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PHOTOSYNTHESIS IN PRIMITIVE PLANTS

Stylites, a vascular land plant without stomata absorbs CO₂ via its roots

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Photosynthetic organs of most higher plants normally have access to atmospheric CO₂ through stomatal pores which also serve as variable valves to control the loss of H₂O vapour which accompanies CO₂ uptake¹. The acquisition of stomata is commonly thought to have been a crucial development permitting 'conquest' of land and direct access of plants to atmospheric CO₂. Only in desert stem succulents during drought do stomata remain so tightly closed in the light that the photosynthetic tissues are dependent on internal CO₂ generated through the photosynthetic pathway known as crassulacean acid metabolism². Functional stomata are absent in submerged aquatic plants and in non-vascular land plants (for example, mosses) which are normally covered by a water film. Although it is now clearly established that some aquatic plants assimilate large amounts of CO₂ from the sediment via roots³⁻⁵, terrestrial plants are thought to assimilate only insignificant amounts of CO₂ via this path⁶. Here we report on a terrestrial plant, *Stylites andicola*, which lacks stomata and is unable to exchange gas with the aerial atmosphere. Rather, it derives nearly all of its photosynthetic carbon through its roots. In addition, this species possesses characteristics of crassulacean acid metabolism.

S. andicola (Isoetaceae) is a rare fern ally found in small populations in the high Andes of Peru⁷. We studied a small population in the vicinity of Lago de Junin (4,135 m) in December 1982. There, as well as at other sites⁸, it forms dense colonies of hundreds of plants most commonly on hummocky ground elevated above the water level of the surrounding (seasonal) bog. The substrate is a highly decomposed peat (largely derived from *Stylites*) exceeding 1 m depth and overlying limestone. Interstitial groundwater pH was 6.4-6.6 and alkalinity ranged from 50 to 70 g m⁻³ CaCO₃.

Stylites produces an evergreen rosette (2-3 cm across) of leaves (2-4 cm long) arising from an elongated corm which may be branched (Fig. 1). These plants are embedded in the peat so

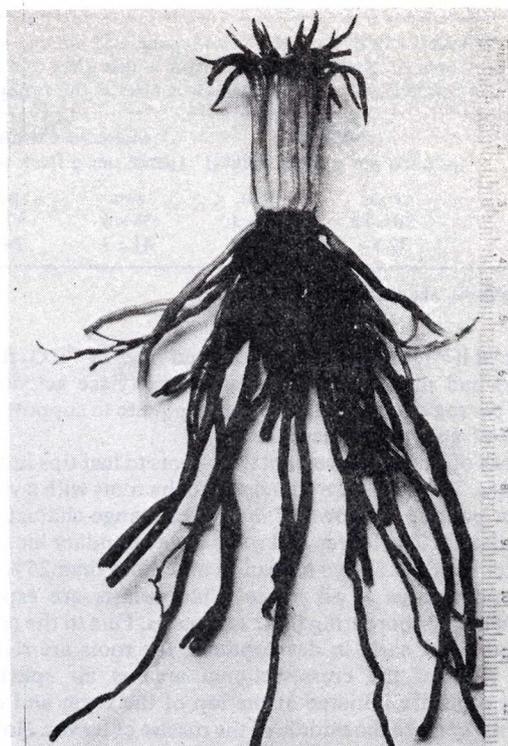


Fig. 1 *Stylites andicola*; scale is a 12-cm ruler.

that the bulk of the plant is underground; the only exposed surfaces are the upper-third chlorophyllous portion of the leaves which constitute a small part of the total biomass. Average plant dry weight = 0.829 g (s.d. = 0.19, $n = 4$); green portions of leaves = 6.6%, white portions = 18.0%, stems = 44.0% and roots = 31.4%.

Stylites leaves lack stomata and are covered with a thick continuous cuticle which is essentially impermeable to CO₂ and H₂O vapour. Measurements with a dual isotope porometer⁹ (on plants maintained as described in Table 1) gave CO₂ conductances (mm s⁻¹) in the morning and afternoon of 0.001 (s.d. = 0.001, $n = 4$) and 0.005 (s.d. = 0.007, $n = 4$), respectively. These conductances are nearly an order of magnitude lower than those observed for desert cacti with closed stomata².

When the photosynthetic tissues were exposed to ¹⁴CO₂ in the light, negligible rates of ¹⁴C incorporation were observed (Table 1). However, when the root system was exposed to ¹⁴CO₂, up to 50 times the amount of ¹⁴C was incorporated in the photosynthetic tissues. In field conditions this difference could be even greater as, during transplanting, the original root systems died and were only partially recovered at the time of these experiments. The root/green tissue ratio for oven-dried plants from the field was 5.5 ± 3.3 ($n = 9$), in contrast to a ratio of 2.5 ± 1.1 ($n = 12$) for the plants in Table 1.

In natural conditions the chlorophyll-containing tissues of *Stylites* showed a nighttime increase and a daytime decrease in titratable acidity and malic acid content (Table 2), characteristic of crassulacean acid metabolism (CAM) which is common in aquatic species in the Isoetaceae¹⁰. However, the amount of CO₂ recycled through CAM each night was only equivalent to ~1 h of photosynthesis when roots were exposed to ¹⁴CO₂. Carbon isotope discrimination, often helpful in identifying photosynthetic pathways and CO₂ sources in CAM, does not help to evaluate these processes in this case. The δ¹³C for green leaves (-25.7‰) was similar to that of the peat (-26.6‰) and groundwater carbonate (-25.0‰). These data are consistent with the assimilation of peat-derived CO₂ in a closed system, either through CAM or C₃ pathways of photosynthesis. The chlorophyllous tips of *Stylites* leaves contain sufficient activities of ribulosebisphosphate carboxylase (70-134 μmol per mg

Table 1 ¹⁴CO₂ uptake by chlorophyllous portions of *Stylites* leaves

		CO ₂ uptake by green leaves*	
		(μmol CO ₂ per mg chlorophyll h ⁻¹)	
		¹⁴ CO ₂ fed to leaves	¹⁴ CO ₂ fed to roots
Light	(Moist)	0.74 ± 0.14 (3)	39.75 ± 6.14 (3)
	(Wet)	1.12 ± 1.05 (2)	18.78 ± 0.79 (2)
Dark	(Moist)	0.26 ± 0.16 (2)	0.51 ± 0.07 (2)

Leaves and roots were isolated in separate chambers similar to those used in aquatic plant studies^{3,4}. In all trials green portions of leaves were kept in air and roots in 25 mM NaH₂PO₄, 2 mM NaHCO₃ buffer at pH 6.5, which approximated field carbon and pH conditions. NaH¹⁴CO₃ (4.7 mCi μmol⁻¹) was injected into either the root medium or a small tray of HCl in the leaf chamber to produce a final activity of 2 μCi ml⁻¹. Plants were incubated for 2 h at 25 °C with 1,000 μeinsteins m⁻² s⁻¹. Gentle stirring was applied in both the top and bottom of the chamber. Tissues were killed in boiling 80% ethanol, ground in a glass homogenizer, dried and resuspended in distilled H₂O and counted. Values are the mean ± s.d. (n = number of experiments). Before experiments, the plants had been maintained in either moist but drained peat or water-saturated peat at 13 °C during the day and 7 °C during the night on a 12-h photoperiod.

* These tissues contained 1.0 ± 0.2 mg chlorophyll per g fresh weight.

Table 2 Titratable acidity (to pH 6.4) and malic acid content in green portions of leaves of *Stylites* collected from moist sites and wet or temporarily submerged conditions in December at the Junin site

Site	Acidity ($\mu\text{equiv. per g fresh weight}$)		Malic acid content ($\mu\text{mol per g fresh weight}$)	
	a.m.	p.m.	a.m.	p.m.
Moist	20 \pm 10	9 \pm 1	34 \pm 6	17 \pm 1
Wet	32 \pm 4	10 \pm 2	41 \pm 7	24 \pm 5

For methods, see ref. 10 ($n = 2$).

chlorophyll h^{-1}) to support the measured rates of $^{14}\text{CO}_2$ fixation (Table 1) and phosphoenolpyruvate carboxylase activity (24–34 $\mu\text{mol per mg chlorophyll h}^{-1}$) was adequate to support observed rates of acid accumulation.

The path of carbon movement from roots to leaf tips in *Stylites* is unknown. The leaves are connected to the roots with a vascular trace⁸ but nothing is known of the gas exchange characteristics of these tissues. The leaves and roots have abundant air spaces; the former have four large air canals and more than 25% of the cross-sectional area is air space. Chloroplasts are especially abundant in cells bordering these air spaces. Due to the collapse of cortical tissue early in development, the roots are relatively hollow; 75% of the cross-sectional area is air space. The youngest roots are initiated at the top of the corm and extend through the corm to the middle of the rosette of leaves. However, older roots may be separated from leaves by a centimetre or more of spongy corm tissue.

This study raises intriguing questions concerning the photosynthetic physiology, as well as ecological and phylogenetic relationships of these primitive land plants. In the natural habitat, colonies of *Stylites* are interspersed amongst a dense array of other plants. Some degree of convergence is evident in the superficially similar rosette arrangement of photosynthetic parts of *Stylites* and associated species. However, in the major aspects discussed here, *Stylites* is unique; all the other rosette-forming

species associated with *Stylites* possess stomata and finely divided roots with a dense cortex.

Whether the morphology and photosynthetic physiology of *Stylites* represents an important link between aquatic plants and primitive land plants, or whether it is merely an interesting variant, merits further careful assessment. Although cuticles of leaf-like structures which lack stomata are known from the Devonian/Silurian, it is not always easy to find stomata even in well preserved fossils¹¹. Organisms such as *Stylites* may represent relics of many marginally successful compromises from which present day terrestrial photosynthetic systems were selected. With their principal functional counterpart, the seasonally sealed stem succulents, *Stylites* share an ability to carry out CAM. Their capacity for CAM supports the notion that this pathway of photosynthetic carbon assimilation has long been part of the metabolic background of higher plants¹². Because *Stylites* lacks stomata and exhibits CAM, this unusual organism does not conform to the hypothesis that CAM may have evolved from stomatal guard cell metabolism¹³.

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1. Farquhar, G. D. & Sharkey, T. D. A. *Rev. Pl. Physiol.* **33**, 317–345 (1982).
2. Kluge, M. & Ting, I. P. *Crassulacean Acid Metabolism* (Springer, Berlin, 1978).
3. Wiium-Andersen, S. *Physiologia Pl.* **25**, 245–248 (1971).
4. Sondergaard, M. & Sand-Jensen, K. *Aquat. Bot.* **6**, 1–12 (1979).
5. Richardson, K., Griffiths, H., Reed, M. L., Raven, J. A. & Griffiths, N. M. *Oecologia* **61**, 115–121 (1983).
6. Berquist, N. O. *Bot. Notiser* **117**, 249–258 (1964).
7. Amstutz, E. *Ann. Mo. bot. Gdn* **44**, 121–123 (1957).
8. Rauh, W. & Falk, H. *Sber. heidelb. Akad. Wiss.* **1**, 1–83; **2**, 1–160 (1959).
9. Johnson, H. B., Rowlands, P. G. & Ting, I. P. *Photosynthetica* **13**, 409–418 (1979).
10. Keeley, J. E. *Am J. Bot.* **69**, 254–257 (1982).
11. Edwards, E., Edwards, D. S. & Rayner, R. In *The Plant Cuticle* (eds Cutler, D. F., Alvins, K. L. & Price, C. E.) 341–361 (Academic, New York, 1982).
12. Osmond, C. B. in *Proc. VI int. Photosynth. Congr.* Vol III, 557–564 (Junk, The Hague, 1984).
13. Cockburn, W. *Pl. Cell Envir.* **4**, 417–418 (1981).