CARBON FIXATION IN ORCHID AERIAL ROOTS

BY C. J. GOH, J. ARDITTI* AND P. N. AVADHANI

Department of Botany, National University of Singapore, Singapore 0511

(Accepted 25 June 1983)

SUMMARY

Orchid aerial roots contained chlorophylls and were shown to be capable of photosynthesis. 14 CO₂ feeding in the light showed that the C₃ 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 CO₂ feeding. CO₂ exchange patterns in aerial roots were consistent with CAM CO₂ fixation during the night hours. The rate of photosynthetic CO₂ fixation in the light was much greater than the rate of CAM CO₂ fixation in the night. Measurements of CO₂ exchange, however, showed little or no net uptake indicating that these roots were not completely autotrophic.

INTRODUCTION

Fixation of CO₂ 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 (C₃ pathway) (Avadhani et al., 1982). Recent studies on carbon fixation as well as acidity and CO₂ production rhythms in orchid flowers showed that flowers of succulent orchids could exhibit both CAM and C₃ 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 CO₂ fixation. Similar results were also shown by Erickson (1957) with an unnamed Cattleya hybrid and Hew (1976) in Aranda Wendy Scott.

Fixation of labelled CO₂ 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.

* On sabbatical leave at National University of Singapore during April–May 1981 and July 1982. Permanent address: Department of Developmental and Cell Biology, University of California, Irvine, CA 92717, USA.

© 1983 The New Phytologist
Measurement of CO₂ production. Production of CO₂ 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 cm³ 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 cm³ 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-18SB 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).

¹⁴CO₂ feeding experiments. Feeding of ¹⁴CO₂ was conducted with excised root segments (both apical and sub-apical segments) in 50 ml Erlenmeyer 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 ¹⁴CO₃ (20 µCi/2.5 µmol). The flasks were then capped with subaseal serum caps. ¹⁴CO₂ 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
Carbon fixation in orchid aerial roots

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 CO₂.** The pattern of CO₂ 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)], CO₂ output was slightly more than doubled when the light was turned 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 CO₂ g⁻¹ fresh weight h⁻¹ until 06.00 h when light was turned on again. 'Light-on' caused a sharp drop in CO₂ output to zero within an hour and for the next hour or so, there was some CO₂ uptake by the root tissues. Output of CO₂ 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.

![Graphs showing CO₂ exchange](image-url)
When the intact aerial root was kept in continuous darkness from around noon, the rate of CO₂ 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 CO₂ 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 CO₂ output was about half that of the intact roots. There was, however, no indication of net CO₂ 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 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

<table>
<thead>
<tr>
<th>Orchid hybrid</th>
<th>Time excised (h)</th>
<th>Acidity* (µequiv g(^{-1}) fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>09.00 h</td>
<td>15.00 h</td>
</tr>
<tr>
<td>Arachnis Maggie Oei</td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.00</td>
<td>21.93±4.32</td>
<td>10.10±1.26</td>
</tr>
<tr>
<td>15.00</td>
<td>10.69±2.33</td>
<td>10.90±1.90</td>
</tr>
<tr>
<td>Aranda Deborah</td>
<td>09.00</td>
<td>17.26±2.33</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>8.28±0.55</td>
</tr>
<tr>
<td></td>
<td>15.00+</td>
<td>18.78±0.60</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Orchid hybrid</th>
<th>Root region</th>
<th>Period of exposure</th>
<th>Total radioactivity fixed (d.p.m. g$^{-1}$ fresh weight)</th>
<th>Rate of fixation* (d.p.m. g$^{-1}$ fresh weight h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethanolic extract</td>
<td>Residue</td>
</tr>
</tbody>
</table>
| *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 CO₂ production, the rate was however not maintained but decreased during the night [Fig. 1(a)]. A decrease in the rate of CO₂ 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 CO₂ 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 (Lütge et al., 1982).

As mentioned earlier, CO₂ 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 CO₂ 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 CO₂ fixation in the light was much greater than the rate of CAM CO₂ fixation in the dark. In the mature sub-apical segments of the aerial roots, the rate of CAM CO₂ 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 CO₂ fixation. Obviously the roots fixed more carbon via C₃ photosynthesis than CAM. Nonetheless, measurements of CO₂ exchange showed little or no uptake but CO₂ output from the root tissues (Fig. 1), it indicates that the leaves are not completely autotrophic and are still dependent on the leaves for nutritional support. This is consistent with the observation that attachment of CO₂ exchange 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 C₃ and CAM to varying proportions. The extreme examples of leafless orchids with only vestigial stems such as Campylocentrum fasciola, C. pachyrhizum, Dendrophylax fulvalis, Doritis taenialis, Harrisella 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.
ACKNOWLEDGEMENTS

We thank Mrs T. Gan for help in the determination of chlorophyll content and Mr T. K. Ong for development of autoradiographs.

REFERENCES


