The photosynthetic pathway of the roots of twelve epiphytic orchids with CAM leaves

C.E. MARTIN[*,+], E.J. MAS[**], C. LU[**], and B.L. ONG[**]

Department of Ecology & Evolutionary Biology, University of Kansas, Lawrence, Kansas, 66045, U.S.A.[*]
Department of Biological Sciences, National University of Singapore, Singapore[**]

Abstract

The photosynthetic pathway of the roots (both the white velamentous main portions and the green, nonvelamentous tips) was investigated in twelve taxa (natural species and intergeneric hybrid cultivars) of epiphytic orchids having CAM leaves. All organs contained chlorophyll, and the *a*/*b* ratios indicate that the organs, especially the roots, are likely shade-adapted. Stable carbon isotope ratios of the tissues were near –15‰ for all organs, a value typical of obligate (constitutive) CAM plants. Values for root tissues were slightly lower (more negative) than those of the leaves. The presence of CAM in the leaves of these orchids did not ensure that their roots performed CAM photosynthesis. Further work is needed to address the questions raised in this study and to determine if the photosynthetic roots of these taxa are capable of assimilating atmospheric CO_2.

Additional key words: chlorophyll; CO_2 exchange; $\delta^{13}C/^{12}C$; velamen.

Introduction

Although only an exceedingly small percentage of species in the species-rich Orchidaceae have been examined, the proportion of epiphytic orchids in tropical regions with Crassulacean acid metabolism (CAM) appears to be quite high (Nuernbergk 1960, Coutinho 1963, Milburn *et al*. 1968, Goh *et al*. 1977, Hew 1976, Avadhani *et al*. 1978,1982, Sanders 1979, Medina *et al*. 1986, Winter *et al*. 1986, Hew 1989, Goh and Kluge 1989, Hew and Yong 1997, Zotz and Ziegler 1997, Zotz 2004). This is not surprising, as CAM is a common feature of many other tropical epiphytes, especially those in the Bromeliaceae (Medina *et al*. 1986, Smith 1989, Martin 1994). Although some claims exist that CAM benefits tropical epiphytes by allowing capitalization on the increased availability of CO_2 at night in the host canopy (Knauft and Arditti 1969, Hsu e*t al*. 2006, Martin *et al*. 2005), little evidence exists for this claim (Hsu *et al*. 2006), and it is assumed that the primary adaptive benefit of CAM in such epiphytes is the same as that claimed for terrestrial CAM plants, *i.e*., water conservation (Osmond 1978, Kluge and Ting 1978, Winter and Smith 1996a). Although the majority of terrestrial CAM plants grow in temperate arid regions, most epiphytic CAM plants are found in tropical regions characterized by high rainfall (Kluge and Ting 1978, Goh and Kluge 1989, Smith 1989, Martin 1994, Winter and Smith 1996a). Despite the latter, evidence from several studies indicates that many tropical epiphytes are subject to and often well-adapted to frequent periods of drought stress between precipitation events (Benzing *et al*. 1982, Ong *et al*. 1986, Smith 1989, Sekizuka *et al*. 1995, Stiles and Martin 1996, Hew and Yong 1997, Nowak and Martin 1997, Stancato *et al*. 2001), in addition to stress from other environmental factors (Griffiths *et al*. 1989, Hew and Yong 1997, Benzing and Pockman 1989, He *et al*. 1998, Konow and Wang 2001).

The CAM photosynthetic pathway conserves water by limiting stomatal opening to the nighttime when air temperatures are lower and humidities higher, relative to the daytime (Osmond 1978, Kluge and Ting 1978, Winter and Smith 1996a). Carbon dioxide is absorbed from the atmosphere during the night, resulting in the formation of malic acid, which accumulates in the vacuoles of the chlorenchyma cells throughout the night. During the day, the malic acid is released from the vacuoles and decarboxylated. The consequent high CO_2 concentrations

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[+]Corresponding author; fax: 785-864-5801, e-mail: ecophys@ku.edu
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Abbreviations: CAM – crassulacean acid metabolism; Chl – chlorophyll; DM – dry mass; FM – fresh mass; PDB – Pee Dee belemnite.

in the photosynthetic tissue effect stomatal closure during the day, minimizing water loss from the tissue. As the day proceeds, this CO_2 is slowly fixed by Rubisco and reduced to carbohydrate. The diel fluctuation in tissue acidity is a diagnostic feature of CAM, as are the restriction of stomatal opening to the night and nighttime fixation of CO_2 (Kluge and Ting 1978, Osmond 1978, Winter and Smith 1996*a*).

Highly conservative water use resulting from CAM is accompanied, however, by a comparatively severe cost in growth rate. Limitations on acid and carbohydrate storage in the cells of CAM tissue, coupled with the high carbohydrate demands of the diel CAM biochemical cycle, permit only small amounts of reduced carbon for plant growth (Osmond 1978, Kluge and Ting 1978, Winter and Smith 1996*a,b*). Thus, CAM plants are typically found in habitats characterized by high levels of abiotic stress, but low levels of biotic stress, *e.g*., competition.

Although many epiphytic orchids have aerial, green, photosynthetic roots (Hew and Yong 1997), studies of photosynthesis in orchids have focused primarily on their leaves (Nuernbergk 1960, Coutinho 1963, Knauft and Arditti 1969, Neales and Hew 1975, Goh *et al*. 1977, Avadhani *et al*. 1978, 1982, Ando 1982, Miura 1984, Winter *et al*. 1986, Sinclair 1984, Medina *et al*. 1986, Miura *et al*. 1989, Zimmerman and Ehleringer 1990, Ota *et al*. 1991, Hew and Yong 1994, Kluge *et al.* 1995, Sekizuka *et al*. 1995, He *et al*. 1998, Lootens and Heursel 1998, Konow and Wang 2001, Stuntz and Zotz 2001). In only a limited number of studies has the photosynthetic pathway of the aerial roots been examined (Benzing and Ott 1981, Goh *et al*. 1983, Ho *et al*. 1983, Hew *et al*. 1984, 1991, 1996; Cockburn *et al.* 1985, Winter *et al.* 1986, Endo and Ikusima 1989, Goh and Kluge 1989, *see* review by Hew 1989, Kluge *et al*. 1995, Hew and Yong 1997, Gehrig *et al*. 1998). In several such cases, the orchids examined were aphyllous or shootless taxa, in which the roots comprise the sole source of photosynthetic carbon for the plant. Although such roots lack stomata, they utilize the CAM photosynthetic pathway (Benzing and Ott 1981, Cockburn *et al.* 1985, Winter *et al.* 1986, Benzing and Pockman 1989, Kluge *et al*. 1995). The photosynthetic pathway of the roots of epiphytic orchids with CAM leaves has seldom been examined, although Hew *et al*. (1984, 1991) reported CAM in the aerial roots of a hybrid species of *Arachnis*, one of *Aranthera,* and a hybrid species of *Aranda,* all of which had CAM leaves, and Benzing and Ott (1981) included nine leafy species in their investigation of root photosynthesis in epiphytic orchids. In addition, the aerial, photosynthetic roots of the orchid vine *Vanilla planifolia* are C_3, while the leaves are CAM (Gehrig *et al*. 1998). Despite the latter example, one might assume that the photosynthetic pathway of the roots would always match that of the leaves; however, the cost of CAM in terms of growth, as well as the astomatous nature of orchid aerial roots, makes this assumption questionable. The lack of stomata in orchid roots (Benzing and Ott 1981, Cockburn *et al*. 1985, Hew and Yong 1997, but *see* Benzing *et al*. 1982) obviates the water-conservation benefit of CAM. Thus, it appears equally likely, if not more so, to expect the green aerial roots of some CAM orchids, if not most, to exhibit C_3 photosynthesis. Indeed, Benzing and Ott (1981) reported nocturnal CO_2 uptake in the roots of only one of nine species of orchids with CAM leaves (the roots of all three leafless taxa exhibited nocturnal CO_2 uptake), and Endo and Ikusima (1989) found no evidence of CAM in the photosynthetic roots of a hybrid taxon with CAM leaves in the epiphytic orchid genus *Phalaenopsis*. Given that CO_2 uptake by green orchid roots is often obscured by high rates of respiration (Hew *et al*. 1984, 1991, Gehrig *et al*. 1998), the best measure of CAM might be the presence of tissue diel acid fluctuations. This experimental approach is also critical given the common occurrence of CAM-cycling among plants (Ting 1985, Martin *et al*. 1988, Griffiths 1988b), including some orchid roots (Hew and Yong 1997). In this variant of C_3-CAM intermediacy, diel fluctuations in acidity in the photosynthetic tissue are accompanied by daytime stomatal opening and atmospheric CO_2 uptake, while stomata are closed at night (Ting 1985, Griffiths 1988a,b; Martin 1996). Malic acid is formed at night presumably as a result of the utilization of respiratory CO_2 (Patel and Ting 1987). Recently, Motomura *et al*. (2008) measured diel acid fluctuations and stable carbon isotope ratios in the leaves and roots of eight species of *Phalaenopsis*. They found evidence for CAM in the leaves of all species, yet only several species also exhibited CAM in the roots. Given their intriguing findings, coupled with those described above, it was the purpose of the present study to determine the photosynthetic pathway, based on diel changes in tissue acidity, of the roots of twelve different taxa of epiphytic orchids having CAM leaves.

Materials and methods

Plant materials: Leaf and root tissues were sampled from four (occasionally the sample size was three due to loss of a sample) individuals of each of 12 taxa of epiphytic orchids in a commercial greenhouse (T S Orchid Laboratory at the Woon Leng Nursery in Singapore), in which the plants had been grown from seed or cuttings in partial shade and provided with abundant water throughout their growth and at the time of the study. Eight of the taxa were derived as intergeneric or interspecific hybrids, while four were naturally occurring orchid species (Table 1). All plants were flowering at the time of this study (late May/early June 2002).

Leaf discs (0.7 cm in diameter) were taken from the central portion (avoiding the mid-rib) of mature leaves; evening and morning samples were taken from opposite sides of the same leaf. Root tissue was taken from two locations; the entire green (nonvelamentous) apical tips (usually 1–2 cm long) were excised, as well as the subtending 1–2 cm of the white, velamentous portion of the roots. In several species with particularly thin roots, numerous roots were included at each sampling time, and the tissue was pooled for analysis. In all taxa, different roots were sampled in the evening and morning. In all cases, evening and morning tissues were sampled from the same individuals.

For all analyses, tissue was sampled from leaves and roots approximately one hour before sunset and one hour after sunrise on the following day. After excision, tissue was frozen on dry ice and transported to the laboratory, whereupon the samples were stored in a freezer (–10°C) until analysis several days later.

Determination of chlorophyll (Chl) concentration: Frozen leaf or root tissue [approximately 0.01–1.0 g dry mass (DM)] was thawed, sliced, and ground in approximately 5–50 ml of acetone at a temperature near 5°C. Following extraction, most tissues lacked visibly green portions. Absorption of the extracts was measured spectrophotometrically at 645 nm and 663 nm. Calculation of Chl concentrations followed Šesták (1971). The tissue remaining after extraction was dried at 70°C until constant weight, and all Chl concentrations are expressed on a dry mass basis.

Determination of titratable acidity: Frozen leaf or root tissue (approximately 0.1–1.0 g DM) was thawed and ground in a mortar and pestle with a small amount (typically 5–10 ml) of distilled water. The resultant slurry was titrated to pH 7.0 with 0.01 N NaOH, then evaporated and dried at 70°C until constant mass. Acidities are expressed on DM basis.

Determination of $\delta^{13}C/^{12}C$ ratio: Following determinations of DM for the Chl analyses, subsamples were oxidized and the $\delta^{13}C$ of the resultant CO_2 determined with a *Finnigan MAT Delta-Plus* (Bremen, Germany) mass spectrometer at the Mass Spectrometer Laboratory at Kansas State University, Manhattan, KS, USA and compared with the PDB standard [$\delta^{13}C$ (‰) = $(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard}) - 1)$ 1000]. The precision of the mass spectrometer for $\delta^{13}C$ analyses was 0.05‰.

Statistical analyses: If the data met the requirements for the use of parametric statistics, means were compared with a paired *t*-test (two means) or an analysis of variance (three means), followed by a *Tukey* pairwise multiple comparison-of-means test when differences among the three means were found. Data for some pairs of means did not meet the requirements for the use of parametric statistics, so the Mann-Whitney *U*-test was used. In all cases, analyses followed Sokal and Rohlf (1981) and were performed by the *SigmaStat* (*SPSS, Inc*., Chicago, IL, USA) statistical software package. Statistical differences among the means were inferred when $P \leq 0.05$.

Results and discussion

All tissues contained Chl (Fig. 1); concentrations in the leaves usually exceeded those in the roots, as was found in eight of nine species examined by Benzing and Ott (1981) and in *Oncidium* 'Goldiana" by Hew *et al*. (1996). Unexpectedly, given the DM basis of the data, Chl concentrations of the velamentous (white) roots were often greater than those of the nonvelamentous, green root tips. Thus, all orchid organs examined in this study were presumed to be capable of photosynthesis (*see* also Ho *et al*. 1983), although gas exchange measurements are required to determine the ability of the organs to assimilate atmospheric CO_2.

Chl *a*/*b* ratios of the orchid organs were quite low, around 2.0 or less (Fig. 2), which may reflect an adaptation to shade (Boardman 1977) from the host tree canopy in the epiphytic habitat of these taxa or their progenitor taxa. Similarly low Chl *a*/*b* ratios were reported for the leaves of *Oncidium* 'Goldiana' by Hew and Yong (1994), who found other evidence for shade adaptation of the photosynthetic apparatus in this epiphytic orchid, as did Ota *et al*. (1991) and Lootens and Heursel (1998) for several *Phalaenopsis* hybrids, although another *Phalaenopsis* hybrid appeared to be adapted to high light (Konow and Wang 2001). In addition, He *et al*. (1998) found evidence of shade adaptation in two *Dendrobium* cultivars. In the twelve orchid taxa investigated here, Chl *a*/*b* ratios were usually lowest in the nonvelamentous, green root tips and highest in the leaves, which may reflect the exposure levels of the three organ types when growing as epiphytes in their natural habitats (leaves most exposed; roots shaded). The finding of higher Chl *a*/*b* ratios in the white, velamentous roots, relative to values for the nonvelamentous, green tips of these roots was unexpected, given the reflective nature of the velamen, which presumably shades the underlying green tissue in the velamentous portions of the roots.

In all but one (*Kagawara* Christie Low) of the twelve orchid taxa, morning acidities of the leaves were significantly greater than the acidities measured the previous evening (Fig. 3), indicative of CAM in the leaves. Furthermore, the acid content of the leaves of *Kagawara* Christie Low was substantially higher in the morning than that in the evening, indicating that the lack of a statistically significant diel difference in tissue acidity likely reflected the low sample size in this study.

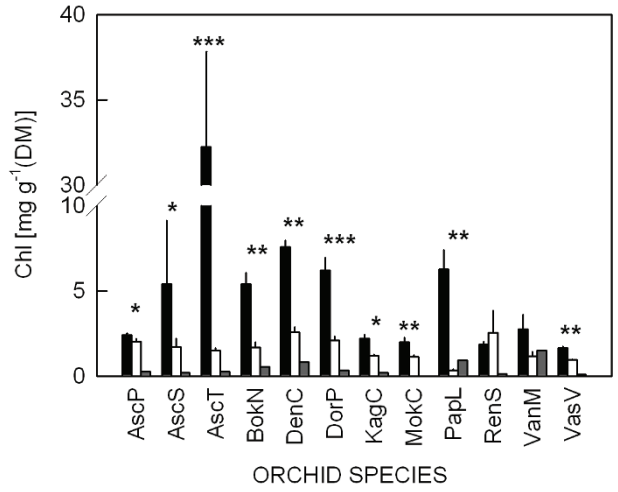

Fig. 1. Mean total chlorophyll (Chl) concentrations of leaves (*black bars*), white (velamentous) portions of the roots (*white bars*), and green (nonvelamentous) root tips (*gray bars*) of four (occasionally three) different individuals of each of 12 orchid taxa (*see* Table 1 for taxa names and origins) Lines projecting from the bars represent one standard deviation, when large enough to be shown. Data are expressed on a dry mass (DM) basis. For each taxon, differences among the three organ means are indicated by * ($P \leq 0.05$), ** ($P \leq 0.01$), or *** ($P \leq 0.001$). Lack of these symbols indicates that the three organ means are not significantly different ($P > 0.05$).

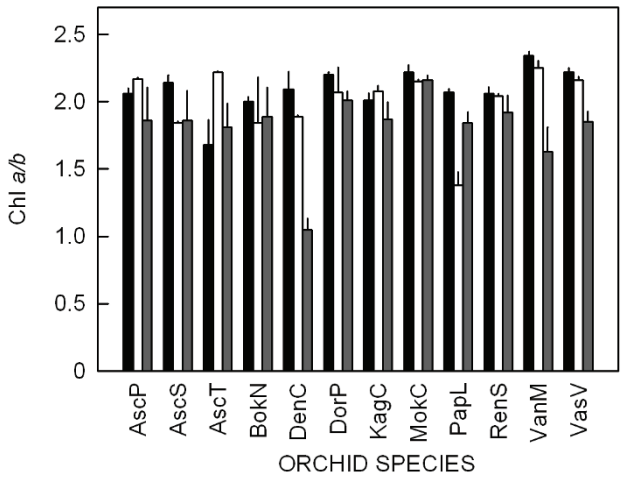

Fig. 2. Mean chlorophyll *a/b* ratios of leaves (*black bars*), white (velamentous) portions of the roots (*white bars*), and green (nonvelamentous) root tips (*gray bars*) of four (occasionally three) different individuals of each of 12 orchid taxa (*see* Table 1 for taxa names and origins). Lines projecting from the bars represent one standard deviation, when large enough to be shown. For all taxa, the three organ means are significantly different at the $P \leq 0.001$ level.

In contrast with the results for the leaves, nocturnal increases in acidity of the velamentous white portions of the roots were found in only three taxa (*Doritis (*now *Phalaenopsis) pulcherimma* var. *alba, Renanthera stolata, Vascostylis* Veerewan; Fig. 4), and a day/night CAM acid fluctuation occurred in the green tips of the roots in only one taxon (*Renanthera stolata*; Fig. 5).

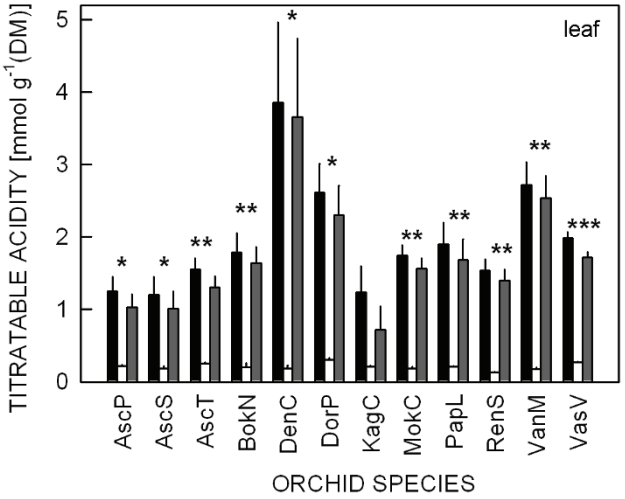

Fig. 3. Mean morning (*black bars*), evening (*white bars*), and diel change (morning minus evening, *gray bars*) in titratable acidity of leaves of four (occasionally three) different individuals of each of 12 orchid taxa (*see* Table 1 for taxa names and origins). Lines projecting from the bars represent one standard deviation, when large enough to be shown. Data are expressed as mmol H^+ per g dry mass (DM). For each taxon, differences between the morning and evening means are indicated by * ($P \leq 0.05$), ** ($P \leq 0.01$), or *** ($P \leq 0.001$). Lack of these symbols indicates that the two means are not significantly different ($P > 0.05$).

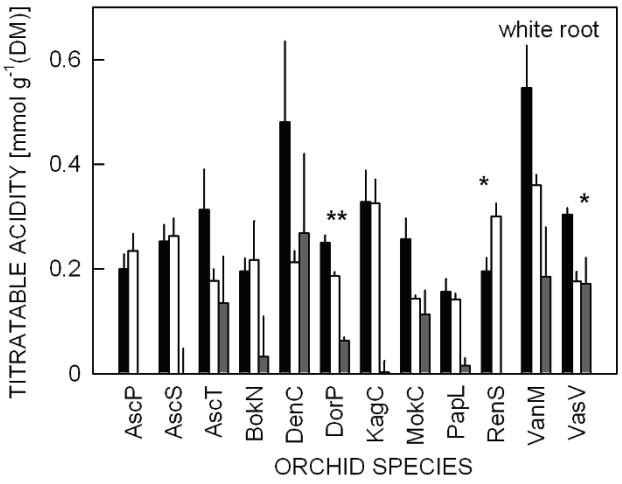

Fig. 4. Mean morning (*black bars*), evening (*light gray bars*), and diel change (morning minus evening, *dark gray bars*) in titratable acidity of the white (velamentous portions) of the roots of four (occasionally three) different individuals of each of 12 orchid taxa (*see* Table 1 for taxa names and origins). Lines projecting from the bars represent one standard deviation, when large enough to be shown. Data are expressed as mmol H^+ per g dry mass (DM). For each taxon, differences between the morning and evening means are indicated by * ($P \leq 0.05$) or ** ($P \leq 0.01$). Lack of these symbols indicates that the two means are not significantly different ($P > 0.05$).

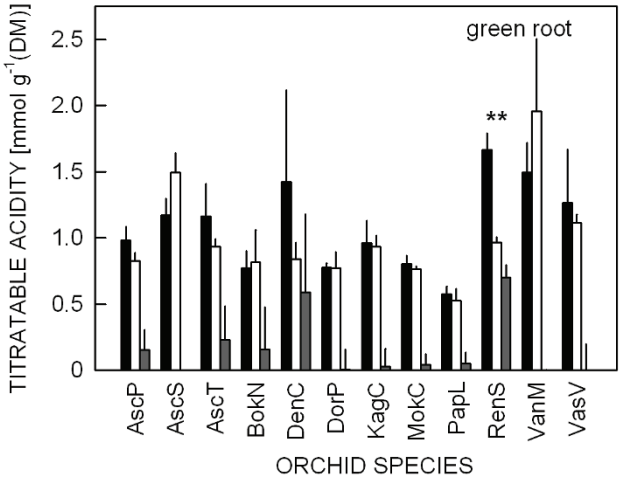

Fig. 5. Mean morning (*black bars*), evening (*white bars*), and diel change (morning minus evening, *gray bars*) in titratable acidity of the green (nonvelamentous) root tips of four (occasionally three) different individuals of each of 12 orchid taxa (*see* Table 1 for taxa names and origins). Data are expressed as mmol H^+ per g dry mass (DM). Lines projecting from the bars represent one standard deviation, when large enough to be shown. The difference between the morning and evening means was significantly different ($P \leq 0.01$) only for RenS. In all other taxa, the two means are not significantly different ($P > 0.05$).

Thus, the correlations between the presence and magnitude of diel acid fluctuations in the leaves and in both types of roots for the 12 orchid taxa were weak (white roots: $R^2 = 0.55$, $P < 0.01$; green roots: $R^2 = 0.33$, $P < 0.01$). In the three taxa showing evidence of CAM in their roots, the degree of CAM (amount of acid accumulated overnight) was substantially greater in the leaves than in either portion of the roots (Fig. 3). Greater CAM activity in the leaves than in the roots (both white and green portions) was also found by Hew *et al*. (1984) in the epiphytic orchid hybrids *Arandis* 'Maggie Oei' and *Aranthera*' James Storie,' as well as by Motomura *et al*. (2008) in several species of *Phalaenopsis*.

The degree of CAM, based on the amount of acid accumulated overnight, was greatest among the twelve taxa in the leaves of *Dendrobium crumenatum* (Figs. 3,4). Although large amounts of acid were also found in the velamentous (white) roots and green root tips of this species (Fig. 5), morning values were not significantly greater than evening values, most likely a result of the small sample size and high variability of the data.

All stable carbon isotope ratios, regardless of taxon or type of tissue, were within several per mil of –15‰ (Table 1), a value typical of plants with CAM photosynthesis (Kluge and Ting 1978, Griffiths 1992, 1993). The lowest (most negative) value, –19‰, was found in the leaves of *Bokchoonara nodata maili*, possibly indicating a small contribution of daytime, Rubisco-mediated CO_2 uptake (Griffiths 1992, 1993) by the leaves or roots of this taxon. In most taxa, $\delta^{13}C$ values of the roots were lower (more negative) than those of the leaves; however, differences were typically only 1–2‰ (Table 1). Such differences are opposite the finding for most C_3 terrestrial plants (Bowling *et al*. 2008) and might be attributable to daytime atmospheric CO_2 fixation by the leaves (or roots) or to a greater lipid content of the roots (Griffiths 1992, 1993), or possibly further isotopic fractionation related to translocation of carbohydrate from the leaves to the roots. Further studies are required to differentiate among these hypotheses, although the third hypothesis appears unlikely, given the results of many studies of nonorchid taxa as reviewed by Bowling *et al*. 2008). In one species, *Kagawara* Christie Low, CAM-like $\delta^{13}C$ values were measured in roots, yet neither the roots or the leaves showed statistically significant diel changes in tissue acidity (but *see* discussion of the leaf data above). If CAM were to be shown in the leaves of this taxon with a greater sample size, however, these findings would lend support to the suggestion that the $\delta^{13}C$ value of the roots reflects the translocation of carbon from the leaves to the roots. As in the current study of CAM orchids, the $\delta^{13}C$ of roots of the C_3 epiphytic orchid *Oncidium* 'Goldiana' (Hew *et al.* 1996) and of nine CAM species of *Phalaenopsis* were also several per mil lower (more negative) than leaf values. As noted above, it is not known if the roots of these taxa are capable of net CO_2 uptake from the atmosphere. Hew *et al.* (1984) found similar $\delta^{13}C$ values and similar leaf-root differences in the epiphytic orchid hybrid *Arachnis* 'Maggie Oei', and they also found evidence of atmospheric CO_2 fixation at night in the roots of this taxon, although rates of CO_2 uptake were obscured by high rates of CO_2 release [also found in the CAM roots of *Aranda* 'Tay Swee Eng' by Hew *et al*. (1991) and in the photosynthetic roots of a cultivar of *Laeliocattleya* by Miura (1984)]. Ho *et al*. (1983) reported the presence of similar photosynthetic machinery in both the leaves and green roots of *Aranda* 'Christine 130' and *Vanda suavis*, and Erickson (1957) reported high rates of photosynthetic oxygen evolution, along with high rates of respiratory oxygen consumption in the velamentous portions of the photosynthetic, aerial roots of a species of *Cattleya*. Furthermore, Benzing and Ott (1981) reported little or no atmospheric CO_2 uptake by the photosynthetic roots of eight of nine species of leafy orchids, and Benzing and Pockman (1989) argued that elemental nutrient limitations in epiphytic orchids might restrict the function of photosynthesis in these organs to reducing CO_2 losses, instead of assimilating carbon on a net positive basis. In contrast to many of the above studies, Arditti and Dueker (1968) reported greater atmospheric CO_2 uptake by roots than leaves in three orchid taxa. Thus, interpretation of the root $\delta^{13}C$ values in the current study must await further examination of the physiology of the green aerial roots of these epiphytic orchids to differentiate the comparative importance of photosynthetic discrimination against ^{13}C versus possible

Table 1. Names, parentage, and mean (± one standard deviation) $\delta^{13}C$ ratios of leaves, white (velamentous) portions of the roots, and green (nonvelamentous) root tips of four (occasionally three) different individuals of each of the 12 orchid taxa included in the study. Parentage information was taken from the International Orchid Register (Royal Horticultural Society; http://www.rhs.org.uk/plants/registerpages/orchidsearch.asp). Mean $\delta^{13}C$ ratios are significantly different ($p \leq 0.05$) unless sharing the same letter as superscript.

Taxon	Immediate parentage	Genera used to create genus	Abbreviations used in the figures	Leaf $\delta^{13}C$ [‰] mean ± SD	White Root $\delta^{13}C$ [‰] mean ± SD	Green Root $\delta^{13}C$ [‰] mean ± SD
Ascocenda Thai Beauty	*Vanda* Blue Kinzweiler X *Ascocenda* Fuchs Joy	*Ascocentrum, Vanda*	AscT	$-14.9^{a} \pm 3.9$	$-15.1^{a} \pm 1.7$	$-14.7^{b} \pm 2.6$
Ascocenda Princess Mikasa	*Ascocenda* Royal Sapphire X *Vanda coerulea*	*Ascocentrum, Vanda*	AscP	$-13.9^{a} \pm 3.9$	$-14.2^{a} \pm 8.2$	$-13.9^{b} \pm 3.1$
Ascocenda Su-Fun Beauty	*Ascocenda pralor* X *Vanda bangkapi*	*Ascocentrum, Vanda*	AscS	$-13.9^{a} \pm 5.3$	$-13.7^{a} \pm 2.0$	$-13.3^{b} \pm 2.2$
Bokchoonara nodata maili	unknown	*Arachnis, Ascocentrum, Phalaenopsis, Vanda*	BokN	$-15.8^{a} \pm 5.0$	$-15.8^{b} \pm 6.7$	$-13.4^{b} \pm 3.2$
Dendrobium crumenatum Sw.	naturally occurring species	not applicable	DenC	$-13.6^{a} \pm 4.1$	$-12.9^{ab} \pm 1.7$	$-11.8^{b} \pm 1.5$
*Doritis (*now *Phalaenopsis) pulcherimma* var *alba*	naturally occurring variety	not applicable	DorP	$-14.7^{a} \pm 5.0$	$-15.7^{a} \pm 8.2$	$-15.2^{a} \pm 5.2$
Kagawara Christie Low	*Renanthera* Nancy Chandler X *Ascocenda* Memoria Choo Laikeun	unknown	KagC	$-15.4^{a} \pm 1.2$	$-15.1^{a} \pm 4.8$	$-14.2^{a} \pm 2.8$
Mokara Chao Praya Gold	*Mokara* Bangkok Gold X *Ascocenda* Madame Kenny	*Arachnis, Ascocentrum, Vanda*	MokC	$-15.8^{a} \pm 5.5$	$-15.1^{a} \pm 4.7$	$-14.6^{a} \pm 2.9$
Paphiopedilum Lost Horizon	*Paphiopedilum* Wend Valley X *Paphiopedilum* White Legacy	not applicable	PapL	$-18.8^{a} \pm 1.4$	$-16.1^{ab} \pm 5.7$	$-15.6^{b} \pm 9.6$
Renanthera stolata	naturally occurring species	not applicable	RenS	$-14.7^{a} \pm 4.6$	$-14.4^{a} \pm 3.0$	$-14.3^{a} \pm 4.2$
Vanda Miss Joaquim	*Vanda hookeriana* X *Vanda teres*	not applicable	VanM	$-13.4^{a} \pm 2.8$	$-12.2^{a} \pm 1.2$	$-12.9^{a} \pm 2.6$
Vascostylis Veerewan	unknown	unknown	VasV	$-17.1^{a} \pm 9.3$	$-15.9^{a} \pm 5.8$	$-15.2^{a} \pm 5.3$

discrimination associated with the movement and/or subsequent metabolism of translocated carbon. Overall, the finding of high (not very negative) $\delta^{13}C$ values in the leaves and roots of the taxa examined in this study most likely indicates that the roots are assimilating atmospheric carbon at night or that they import the majority of their carbon from the CAM leaves. The latter is most likely in the nine taxa lacking a diel change in tissue titratable acidity reported here.

In most of the taxa examined here, fresh mass (FM)/DM ratios were highest in the green, non-velamentous root tips and lowest in either the leaves or the white, velamentous portions of the roots (Table 2). These findings clearly reflect the greater proportion of rigid, structural support tissues in the latter organs, relative to the former. Although tissue succulence, one measure of which can be the leaf FM/DM ratio, often correlates well with CAM (Hew 1976, Avadhani *et al*. 1976, 1982, Kluge and Ting 1978, Teeri *et al*. 1981, Earnshaw *et al*. 1987, Kluge *et al*. 1995, but *see* Martin *et al.* 2009), this correlation was weak ($R^2 = 0.12$; $P<0.05$) in the current study, most likely a result of the over-riding importance of structural support in the leaves and velamentous roots of these epiphytic orchids.

In summary, using diel fluctuations in tissue acidity as an indicator of CAM, the photosynthetic pathway of the

Table 2. Mean (± one standard deviation) fresh mass (FM)/dry mass (DM) ratios of leaves, white (velamentous) portions of the roots, and green (nonvelamentous) root tips for four (occasionally three) different individuals of each of 12 orchid taxa (*see* Table 1 for more information about the taxa). For each taxon, FM/DM ratios are significantly different ($p \leq 0.05$) unless sharing the same letter as superscript.

Taxon	Leaf FM/DM	White root FM/DM	Green root FM/DM
Ascocenda Thai Beauty	$8.56^{a} \pm 0.47$	$10.74^{ab} \pm 1.13$	$13.64^{b} \pm 0.47$
Ascocenda Princess Mikasa	$6.22^{a} \pm 0.24$	$9.20^{a} \pm 1.18$	$13.25^{a} \pm 0.47$
Ascocenda Su-Fun Beauty	$6.53^{a} \pm 0.33$	$7.61^{b} \pm 1.25$	$10.80^{b} \pm 0.47$
Bokchoonara nodata maili	$9.55^{a} \pm 0.76$	$11.03^{b} \pm 1.26$	$19.76^{b} \pm 0.47$
Dendrobium crumenatum Sw.	$12.85^{a} \pm 1.52$	$6.26^{b} \pm 0.59$	$10.21^{ab} \pm 0.47$
*Doritis (*now *Phalaenopsis) pulcherimma* var *alba*	$15.55^{a} \pm 0.97$	$12.46^{b} \pm 0.40$	$16.51^{ab} \pm 0.47$
Kagawara Christie Low	$6.23^{a} \pm 0.16$	$6.40^{a} \pm 0.74$	$11.82^{b} \pm 0.47$
Mokara Chao Praya Gold	$6.89^{a} \pm 0.70$	$11.51^{b} \pm 0.61$	$10.14^{b} \pm 0.47$
Paphiopedilum Lost Horizon	$9.68^{a} \pm 0.47$	$5.52^{b} \pm 0.41$	$13.36^{b} \pm 1.58$
Renanthera stolata	6.47 ± 0.15	9.40 ± 0.39	11.29 ± 0.47
Vanda Miss Joaquim	$8.35^{a} \pm 0.53$	$5.21^{a} \pm 0.82$	$12.82^{a} \pm 0.47$
Vascostylis Veerewan	8.79 ± 0.30	8.59 ± 1.03	14.12 ± 0.47

green roots of nine of 12 epiphytic orchid taxa having CAM leaves did not match that of the leaves on the same plant. Similar findings were reported for nine species of the orchid genus *Phalaenopsis* (Motomura *et al*. 2008). Perhaps the C_3 roots of these CAM epiphytes are typically too shaded to support the high energetic demand of CAM (Winter and Smith 1989b). This suggestion is supported by the Chl data reported here, *i.e.*, the Chl concentrations of the roots were higher, and their *a*/*b* ratios lower than the values found for the leaves. In addition, because the photosynthetic roots of orchids typically lack stomata, the primary adaptive benefit of CAM, *i.e*., high water-use efficiency, is difficult to envision for the green, aerial roots of orchids.

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