths (Tables 1, 2, 3; Fig. 2). Combined with estimates of absorptance in green light (525 nm) it is possible to estimate average absorptances in the PAR range ($Abt_{400–700}$). Photosynthesis using green light is often underestimated or neglected (Terashima *et al.* 2009, Johkan *et al.* 2012). By adjusting the intensity of the three channels of an RGB-LED diode an approximate 'white' light source can be obtained and used to estimate $Abt_{400–700}$ directly. Where Taylor sphere data are available, the fit between absorptances measured using the RAT meter (Tables 1, 2, 3) and the Taylor integrating sphere determinations are within a few % of one another but Fig. 2 shows a consistent trend for the RAT readings at both 465 and 625 nm to underestimate reflectance (R) and hence Abt is a few percent higher than determined using the Taylor sphere. One reason for this is that the RAT measurements are not corrected for nonspecific absorptance by zeroing on measurements made at 750 nm (Cummings and Zimmerman 2003), but we zeroed our absorptance readings using the Taylor sphere on 750 nm. An average absorptance at 750 nm of 6.63 ± 1.03 % can be calculated from the data of McCree (1972). Schultz (1996) noted a similar discrepancy in grape vine leaves (*V. vinifera* L.) in his RAT apparatus when compared to results using an Ulbricht integrating sphere. Schultz (1996) used natural sunlight as the light source for his RAT meter measurements and had access to an Ulbricht sphere that used natural sunlight as the light source. Schultz (1996) shows an almost direct proportionality of $Abt_{400–700}$ calculated from his quantum sensor setup and the Ulbricht sphere. Schultz (1996) found that the $Abt_{400–700}$ of mature grapevine leaves was about 0.85.

The RAT can be used to estimate an average absorptance over the PAR range. Such an averaged absorptance (Abt_F) is needed when using PAM fluorometers or IRGA machines that use a halogen 6,000 K actinic light source. The average Abt_{465} of the vascular plants we used was about 96% and about 89% for 625 nm light and about 86–89% for 'white' light (Table 3). Vascular plants have relatively low Abt in green light (≈ 550 nm) (Fig. 2) and so the average Abt over the entire PAR range (400–700 nm) for the 22 crop plants used by McCree (1972) would be about 87.1 ± 0.36 % (average Abt for all wavelengths 400–700 nm). This is in good agreement with our findings (Table 3). Bauerle *et al.* (2004) working on various cultivars of red maple (*Acer rubrum* L.) found very similar R, T, and Abt using a Taylor sphere and also found that the average absorptance over the PAR range ($Abt_{400–700}$) was about 85 to 90%. Taylor sphere scans on leaves of forest trees by Merzlyak *et al.* (2008) show very low green light absorptances (< 60%) by young spring leaves but in more

mature leaves the green absorptance is much higher due to the presence of anthocyanins. Average absorptances in blue and red light found in the present study (Tables 1, 2) are nearly all above the standard default absorptance value (Abt_F = 0.84) commonly used as the default in PAM studies. We found that the xerophytic CAM plant *Kalanchoe* had a very high absorptance in blue light (Abt_{465} = 95.3 ± 0.406 %, Table 1) but only a slightly lower absorptance in red light (Abt_{625} = 89.9 ± 0.645 %, Table 2). We used well-watered specimens that might not be comparable to xerophytic plants under arid conditions (Gates *et al.* 1965, Ehleringer *et al.* 1976, Ehleringer 1981, Stemke and Santiago 2011). Overall, from our study we conclude that using the default absorptance (Abt_F) of 0.84 would generally underestimate photosynthesis if a blue- or red-light source PAM machines were used.

The equation used to calculate absorptance assumes that the illuminated object (test card or specimen) are both perfect Lambertian surfaces (reflected light is uniformly scattered in all directions). The reflectance at 45º is not representative of scattered reflected light at all angles in a real system and the surfaces of a specimen (leaf or a film of cells on a filter) and a Lambertian test card are only approximately comparable. There is also a partial polarisation of the reflected light (Schultz 1996). Thus reflectance values for leaves that have parallel venation were expected to be significantly different if the leaf is set in line to the plane of the line-of-sight of the reflectance detector or if the leaf is set at 90º to it (longitudinal and latitudinal arrangement of leaves on the measuring platform of the RAT). Abt_{465} and Abt_{625} measurements were made on latitudinally and longitudinally arranged leaves of bird's nest fern, banana, *Doryanthes*, oil palm, orchid, pineapple, rice, and sugarcane leaves (Tables 1, 2). In all cases, at least 16 latitudinally and longitudinally arranged leaves were measured but no significant differences in Abt_{465} were found in all the angiosperms. It was concluded that parallel venation had little effect on measured absorptance except in the bird's nest fern. A Taylor sphere will detect all reflected light, whether as a reflected beam like in the case of a mirror, or as uniformly scattered light as for a Lambertian surface. The detector geometry used by Schultz (1996) and the current RAT machine tends to underestimate reflected light and hence overestimate Abt by a few percent but we concluded that this was not primarily a polarisation effect because orientation of the leaves had so little effect.

A major motivation for the development of the RAT was the unsatisfactory nature of relative ETR (rETR) measurements using PAM machines. The results of this study confirm the unsatisfactory nature of the default absorptance value or Genty factor (Abt_F = 0.84) currently in common use (Tables 1, 2, 3). If a default value for absorptance needs to be adopted, a value of Abt_{465} of ≈0.96 would be more representative for blue light sources (Table 1) in agreement with McCree (1972), Gausman and Allen (1973), Knapp and Carter (1998), Carter and Knapp (2001), and Bauerle *et al.* (2004). This implies that currently quoted rETR values underestimate actual ETR by an average factor of about 1.14 for a blue-LED diode based PAM. One of the authors has used the default Abt_F for estimating photosynthesis in a *Dendrobium* orchid, pineapple, and water lily (Ritchie and Bunthawin 2010a, b; Ritchie 2012) leading to underestimations of ETR by factors of 1.13, 1.14, and 1.17, respectively. Ritchie (2012) reported that blue water lily was apparently capable of fixing about 5.3 g(C) m^{-2} d^{-1}, based on an Abt_F value of 0.84: since the actual Abt_{465} is 0.979 (Table 1) then the actual daily gross photosynthetic rate is probably more like 6.2 g(C) m^{-2} d^{-1}. Most red-light absorptances are also higher than the default value of 84% and average about 90% (Table 2) which implies that use of the default value tends to underestimate ETR by a factor of about 1.055 in red-LED diode based PAM machines. The Abt_{625} in the species used in the present study span a considerably wider range than Abt_{465} measurements (Tables 1, 2, 3). Calculations of average absorptances for the PAR range in the present study ($Abt_{400\text{-}700}$) are also ≈ 86 to 89 (Table 3) and so the standard Genty absorptance factor (Abt_F = 84) is about 2–5% too low if used for a 'white' light source.

The RAT was designed to provide measurements of the absorptance of photosynthetic material but it also measures reflectance as part of the procedure. Reflectance of leaves in the PAR range (400–700 nm) and at more defined blue (465 nm), green (525 nm), and red (625 nm) wavelengths are important parameters of value to remote sensing studies and so the RAT would be valuable for providing ground-truth values for crop, horticulture, forestry, and ecological studies where reflectance information on vegetation is needed (Jones and Vaughan 2010). Table 4 shows that although average PAR light reflectances ($R_{400–700}$) are about 6%, different species have different reflectance signatures in blue, green, and red light (Table 4). In canopy shading studies, RAT transmittance data would be useful for determining the optical environment for understory species but under such conditions light is diffuse rather than predominantly as a direct beam. Gorton *et al.* (2010) have addressed the difficult problem of absorptance of plants under diffuse light using a pair of Taylor spheres. They found that under diffuse light the absorbances of *Helianthus annuus* leaves were lower than in direct light as a result of reflectances being higher in diffuse light.

In summary, our investigation shows that the widely used Abt_F value of 0.84 would be better replaced with a value of Abt_{465} ≈ 0.96 when used with photosynthetic quantum yield estimates obtained from blue diode fluorometers, and Abt_{625} = 0.89 for red-diode fluorometers respectively. The consequence of using these more accurate absorptance values is a significant increase in calculated electron transport rates, which has important implications for the assessment of photosynthetic rates when calculated using chlorophyll fluorescence measurements of photosynthetic material. There is, however, a

great advantage in having a simple portable machine to experimentally measure absorptances of plants under the actual conditions under which they are growing and at different stages of development rather than having to resort to default values.

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