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CARBON FIXATION IN ORCHID AERIAL ROOTS

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SUMMARY

Orchid aerial roots contained chlorophylls and were shown to be capable of photosynthesis. $^{14}CO_2$ feeding in the light showed that the C_3 pathway was operating during the day. Aerial roots also exhibited diurnal acidity fluctuations typical of crassulacean acid metabolism (CAM) and malate was the only labelled compound isolated after night $^{14}CO_2$ feeding. CO_2 exchange patterns in aerial roots were consistent with CAM CO_2 fixation during the night hours. The rate of photosynthetic CO_2 fixation in the light was much greater than the rate of CAM CO_2 fixation in the night. Measurements of CO_2 exchange, however, showed little or no net uptake indicating that these roots were not completely autotrophic.

INTRODUCTION

Fixation of CO_2 in orchid leaves has been widely studied. These studies indicate that thick-leaved succulent orchids are basically crassulacean acid metabolism (CAM) plants while thin-leaved orchids photosynthesize via the Calvin–Benson cycle (C_3 pathway) (Avadhani *et al.*, 1982). Recent studies on carbon fixation as well as acidity and CO_2 production rhythms in orchid flowers showed that flowers of succulent orchids could exhibit both CAM and C_3 photosynthesis (Goh, 1983).

In contrast, reports on carbon fixation in orchid roots are scanty. Orchid aerial roots are green and can photosynthesize. Indeed, gaseous exchange experiments showed that green roots of *Cattleya gigas*, *Epidendrum xanthium*, *Phalaenopsis* hybrid and *Vanda suavis* could carry out photosynthesis but the rate of respiration was even higher (Dycus and Knudson, 1957) so that there was no net CO_2 fixation. Similar results were also shown by Erickson (1957) with an unnamed *Cattleya* hybrid and Hew (1976) in *Aranda* Wendy Scott.

Fixation of labelled CO_2 in the light was demonstrated by Dycus and Knudson (1957) in the aerial roots of the same four orchids they studied. However, the exposure period was long (96 h) and the mechanism of fixation was not investigated. The present experiments were conducted to study carbon fixation in aerial roots of succulent orchids.

MATERIALS AND METHODS

Plant material. Two terrestrial orchid hybrids, *Arachnis* Maggie Oei and *Aranda* Deborah were used in the present study. Both orchids have succulent leaves.

In all experiments, aerial roots were harvested from plants grown in garden beds of the Botany Department, National University of Singapore.

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Measurement of CO_2 production. Production of CO_2 by aerial roots was measured with a Beckman model 865 infra-red gas analyser connected to a recorder in an open gas flow system. For measurements with intact aerial roots, established cuttings with terrestrial roots partly immersed in a basin of distilled water were used. The aerial root was inserted through tight-fitting rubber stopper of a cylindrical glass respiratory chamber 200 cm^3 in volume. For excised aerial roots, seven roots of about 10 g fresh weight were allowed to stand in a small amount of distilled water within the respiratory chamber. The flow rate of air was maintained at 500 cm^3 min^{-1} by a Gilmont ball-type flow meter. When scheduled, light was provided with a cool mercury lamp at 300 μE s^{-1} m^{-2} (photosynthetically-active radiation, PAR, measured with a LI-COR LI-185B light meter connected to a LI-190SB quantum sensor). All measurements were conducted in an air-conditioned laboratory at 24 to 25 °C.

Acidity measurement. Aerial roots of about 10 g fresh weight were extracted in boiling water and their acidity determined by titration with 0·01 N NaOH using phenolphthalein as indicator (Goh *et al.*, 1977). Comparable samples were used for dry weight determinations. The results are expressed as microequivalents of acid per gram fresh or dry weight of tissue (μequiv g^{-1} fresh or dry weight).

Chlorophyll content. Freshly harvested roots each of about 5 cm length were divided into tip portions (apical segment without the velamen) and sub-apical mature portions (with mature velamen layers). They were extracted in 80 % (v/v) acetone and chlorophyll contents determined by the method of Arnon (1949).

$^{14}CO_2$ feeding experiments. Feeding of $^{14}CO_2$ was conducted with excised root segments (both apical and sub-apical segments) in 50 ml Erlenmyer flasks. Root samples (3 to 4 g fresh weight) were placed on moist filter paper in flasks within which were small vials containing 0·2 ml of NaH $^{14}CO_3$ (20 μCi/2·5 μmol). The flasks were then capped with subaseal serum caps. $^{14}CO_2$ was generated at the scheduled time by injection of 1·0 ml lactic acid into the bicarbonate solution with a hypodermic syringe. The flasks were then kept under dark or light (150 μE s^{-1} m^{-2}) conditions for the required feeding period. The flasks were then evacuated and the root tissues killed and extracted in boiling 80 % (v/v) ethanol followed by distilled water. The extracts were concentrated and the radioactivity measured with a Packard Tri-carb 240 CL/D liquid scintillation counter using a premixed emulsifier/scintillator 299 cocktail. Radioactivities of the residues from samples in light fixation were also measured after digestion with Soluene 350.

The extracts were further analysed by two-dimensional thin-layer chromatography (t.l.c.) with the following solvent systems: (1) phenol:water (4:1 v/v) and (2) tertiary amylalcohol:formic acid:water (3:1:3 by volume) (TAW). Organic acids were detected by spraying chromatograms with 0·04 % (w/v) bromocresol purple solution (Ranson, 1955). Radioautographs of chromatograms were prepared with Kodak X-Omat/s rapid films.

OBSERVATIONS AND RESULTS

Both the orchid hybrids, *Arachnis* Maggie Oei and *Aranda* Deborah, are monopodial and terrestrial. Aerial roots are produced at intervals around the nodes along the erect stem. In an actively growing aerial root, the tip region, ranging from 0·5 to

about 2·0 cm, remains green and fleshy; beyond this region, the velamen tissues become well differentiated and the root appears white when dry but green when moist.

Many aerial roots of *Aranda* Deborah appeared purplish-green indicating the presence of anthocyanin pigments in addition to chlorophyll.

When an aerial root enters the substratum, it branches repeatedly to form the terrestrial roots. These terrestrial roots are white or yellowish in colour.

Chlorophyll content. The total chlorophyll content of the apical and sub-apical (mature) tissues of the aerial roots of *Arachnis* Maggie Oei was about the same, around 66 μg g^{-1} fresh weight. In *Aranda* Deborah, the apical region appeared to have a higher chlorophyll content (78 μg g^{-1} fresh weight) than the mature region (58 μg g^{-1} fresh weight).

Production of CO_2. The pattern of CO_2 production was similar in aerial roots of *Arachnis* Maggie Oei and *Aranda* Deborah. In both cases, the rate of output was low, around 15 to 20 μg g^{-1} fresh weight h^{-1}. With intact root of *Arachnis* [Fig. 1(a)], CO_2 output was slightly more than doubled when the light was turned

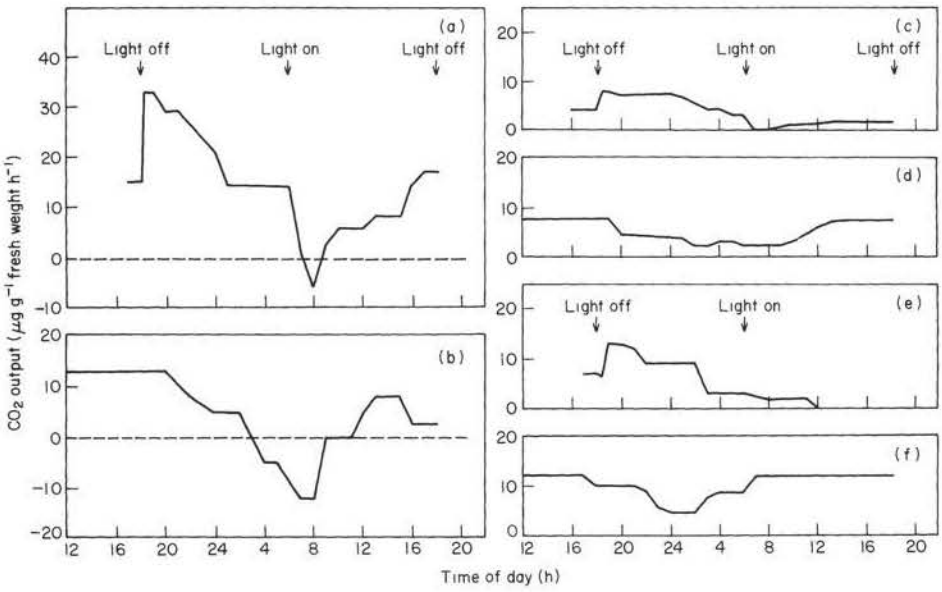

Fig. 1. Exchange of CO_2 in orchid aerial roots. (a) *Arachnis* Maggie Oei, intact root in light/dark cycle. (b) *Arachnis* Maggie Oei, intact root in continuous dark. (c) *Arachnis* Maggie Oei, excised roots in light/dark cycle. (d) *Arachnis* Maggie Oei, excised roots in continuous dark. (e) *Aranda* Deborah, excised roots in light/dark cycle. (f) *Aranda* Deborah, excised roots in continuous dark.

off at 18.00 h. However, within 2 h, the rate began to decrease and by 01.00 h, it reached a steady rate of around 15 μg CO_2 g^{-1} fresh weight h^{-1} until 06.00 h when light was turned on again. 'Light-on' caused a sharp drop in CO_2 output to zero within an hour and for the next hour or so, there was some CO_2 uptake by the root tissues. Output of CO_2 then gradually increased to around 10 μg g^{-1} fresh weight h^{-1} over 7 or 8 h and further increased to about 15 μg in the late afternoon.

When the intact aerial root was kept in continuous darkness from around noon, the rate of CO_2 output remained steady at 13 μg g^{-1} fresh weight h^{-1} for about 8 h [Fig. 1(b)]. From 20.00 h it decreased steadily to zero by 03.00 h and CO_2 uptake was recorded for the next 5 to 6 h before output increased again.

Results obtained with excised aerial roots of *Arachnis* [Fig. 1(c), (d)] as well as those of *Aranda* Deborah [Fig. 1(e), (f)] showed essentially the same rhythm although the rate of CO_2 output was about half that of the intact roots. There was, however, no indication of net CO_2 uptake throughout the periods of measurement.

Acidity. Titratable acidity in the roots of both *Arachnis* Maggie Oei and *Aranda* Deborah started to increase before 18.00 h and reached a peak at 09.00 h after which it decreased to a minimum at 15.00 h (Fig. 2) When fluctuations in

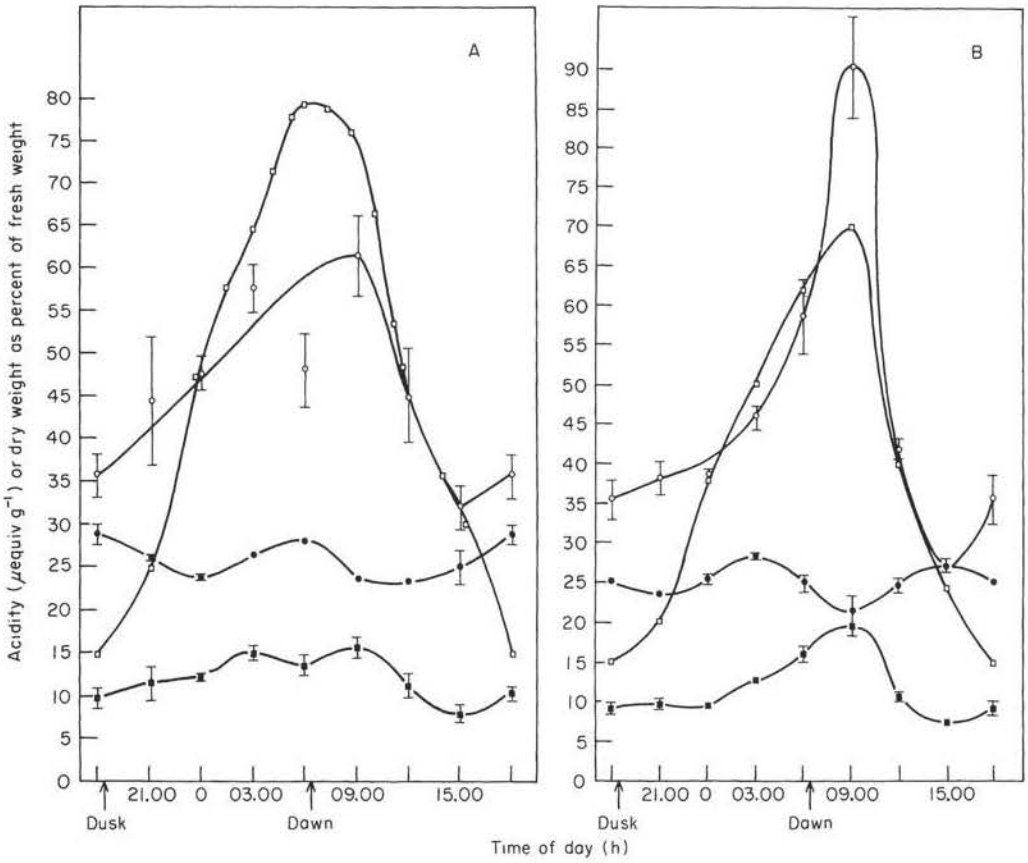

Fig. 2. Acidity and fresh weight changes in orchid aerial roots. (a) *Arachnis* Maggie Oei, (b) *Aranda* Deborah. The acidity changes of leaves (□, data from Goh *et al.*, 1977) were included for comparison. ○, μequiv g^{-1} dry weight; ■, μequiv g^{-1} fresh weight; ●, (dry weight/fresh weight) × 100. Vertical bars indicate standard deviation.

water content was taken into consideration (see below), the maximum acidity at 09.00 h was at least 7 μequiv g^{-1} fresh weight higher than the minimum level at 15.00 h (around 8 μequiv g^{-1} fresh weight). This trend of diurnal fluctuation was even more evident when the acidity was expressed in terms of microequivalents

per gram dry weight. However, acidity fluctuations in the roots were of a much smaller magnitude compared to those in the leaves.

When aerial roots were excised at high acidity (09.00 h) or low acidity (15.00 h) levels, deacidification or acidification proceeded to similar extent as intact roots (Table 1).

Table 1. *Titratable acidity in orchid aerial roots*

Orchid hybrid	Time excised (h)	Acidity* (μequiv g^{-1} fresh weight) 09.00 h	15.00 h
Arachnis Maggie Oei	09.00	21·93 ± 4·32	—
	09.00	—	10·10 ± 1·26
	15.00	—	10·69 ± 2·33
	15.00†	17·97 ± 1·90	—
Aranda Deborah	09.00	17·26 ± 2·33	—
	09.00	—	8·28 ± 0·55
	15.00	—	8·25 ± 0·14
	15.00†	18·78 ± 0·60	—

* Averages of four replicates ± standard deviation.

† These samples were kept overnight in the dark and their acidity determined the next morning at 09.00 h.

Dry weight/fresh weight content. The water content of aerial roots was found to be relatively low, ranging from 70 to 80 % of fresh weight. It did not remain constant but fluctuated throughout the day (Fig. 2). Dry weight as percentage of fresh weight exhibited a rhythm with two maxima/minima points. In *Arachnis* Maggie Oei, the two peaks were observed at 06.00 and 18.00 h and the minima at midnight and 09.00 to 12.00 h. In *Aranda* Deborah, one peak (the highest) occurred at 03.00 h and the second (lower) peak at 15.00 h; one minimum (the higher) occurred at 21.00 h and the second (lower) at 09.00 h. In both *Arachnis* and *Aranda*, maximum acidity at 09.00 h coincided with the highest level of water content (lowest dry weight/fresh weight ratio) in the aerial roots.

Fixation of $^{14}CO_2$. In the first series of experiments, short-term feeding periods ranging from 8 s to 3 min in the light showed very poor fixation. Thus, relatively long-term fixation (3 h in light or 12 h in dark) was conducted in the second series of experiments. The results (Table 2) showed orchid aerial roots fixed CO_2 both in the light and in the dark. Fixation occurred in the green apical region as well as the region with mature velamen layers (sub-apical).

In *Aranda* Deborah, the mature segment of the aerial root fixed relatively greater amount of CO_2 than the green apical segment. This was also observed in *A.* Maggie Oei in the dark. Unfortunately no data were obtained for apical segment in the light for similar comparison. In all cases, the rate of fixation in the dark was only a fraction of that in the light, amounting to less than 15 % in soluble fraction and less than 9 % when residues were considered (Table 2).

The amount of radioactivity remained in the residual matter of roots after 3 h of light fixation was quite substantial, ranging from 29 to 36 % of total fixation (Table 2). Residues from night (dark) $^{14}CO_2$ fixation experiments contained only negligible amounts of radioactivity.

The products of $^{14}CO_2$ fixation in the extracts were analysed by t.l.c. and

Table 2. *Radioactivity incorporated by orchid aerial roots exposed to $^{14}CO_2$-enriched atmosphere*

Orchid hybrid	Root region	Period of exposure	Total radioactivity fixed (d.p.m. g^{-1} fresh weight)		Rate of fixation* (d.p.m. g^{-1} fresh weight h^{-1})
			Ethanolic extract	Residue	
Arachnis Maggie Oei	Apical	20.00–08.00 (dark)	108320	—	9030
	Sub-apical	11.00–14.00 (light)	356550	146080	167540
		20.00–08.00 (dark)	174190	—	14520
Aranda Deborah	Apical	11.00–14.00 (light)	158930	65600	74840
		20.00–08.00 (dark)	6620	—	550
	Sub-apical	11.00–14.00 (light)	221570	124650	115410
		20.00–08.00 (dark)	116600	—	9720

* Assuming CO_2 incorporation was at constant rate during the feeding period.

radioautography. Dark fixation in all cases resulted in only one labelled spot identified as malate (confirmed by co-chromatography with authentic malate in TAW system). This was true for both the apical as well as the sub-apical segments. On the other hand, light fixation yielded phosphoglyceric acid and related compounds with very little movement in either of the solvent systems used.

DISCUSSION

Orchid aerial roots contain chlorophylls and are capable of photosynthesis. Our present data support earlier suggestions by Dycus and Knudson (1957) and others. The rate of CO_2 production was more or less doubled soon after the light was turned off; and 'light-on' caused a decrease in CO_2 output. This was observed in both intact as well as excised roots (Fig. 1). Feeding with $^{14}CO_2$ in the light showed incorporation of radioactivity in C_3 photosynthetic intermediates indicating that the C_3 pathway was operating in the aerial roots during the day (light) period.

More interestingly, aerial roots showed diurnal acidity fluctuations typical of CAM (Fig. 2, Table 1). The actual acidity changes were comparatively small, but there is no doubt that CO_2 was being fixed into malate during the night. Indeed, male was the only labelled compound isolated after 12 h of night (dark) ^{14}CO feeding. This was shown for both the apical and sub-apical segments of the roots of both the hybrids. In this connection, it is of relevance that phospho-enol pyruvate (PEP) carboxylase activity has been demonstrated in aerial roots of *Aranda* Christine, another succulent monopodial orchid (Eng, 1982).

That CAM operates in these aerial roots is also supported by their CO_2 production rhythms. In aerial roots subjected to light/dark cycles, while light-off

increased CO_2 production, the rate was however not maintained but decreased during the night [Fig. 1(a)]. A decrease in the rate of CO_2 output during the night period was also observed in roots kept under continuous dark from noon. This was shown even more clearly in excised roots [Fig. 1(d), (f)]. Furthermore, the timing of the decrease and subsequent increase roughly coincided with the normal diurnal rhythm of acidification and deacidification, indicating a circadian (though weak) phenomenon. These observations are consistent with CAM CO_2 fixation during the night hours.

It may be noted that the magnitude of acidity fluctuations in aerial roots is comparable to that observed in orchid flowers and about one-tenth that of the leaves (Fig. 2). Chlorophyll content of aerial roots was four to six times that of flowers and about one-third that of the leaves (Goh *et al.*, 1977; Goh, 1983). In *Aranda* Christine, crude enzyme preparations from aerial roots contained as much as one-half the activity of PEP carboxylase as from the leaves in terms of chlorophyll content (Eng, 1982). The capacity for CAM in these aerial roots might therefore not be limited by PEP carboxylase levels and chlorophyll contents (energy levels) but more likely by the inadequate removal of malate from the site of synthesis as well as the size of the sink (Lüttge *et al.*, 1982).

As mentioned earlier, CO_2 output from aerial roots was not constant throughout the night (or dark) period but decreased from around 20.00 h to morning hours (Fig. 1). This observation was in contrast to that of Hew (1976) on *Aranda* Wendy Scott which showed 'a gradual increase in respiratory rate reaching a steady state rate after three hours in darkness'. *Aranda* Wendy Scott is also a succulent monopodial orchid, and like *Arachnis* Maggie Oei and *Aranda* Deborah, exhibited CAM in both the leaves as well as the flowers (Goh, 1983). It may be expected that their aerial roots would have a similar pattern of CO_2 exchange. The difference is yet to be resolved. However, it is recognized that the operation of CAM can be affected by environmental conditions (Kluge and Ting, 1978).

It has to be pointed out that the rate of photosynthetic CO_2 fixation in the light was much greater than the rate of CAM CO_2 fixation in the dark. In the mature sub-apical segments of the aerial roots, the rate of CAM CO_2 fixation was only 8 to 9% of the light photosynthetic rate; at the apical meristematic region, it was less than 1% (Table 2). Moreover, about one-third of the carbon fixed in light was quickly incorporated into ethanol-insoluble cellular constituents. And higher PAR may result in greater CO_2 fixation. Obviously the roots fixed more carbon via C_3 photosynthesis than CAM. Notwithstanding, measurements of CO_2 exchange showed little or no uptake but CO_2 output from the root tissues (Fig. 1), it indicates that these roots are not completely autotrophic and are still dependent on the leaves for nutritional support. This is consistent with the observation that extension growth in excised aerial roots ceased in 2 days (Goh, unpublished).

As far as we are aware, thin-leaved orchids do not have photosynthetic aerial roots. The thick-leaved succulent orchids are either epiphytic or terrestrial with aerial roots. It is suggested that photosynthetic aerial roots of these succulent orchids (which are CAM plants) exhibit both C_3 and CAM to varying proportions. The extreme examples of leafless orchids with only vestigial stems such as *Campylocentrum fasciola*, *C. pachyrrhizum*, *Dendrophylax funalis*, *Doritis taenialis*, *Harrisela porrecta*, *Polyrrhiza lindenii*, *Taeniophyllum filiforme* and *T. zollingeri* are obviously almost totally dependent on carbon fixation by the roots. Some of these orchids produce long inflorescences with large flowers. Their carbon fixation mechanism(s) could be much more efficient and remained to be studied.

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