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# Anatomy and photosynthetic parameters of roots and leaves of two shade-adapted orchids, *Dichaea cogniauxiana* Shltr. and *Epidendrum secundum* Jacq.

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## Abstract

This study compares photosynthetic and structural features of *Dichaea cogniauxiana* and *Epidendrum secundum* leaves and roots. The diurnal titratable acidity fluctuations indicated crassulacean acid metabolism (CAM) in *E. secundum* leaves, associated with anatomical features like thick cuticle, large and vacuolated cells, and reduced stomata size and frequency. Roots of both species had chloroplasts in their cortical parenchyma. However, neither the roots nor *D. cogniauxiana* leaves did show tissue sap acidity fluctuations. This indicates $C_3$ metabolism in these organs. This lack of oscillation of organic acids in *Epidendrum* roots was at odds with a CAM-like $^{13}C$ ratio, suggesting that in spite of active $CO_2$ fixation in roots during the day, the bulk of carbon is imported from the leaves. Roots of both species showed Fv/Fm, $\Delta F/Fm'$, ETR values similar to reports from other non-foliar photosynthetic organs. Besides reducing root carbon cost, root photosynthesis may also be important by alleviating potential hypoxia, since water-saturated velamen severely impedes the gas exchange between radicular cortex.


***Keywords:*** Orchidaceae; Epiphyte; Root anatomy; CAM; Fv/Fm; Non-foliar photosynthesis

## Introduction

The basic body of a vascular plant is differentiated in a subterranean root system and an aerial shoot system with stems and leaves. This reflects a division of labor with roots specializing on water and nutrient uptake, leaves on photosynthesis, and stems representing a transport system for water, nutrients or organic compounds between roots and leaves. Such a separation is much less obvious among many vascular epiphytes. For example, in many epiphytic bromeliads, roots serve primarily as holdfast, with nutrient uptake by leaves via absorbing scales (Benzing, 2000). Moreover, roots growing exposed on the bark of trees may be able to fix $CO_2$, in the extreme case being the only photosynthetically active organs in the so-called "shootless" orchids (Benzing et al., 1983; Winter et al., 1985). Finally, other organs of orchids, e.g. petals and sepals (Arditti, 1979), fruits (Lemos Filho and Isaias, 2004;

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Zotz et al., 2003), or pseudobulbs (Ng and Hew, 2000) may also be photosynthetically active.

This diversity in function is only partly reflected in the literature (Zotz and Hietz, 2001). Most studies on photosynthesis in epiphytes focus exclusively on leaves, although there is a growing body of studies on the physiology of aerial roots in particular. It has been shown, e.g. that functional chloroplasts are located in cortical parenchyma cells (Benzing et al., 1983; Ho et al., 1983), or that both $C_3$ and crassulacean acid metabolism (CAM) pathways can be found (Benzing and Ott, 1981; Benzing et al., 1983; Cockburn et al., 1985; Dycus and Knudson, 1957; Erickson, 1957; Flores et al., 1993; Hew et al., 1984; Winter et al., 1985).

Carbon dioxide fixation in aerial roots with CAM is similar to the one typically present in leaves of CAM plants with nocturnal $CO_2$ uptake by PEP-carboxylase and daytime $CO_2$ assimilation. The production of organic acids is followed by a reduction during the day, with malate, aspartate, and citrate as the principal products of the nocturnal $CO_2$ fixation (Borland et al., 1998; Bradbeer et al., 1957; Knauft and Arditti, 1969; Medina et al., 1993; Ting, 1985). Noteworthy, aerial roots of leafless orchids cannot control water loss as leaves of typical CAM plants do by stomatal closure, i.e. the CAM pathway cannot promote water efficiency by the reduction of transpiration in roots. On the other hand, nocturnal malic acid production and storage reduces the osmotic potential in aerial roots, which can assist in water absorption (Lüttge, 1989).

Few studies have attempted to compare the function of leaves and roots. However, only such a comparative approach may allow us to put the physiology of individual organs into context, i.e. understand the relative importance within an entire organism. This motivated the present research in which we studied morphological, anatomical, and physiological features of leaves and roots of two species of epiphytic orchids under field conditions in a cloud forest in southeast Brazil.

## Material and methods

The study area, the Serra da Piedade, Minas Gerais, Brazil (ca. 19°49′11″S, 43°40′12″W, at an elevation of approximately 1300 m) is characterized by high humidity, and frequent occurrence of fog. Two common epiphytes were chosen for study: *Dichaea cogniauxiana* Shltr., which is normally growing in the forest at rather low light intensities, and *Epidendrum secundum* Jacq., which was found at the edge of the forest, near a "campo rupestre" area. The second species also occurs on rocks under even higher light intensities. All analyses were done with material collected in the field during sunny days in January 2004. In the following, species are addressed by their genus names.

### Anatomy and morphology

For morphometric data, leaves were counted and one side surface measured. The root surface areas were estimated using the formula $A = h(2\pi r)$, where $h$ corresponds to the total root length, and $r$ is the median of 20 measurements of root radius along the axis (Rossielo et al., 1995). Leaves, stems, and roots were dried at 50 °C for 48 h, and weighed.

Transverse and longitudinal sections were prepared from fresh and $FAA_{50}$ fixed leaf and root fragments (Johansen, 1940). Fresh hand-cut sections were stained with safranin-astra blue (Bukatsch, 1972, modified to 0.5%). Fixed material was embedded in glycol-methacrylate, sectioned (5–7 μm) in a rotatory microtome, and stained with 0.05% toluidine blue O in 0.1 M phosphate buffer (O'Brien et al., 1964). Starch grains were detected using the Lugol reagent (Johansen, 1940). Epidermis was excised from totally expanded leaf samples by immersion in 50% sodium hypochlorite. The fragments were washed in running water, stained with 1% safranin in 50% ethanol (Berlyn and Miksche, 1976) and mounted in Kaiser gelatin (Kraus and Arduin, 1997).

Stomata pore area measurements ($n = 40$) were done on pieces of excised epidermis ($n = 15$) using the image analysis software Motic Images version 1.2 for Windows (Micro Optic Industrial Group Co Hong Kong, Japan). Stomata pore area ($A_{st}$) was estimated by the formula $A_{st} = \pi R_1 R_2$, where $R_1$ and $R_2$ correspond to the larger and smaller radius, respectively, of the stomata pore area. The fraction occupied by the stomata pore area in the leaf was calculated by the formula: $n_{st}A_{st}$, where $n_{st}$ corresponded to stomata number per area.

Succulence and specific mass were estimated using 30 leaf and root fragments per species. Leaf areas were calculated scanning leaves and using Quantikov software (Pinto, 1996). Root areas of both species were obtained according to Rossiello et al. (1995). The succulence (SU) was determined using the difference between fresh (FW) and dry weight (DW), where $SU = (FW-DW)/A$. Specific mass (SM) was determined according to Witkowski and Lamont (1991) by the ratio between dry weight and tissue area ($SM = DW/A$).

### Physiology

To detect diurnal titratable acidity fluctuations ($\Delta H^+$), about 200 mg of leaf and root fragments ($n = 5$ plants) were collected in the field at approximately 8:00 and 18:00 h. After 1 h of transport at low temperatures, the samples were boiled in distilled water