DOI: 10.1007/s11099-011-0026-y PHOTOSYNTHETICA 49 (2): 195-200, 2011

Consequences of light absorptance in calculating electron transport rate of desert and succulent plants

J.A. STEMKE and L.S. SANTIAGO+

Department of Botany and Plant Sciences, University of California, 2150 Batchelor Hall, Riverside, CA 92521, USA

Abstract

The proportional light absorptance by photosynthetic tissue (α) is used with chlorophyll (Chl) fluorescence methods to calculate electron transport rate (ETR). Although a value of α of 0.84 is often used as a standard for calculating ETR, many succulent plant species and species with crassulacean acid metabolism (CAM) have photosynthetic tissues that vary greatly in color or are highly reflective, and could have values of α that differ from 0.84, thus affecting the calculation of ETR. We measured ETR using Chl fluorescence and α using an integrating sphere in 58 plant species to determine the importance of applying a measured value of α when calculating ETR. Values of α varied from 0.55–0.92 with a mean of 0.82 across species. Differences between ETR values calculated with measured α values ranged from 53% lower to 12% greater than ETR values calculated with a standard α value of 0.84 and were significantly different in 39 out of 58 species. While measurements of ETR using Chl fluorescence represent a rapid and effective assessment of physiological performance, the value of α needs to be considered. Measurements of α, especially on species with light-colored or reflective photosynthetic tissue, will allow more accurate determination of photosynthesis in succulent and CAM species.

Additional key words: chlorophyll fluorescence; crassulacean acid metabolism; desert plants; photosynthesis; succulence.

Introduction

Chl fluorescence has become an important tool for determining the level of stress on plant photosynthetic processes (Krause and Weis 1991, Schreiber and Bilger 1993), and comparing photosynthetic performance among contrasting plant species (Brodribb and Feild 2000, Jones *et al.* 2010). The rapid and nondestructive nature of Chl fluorescence measurements allows ease of collecting data and repeated measurements in experiments. The development of portable Chl fluorescence yield analyzers has increased the measurement of effective quantum yield of photosystem II (Φ_{PSII}) under ambient light conditions in the field (Rascher *et al.* 2000), as well as potential quantum yield on dark-adapted samples as an index of photoinhibition (Genty *et al.* 1989). It is also possible to calculate other parameters related to photosynthetic performance, such at the maximum apparent ETR, which represents a measure of the capacity for photosynthetic activity and can be used to compare plant species or treatments in an experimental setting. However, the calculation of ETR requires knowledge of the proportion of light absorbed by photosynthetic tissue (α), but many instruments are preprogrammed with a standard value of 0.84, which was previously shown to be the average for 37 C_3 plant species (Björkman and Demmig 1987). Applying α values of 0.84 could lead to inaccurate ETR calculations in other types of plants if α of photosynthetic tissue differs greatly from this value.

For succulent plants, which may have thick fleshy tissue, it can be difficult to measure photosynthetic processes using traditional gas-exchange cuvettes. Many succulent species exhibit CAM, in which stomata open at night (Winter and Smith 1996), further complicating the determination of photosynthetic parameters. Yet succulent plants often live in arid or semiarid environments necessitating the need to understand how these unique plant species respond to environmental stress

Received 30 September 2010, *accepted* 28 February 2011.
+Corresponding author; tel: (951) 827-4951, fax: (951) 827-4437, e-mail: santiago@ucr.edu
Abbreviations: CAM – crassulacean acid metabolism; Chl – chlorophyll; ETR – electron transport rate; *f* – energy partitioning factor between PSII and PSI; PAR – photosynthetically active radiation; PSI – photosystem I; PSII – photosystem II; SE – standard error; α – absorptance; ρ – reflectance; Φ_{PSII} –quantum efficiency of photosystem II; τ – transmittance.
Acknowledgements: We graciously thank Steven Morgan, Curator of the UC Riverside Botanic Gardens, Doug Holt, Curator of the Botany and Plant Sciences Teaching Collection, and Katia Silvera for use of the orchid species. This project was funded by NSF grant 08-17212 to LSS.

such as high light or limited water availability (Ting 1985). Chl fluorescence measurements have therefore been employed extensively to determine the stress levels and photosynthetic performance of succulent species (deMattos *et al.* 1997, Andrade *et al.* 2006, Ritchie and Bunthawin 2010). In high-light environments, leaves and other photosynthetic tissues are often lighter in color due to the presence of thick waxy layers on the epidermis or a high density of trichomes, hairs or spines (Ehleringer *et al.* 1976, Nobel 1983, Meinzer and Goldstein 1985). Because lighter-colored surfaces and leaf hairs reflect more light than darker-colored surfaces and can cause large variation in α (Ehleringer and Mooney 1978), using a standard value for α when calculating ETR in succulent or desert species could lead to inaccurate values.

We measured ETR and α on 58 species from 21 families, including many commonly studied succulent plant species, to address the following questions: (*1*) What is the variation in α among succulent plant species? (*2*) How sensitive is measurement of ETR to variation in α among succulent plant species? (*3*) How does calculation of ETR using measured values of α compare with using a standard α value of 0.84?

Materials and methods

Measurements were conducted on plants at the University of California, Riverside (UCR) Botanic Gardens and the Teaching Collection of the Botany and Plant Sciences Department. We measured 56 succulent plant species, including 20 of the 34 plant families in which CAM is known to occur (Smith and Winter 1996, Holtum *et al.* 2007) (Table 1). We also included two known C_3 plant species: *Rosa floribunda* which has 'typical' C_3 leaves that are dark green and relatively thin, and *Encelia farinosa*, which has highly reflective pubescent leaves (Ehleringer *et al*. 1976).

ETR was calculated using the equation:

$$ETR = \Phi_{PSII} \times PAR \times f \times \alpha \quad (1)$$

where Φ_{PSII} is the quantum efficiency of PSII measured during a 0.8-s saturating flash (2,000–3,000 μmol m^{-2} s^{-1}) with a pulse amplitude modulated fluorometer (*Mini-PAM, Walz*, Effeltrich, Germany), PAR is photosynthetically active radiation and was set at 1,980 μmol m^{-2} s^{-1} using the *Mini-PAM*, but varied slightly among measurements due to ambient light conditions, f is a factor that accounts for the partitioning of energy between PSII and PSI and is assumed to be 0.5, indicating that excitation energy is distributed equally between the two photosystems (Maxwell and Johnson 2000), and α is the proportion of light absorptance by photosynthetic tissue in decimal format (*e.g*. 0.84).

Following ETR measurements, reflectance and transmittance of PAR (400–700 nm) were measured on the same photosynthetic tissue where Φ_{PSII} was obtained. The device used for leaf optical measurements consisted of a white-light illuminator and grating monochromator, appropriate lenses and optical filters, an integrating sphere (7.5 cm diameter) (*LI-1800-12, Li-Cor Biosciences*, Lincoln, NE, USA) and a quantum sensor (*LI-190S, Li-Cor Biosciences*). The inside of the sphere was coated with multiple coats of highly-reflective barium sulfate. The monochromator used 0.5 mm side slits resulting in a waveband 4 nm wide and a total bandwidth of 8 nm. The light passed through a small elongated hole 1.14 × 2.08 cm and focused on the upper side of the leaf or photosynthetic unit (1.5 cm diameter). The light sources used in fluorescence and absorptance measurements were different, which could affect our ability to parameterize our ETR calculations with our absorptance data. However, this error is probably small and much smaller than the variation in absorption among species. We calculated α as the proportion of light not reflected or transmitted from photosynthetic units as:

$$\alpha = 1 - \rho - \tau \quad (2)$$

where ρ and τ are reflectance and transmittance, respectively. Care was taken to ensure that ambient light did not enter the integrating sphere. For most species, leaves or photosynthetic stems were measured intact and still attached to the plant. If leaves were detached, measurements were made immediately following leaf excision. For some species, it was necessary to remove spines to ensure flat contact against the tissue. In order α to measure reflectance as accurately as possible, care was taken to avoid damage to the waxy coatings on leaf surfaces. On photosynthetic units thicker than 2 cm, transmittance was not measured because it was assumed that the tissue was too thick to transmit light. This assumption was verified on several species, but it was not possible to insert photosynthetic units thicker than 1.5 cm into the integrating sphere.

To determine the sensitivity of ETR measurements to variation in α we used Eq. 1 to calculate ETR using the standard value of 0.84 and compared this to calculations using the measured value of α with paired *t*-tests in *SAS* (*ver. 9.2, SAS Institute Inc*., Cary, NC, USA). We also calculated the percent difference in ETR when the measured value of α was applied instead of 0.84 *α* to evaluate the mean difference for each species.

Results and discussion

Mean α across all of the 58 species studied was 0.82 ± 0.01 (±1 SE) and varied from a low value of 0.55 in *E. farinosa*, a nonsucculent C_3 species with highly pubescent, reflective leaves, to a high value of 0.92 in *Peperomia obtusifolia*, a succulent understory species that occurs as terrestrial and epiphytic forms (Table 1). *R. floribunda*, the nonsucculent species with typical C_3 leaves had an α of 0.86. Thus the mean α of all species and the value for *R. floribunda* were very close to the value of 0.84 that was previously reported for 37 C_3 species and is commonly used as a standard value for photosynthetic tissue of C_3 plants (Björkman and Demmig 1987). Moreover, the range of α data from 0.75–0.90 of C_3 plants reported by Björkman and Demmig (1987) show far less variability than the range of α values found among succulent and desert plants in this study. For example, *Agave chrysantha* (α = 0.62), *Opuntia quimilo* (α = 0.67) and *Kalanchoe thyrsiflora* (α = 0.68) exhibited exceptionally low α values even among succulent species. Therefore, the standard α value of 0.84 appears to be consistent with C_3 leaves, but the considerably larger variability among succulent and desert species, especially in the low range (Table 1), suggests that low α values are the result of adaptations to reduce light absorption in high-light habitats (Ehleringer and Mooney 1978).

When ETR was calculated with an α of 0.84, the mean was 182 ± 15 μmol m^{-2} s^{-1} and the species with the lowest mean value was the orchid *Epidendrum* at 11 μmol m^{-2} s^{-1}, whereas the species with the greatest mean value was *Agave parryi* at 464 μmol m^{-2} s^{-1} (Fig. 1). When ETR was recalculated using measured α values, the overall mean was 172 ± 13 μmol m^{-2} s^{-1} with a range of 11–422 μmol m^{-2} s^{-1} (Fig. 1). Differences between ETR calculated using α values of 0.84 versus using measured values were significantly different

Table 1. List of study taxa with mean (±1 SE, n = 5) values of absorptance of photosynthetic tissue (α), mean (±1 SE, n = 5) percent difference between ETR calculated using a standard value of 0.84 for α *vs.* measured α, and p-value from paired t-test between ETR based on standard and measured α values. Significant differences ($p \leq 0.05$) are indicated *in bold* type.

	α [prop.]	Difference between ETR calculated with 0.84 and measured [%]	p-value
Agavaceae			
Agave americana L.	0.900 ± 0.005	6.3 ± 0.5	**≤0.005**
Agave angustifolia Haw. cv. Marginata	0.869 ± 0.029	1.5 ± 3.4	0.33
Agave celsii Hook.	0.883 ± 0.013	4.8 ± 1.4	**≤0.01**
Agave chrysantha Peebles	0.621 ± 0.070	–35.2 ± 1.6	**≤0.001**
Agave deserti Engelm.	0.700 ± 0.027	–20.7 ± 4.9	**≤0.05**
Agave parryi Engelm.	0.761 ± 0.013	–10.4 ± 1.9	**≤0.001**
Agave picta Salm-Dyck	0.836 ± 0.011	– 0.4 ± 1.4	0.58
Agave scabra Ortega	0.742 ± 0.013	–13.3 ± 2.0	**≤0.0005**
Agave shawii Engelm.	0.857 ± 0.015	2.0 ± 1.7	0.48
Agave victoriae-reginae T. Moore	0.755 ± 0.005	–11.6 ± 4.5	**≤0.005**
Agave wocomahi Gentry	0.813 ± 0.031	–3.9 ± 4.2	0.59
Sansevieria fasciata Cornu ex Gérôme & Labroy	0.771 ± 0.026	–2.7 ± 2.1	0.44
Aizoaceae			
Carpobrotus deliciosus L. Bolus	0.843 ± 0.025	0.1 ± 3.3	0.78
Lampranthus primivernus L. Bolus	0.804 ± 0.006	–4.3 ± 0.8	**≤0.05**
Apocynaceae			
Pachypodium lamerei Drake	0.880 ± 0.013	4.6 ± 1.5	**≤0.05**
Araceae			
Zamioculcas zamiifolia (Lodd.) Engl.	0.911 ± 0.04	8.6 ± 0.3	**≤0.05**
Asphodelaceae			
Aloe arborescens Mill.	0.887 ± 0.005	5.5 ± 0.5	**≤0.05**
Aloe plicatilis Mill.	0.844 ± 0.005	0.7 ± 0.6	0.31
Haworthia truncata Schönland	0.839 ± 0.014	0.4 ± 1.5	0.52
Asteraceae			
Encelia farinosa A. Gray *ex* Torr.	0.551 ± 0.026	–53.5 ± 6.6	≤0.005
Bromeliaceae			
Ananas comosus (L.) Merr.	0.871 ± 0.005	3.7 ± 0.6	≤0.005
Billbergia euphemiae E. Morren	0.886 ± 0.016	5.2 ± 1.8	0.09

Table 1 (continued)

	α [prop.]	Difference between ETR calculated with 0.84 and measured [%]	*p*-value
Commelinaceae			
Rhoeo spathacea (Sw.) Stearn	0.884 ± 0.003	5.1 ± 1.3	0.19
Tradescantia pallida (Rose) D.R. Hunt	0.902 ± 0.005	7.3 ± 0.7	**≤0.05**
Tradescantia zebrina Heynh.	0.896 ± 0.017	6.2 ± 1.8	**≤0.05**
Cactaceae			
Hylocereus undatus (Haw.) Britton & Rose	0.897 ± 0.007	6.4 ± 0.7	**≤0.01**
Opuntia basilaris Engelm. & Bigelow	0.721 ± 0.011	–16.6 ± 0.6	**≤0.005**
Opuntia cochenillifera (L.) Mill.	0.759 ± 0.027	–11.2 ± 3.9	0.06
Opuntia ficus-indica (L.) Mill.	0.836 ± 0.010	–0.5 ± 1.3	0.51
Opuntia littoralis (Engelm.) Cockerell	0.765 ± 0.003	–9.7 ± 0.5	**≤0.0005**
Opuntia quimilo K. Schum.	0.667 ± 0.015	–26.1 ± 3.0	**≤0.01**
Opuntia robusta J.C. Wendl.	0.742 ± 0.020	–11.8 ± 3.4	**≤0.05**
Opuntia stricta (Haw.) Haw.	0.777 ± 0.010	–8.1 ± 1.4	**≤0.01**
Clusiaceae			
Clusia L. sp.	0.901 ± 0.002	6.9 ± 0.2	**≤0.0005**
Crassulaceae			
Aeonium canariense (L.) Webb & Berthel.	0.797 ± 0.015	–5.4 ± 1.7	**≤0.05**
Crassula falcata J.C. Wendl.	0.796 ± 0.004	–5.5 ± 0.6	**≤0.005**
Crassula ovata (Mill.) Druce	0.810 ± 0.004	–3.7 ± 0.6	**≤0.01**
Echeveria agavoides Lem.	0.803 ± 0.014	–4.8 ± 1.9	**≤0.05**
Echeveria pulvinata Rose	0.890 ± 0.016	2.8 ± 1.9	0.27
Graptopetalum paraguayense (N.E. Br.) E. Walther	0.780 ± 0.034	–8.7 ± 5.5	0.15
Kalanchoe fedtschenkoi Raym.-Hamet & H. Perrier	0.864 ± 0.006	2.8 ± 0.6	**≤0.01**
Kalanchoe thyrsiflora Harv.	0.682 ± 0.023	–23.6 ± 4.1	**≤0.005**
Kalanchoe daigremontiana Raym.-Hamet & H. Perrier	0.729 ± 0.004	–15.2 ± 0.6	**≤0.001**
Euphorbiaceae			
Euphorbia grandicornis Goebel *ex* N.E. Br.	0.902 ± 0.007	6.9 ± 0.8	**≤0.05**
Euphorbia milii Des Moul.	0.744 ± 0.013	–12.9 ± 2.0	**≤0.01**
Orchidaceae			
Cattleya patinii Cogn.	0.861 ± 0.011	2.6 ± 1.3	0.10
Oncidium ampliatum Lindl.	0.877 ± 0.004	4.3 ± 0.3	**≤0.005**
Oncidium carthagenense (Jacq.) Sw.	0.823 ± 0.010	–1.7 ± 1.3	0.30
Epidendrum L. sp.	0.853 ± 0.004	2.2 ± 0.7	**≤0.01**
Vanilla planifolia Andrews	0.854 ± 0.009	1.8 ± 1.1	0.15
Oxalidaceae			
Oxalis regnellii Miq.	0.816 ± 0.008	–2.8 ± 1.1	0.21
Piperaceae			
Peperomia obtusifolia (L.) A. Dietr.	0.915 ± 0.006	8.2 ± 0.7	**≤0.01**
Portulacaceae			
Portulacaria afra Jacq.	0.738 ± 0.016	–14.0 ± 2.4	**≤0.005**
Rosaceae			
Rosa floribunda Baker	0.855 ± 0.003	1.8 ± 0.3	**≤0.01**
Ruscaceae			
Nolina bigelovii (Torr.) S. Watson	0.729 ± 0.009	–13.1 ± 2.3	**≤0.001**
Vitaceae			
Cissus L. sp.	0.883 ± 0.006	5.0 ± 0.6	**≤0.01**
Welwitschiaceae			
Welwitschia mirabilis Hooker F.	0.846 ± 0.009	0.8 ± 1.0	0.34
Zamiaceae			
Dioon edule Lindl.	0.774 ± 0.015	–8.6 ± 2.1	**≤0.05**

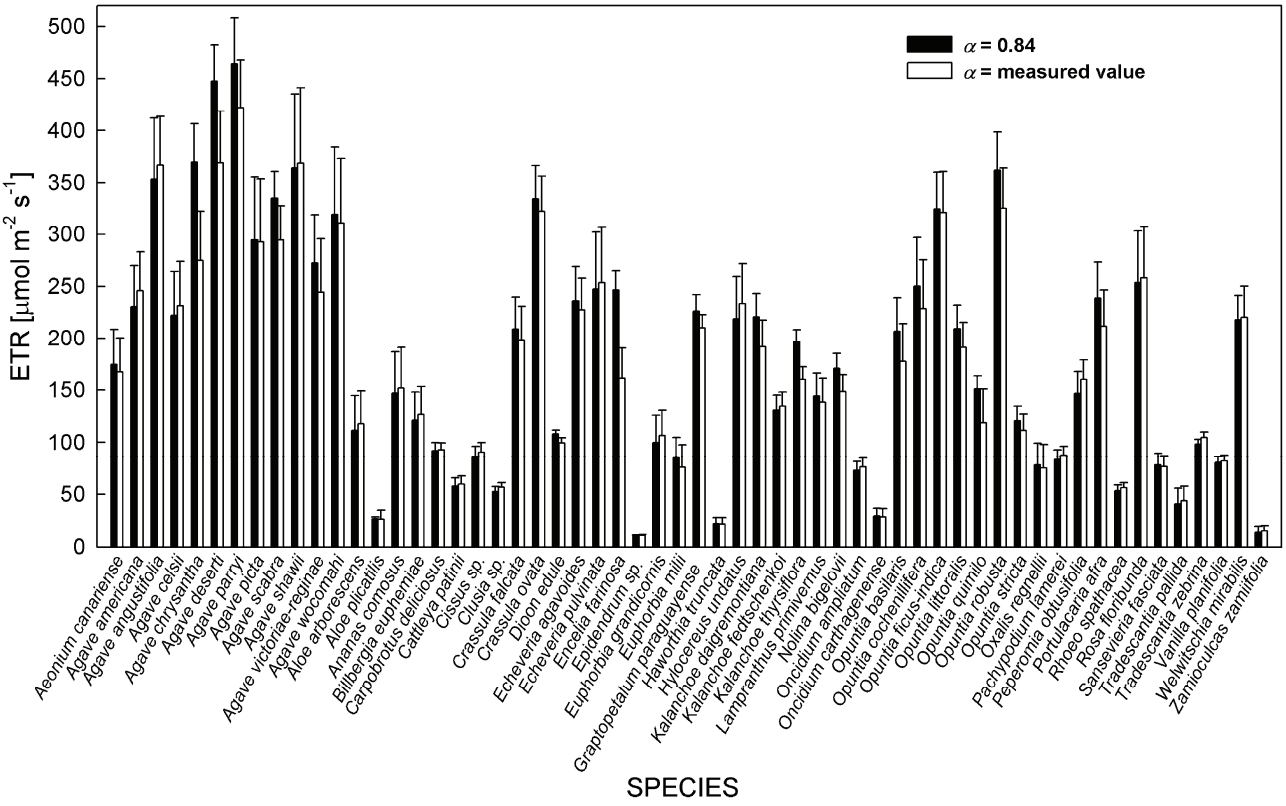

Fig. 1. Mean (±1 SE, n = 5) electron transport rate (ETR) measured with 1,980 μmol m^{-2} s^{-1} white irradiance for 56 species of succulent plant species and two nonsucculent plant species, *Rosa floribunda* and *Encelia farinosa*, calculated using proportional absorptance values of photosynthetic tissue (α) of 0.84 or measured values presented in Table 1. Statistical results are presented in Table 1.

in 39 out of 58 species (Table 1). Such differences were negligible in species with α values close to 0.84 such as *Carpobrotus deliciosus* and *Agave picta*. However, in species with highly reflective photosynthetic tissue such as *A. chrysantha*, ETR values were 35% lower when measured α values were used (Table 1). The largest variation between the two calculated values of ETR was observed in *E. farinosa*, a species specifically chosen because of highly reflective leaf hairs (Ehleringer and Mooney 1978), and showed 53% lower values when measured α values were used. Much of the divergence of ETR values between the two calculations were due to α values that were substantially lower than 0.84 and caused overestimation of ETR when α of 0.84 was used. Roughly half of the species exhibited α values that were greater than 0.84, but these differences were much lower in magnitude than the species whose α values were below 0.84 or dipped into the 0.60 range or lower. For example, the orchid species in this study are known to exhibit CAM (Silvera *et al.* 2009, Silvera *et al.* 2010), but occur as epiphytes in semishaded conditions in tropical forest and thus have α values that are similar to C_3 species (Table 1). Therefore, it appears that using measured values to calculate ETR is more critical in species from high-light habitats which may exhibit light-colored or reflective photosynthetic tissue, whereas when working with species from understory or low-light habitats that may have α values that exceed 0.84, using a standard value of 0.84 for α would generally lead to errors of less than 10%.

The importance of using accurate values of α when calculating ETR is complicated by some of the adaptations exhibited by plants in high-light habitats. We removed spines from several species of cactus in this study so that α could be measured with an integrating sphere, but spines have the potential to shade photosynthetic tissue and reduce incident light. For example, *Opuntia bigelovii* growing in southern California maximizes nocturnal acid accumulation at lower levels of ambient PAR when spines are removed than when shaded by spines, leading to a 60% increase in growth when spines are removed (Nobel 1983). The study by Nobel (1983) suggests that tissue temperature is not strongly affected by spines, so it appears that although spines fulfill an important protective role in cacti, they also cause a reduction in photosynthetic productivity. Another example of a unique adaptation in high-light habitats that can complicate the measurement of α is demonstrated by *Haworthia truncata*, for which we measured α of 0.84. Yet, this species exhibits translucent epidermal tissues

known as "windows" which allow light to penetrate the parenchyma on the interior of photosynthetic tissue (Egbert and Martin 2002). Therefore, the depth to which α can be measured or is relevant for species with very thick photosynthetic tissue may depend on the shapes, sizes, and optical properties of achlorophyllous tissues, which likely vary substantially among the diverse adaptations to high-light habitats. Therefore, even when methods exist for characterizing α and its effect on calculating ETR, complex adaptations for dealing with high light may produce errors in photosynthetic methods that depend on tissue α.

Overall, our data suggest that α varies substantially among succulent and desert species and that utilizing measured values of α can help researchers avoid large errors in calculating photosynthetic parameters. Although the *Li-Cor 1800* integrating sphere that we employed in this study is out of production, several other instruments such as the *ISP-REF* from *Ocean Optics* (Dunedin, Florida, USA) and the *CI-710* from *CID Bio-Science* (Camas, Washington, USA) can be utilized to collect the necessary data to calculate accurate ETR values. Furthermore, integrating spheres and other optical equipment are common in engineering departments, so collaboration between plant biologists and researchers from other disciplines may augment the potential to parameterize physiological data and gain accurate assessments of photosynthetic processes. Future directions in this field may include the development of a database where scientists may enter α values for plant species so that others may consult this database when performing an experiment that requires knowledge of α. Although such a database would not always ensure the highest accuracy because of variation in α within a species, such a database would provide much more accurate information than using a standard value in many cases.

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