DOI: 10.1007/s11099-013-0020-7 PHOTOSYNTHETICA 51 (2): 317-320, 2013

BRIEF COMMUNICATION

Gas exchange of terrestrial and epiphytic orchids from Brazilian Atlantic Rainforest

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Abstract

Leaf gas exchange of terrestrial and epiphytic orchids from the Atlantic Rainforest in northeast Brazil was investigated under artificial growth conditions. The terrestrial orchids showed higher values of all photosynthetic parameters in comparison to epiphytic ones. There was a close relationship between P_N and g_s for both terrestrial and epiphytic orchids. Taken together, our results demonstrated that the photosynthetic parameters were related to the specific growth habits of the orchids under study.

Additional key words: epiphytes; net photosynthetic rate; orchids; photosynthesis; stomatal conductance; terrestrial; transpiration.

The family Orchidaceae, which comprises estimated 20,000 wild species, shows epiphytic, hemi-epiphytic, terrestrial, and lithophytic growth habits (Dressler 1993). However, majority is epiphytic (72%) (Gravendeel *et al.* 2004) and restricted to tropical and subtropical regions. Epiphytic orchids exhibit commonly morpho-physiological traits that enable them to cope with intermittent drought (Benzing 1990). Furthermore, both terrestrial and epiphytic orchids may suffer drastic changes in light conditions in response to formation of treefall gap (Zotz and Hietz 2001). To deal with such environmental variations, this family exhibits a great plasticity in the patterns of CO_2 assimilation with many C_3 and crassulacean acid metabolism (CAM) species, as well as a great morphological variability.

Deforestation caused by agricultural expansion, pasture, and wood extraction in the Atlantic Rainforest in the southern region of the Bahia state (Brazil) affected the native flora, such as terrestrial and epiphytic orchids. However, few studies have been carried out on the physiology of Brazilian orchid species, thus the current knowledge in this field is quite fragmentary. For this reason, this study aimed to evaluate leaf gas-exchange parameters of two terrestrial (*Sobralia* sp. and *Cyrtopodium polyphyllum* Pohet ex. F. Barros) and three epiphytic orchids (*Encyclia fragrans* (Swartz) Dressler, *Miltonia flavescens* (Lindl.) Lindl., and *Gongora quinquenervis* Ruiz & Pavon), to provide a support for conservation programmes and reintroduction of orchids into natural habitats.

Mature plants of the orchids were collected in a reserve of the Atlantic Rainforest at the secondary successional stage, surrounded by an urban matrix in the borders of Itapebi city, Bahia (15°57'S, 39°32'W). Annual rainfall at the site is 1,100–1,700 mm, the mean annual temperature is 24°C and air humidity varies between 65–75% (Araújo 2009), with the dominant vegetation being tropical evergreen forest, which predominates in the Brazilian Atlantic coast. Plants of the terrestrial orchids, *Sobralia* sp. and *C. polyphyllum*, were transplanted into plastic pots with capacity of 10 L, containing soil, organic matter, and washed sand as substrate (3:1:1), while the epiphytic orchids, *E. fragrans*, *M. flavescens*, and *G. quinquenervis*, were transplanted to perforated adobe pots filled with dry seeds of *Spondias mombin* L. as a substrate according to Andrade-Souza *et al.* (2009) and Pires *et al.* (2012). The pots were maintained under greenhouse conditions at a mean

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Received 15 May 2012, *accepted* 20 December 2012.

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Abbreviations: C_i – intercellular CO_2 concentration; C_i/C_a – intercellular to atmospheric CO_2 concentration ratio; CV – coefficient of variation; E – leaf transpiration rate; g_s – stomatal conductance to water vapor; P_{max} – light-saturated photosynthetic rate; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; R_D – dark respiration rate.

Acknowledgements: This research was funded by *Fundação de Amparo à Pesquisa do Estado da Bahia* (FAPESB) and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq).

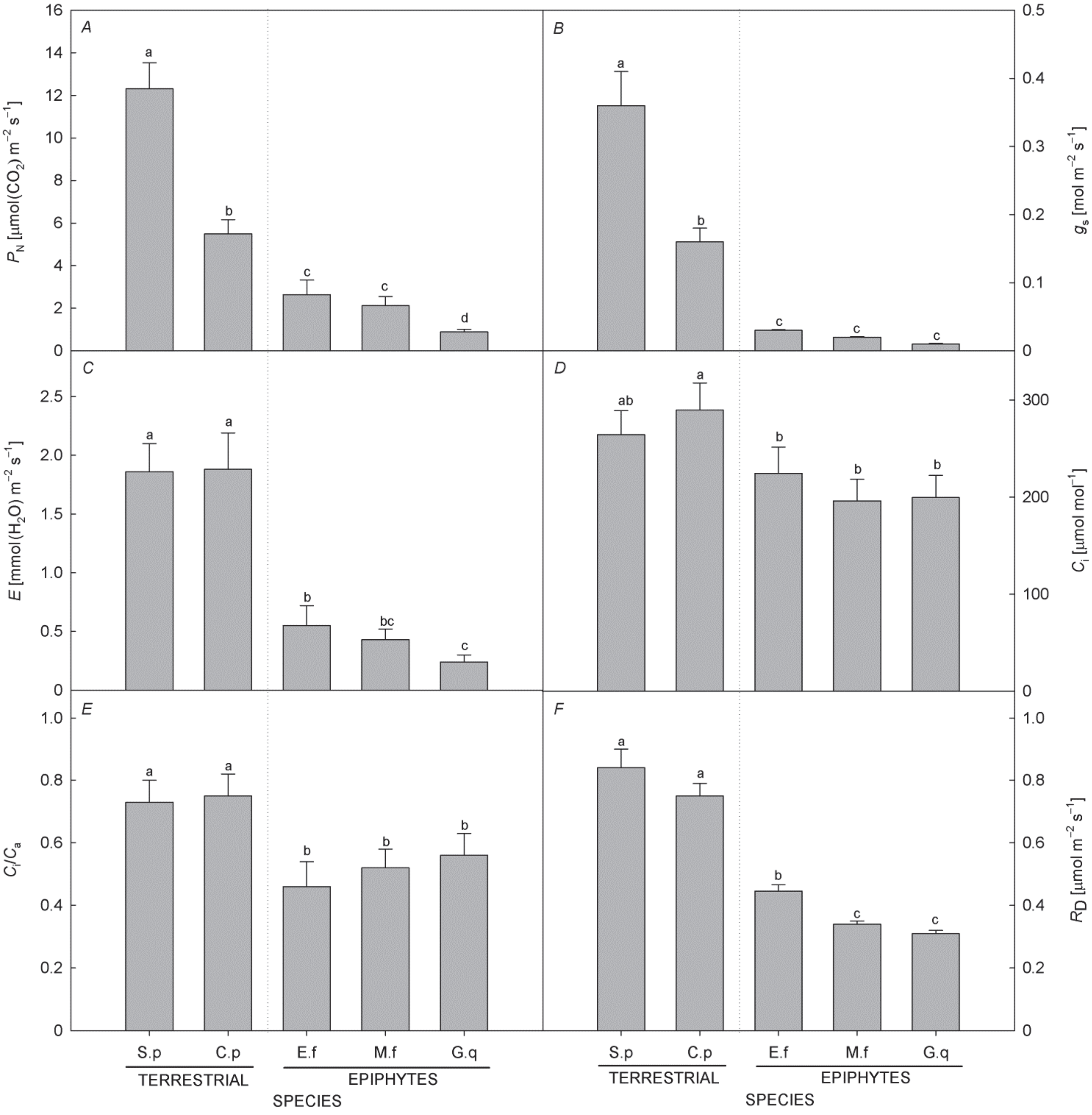

Fig. 1. Net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), transpiration rate, E (*C*), intercellular CO$_2$ concentration, C_i (*D*), intercellular to ambient CO$_2$ concentration ratio, C_i/C_a (*E*), and dark respiration rate, R_D (*F*) of five orchid species. Bars represent means ($n = 5$) and means comparison was done using *Tukey*'s test ($p<0.05$). Vertical bars denote SE. For each variable *lowercase letters* indicate comparison among species. S.p – *Sobralia* sp.; C.p – *Cyrtopodium polyphyllum*; E.f – *Encyclia fragrans*; M.f – *Miltonia flavescens*; G.q – *Gongora quinquenervis*.

temperature of 28 ± 2°C, relative humidity of 70 ± 4%, and 50% of global irradiation [380 ± 75 µmol(photon) m^{-2} s^{-1}], a nonsaturating irradiance. Each pot had 4–5 stumps and represented 1 experimental sample unit. The plants were irrigated daily, fertilized through the leaves (N:P:K ratio of 1:1:1), and sprayed with fungicide once every 15 d.

Leaf gas-exchange parameters were evaluated 90 d after the plants were transferred to artificial conditions, from 8:00 to 12:00 h, on five individual plants per species in a fully expanded, not self-shaded leaf per plant, using a *Portable Photosynthesis System LI-6400* (*Li-Cor Biosciences Inc.*, Lincoln, NE, USA) equipped with an artificial irradiance source *6400-02B RedBlue*. Light-response curves were created with eight levels of photosynthetic photon flux density, PPFD [0, 25, 50, 100, 200, 400, 600, and 800 μmol(photon) m^{-2} s^{-1}] applied in a decreasing order, with 1–2 min intervals between each reading coefficient of variation (CV) < 0.8%. The CO_2 flux was adjusted to maintain a concentration of 380 μmol mol^{-1} inside the chamber; the leaf chamber temperature was maintained at 26°C. The net photosynthetic rate (P_N), leaf transpiration rate (E), stomatal conductance to water vapor (g_s), intercellular CO_2 concentration (C_i), and intercellular to atmospheric CO_2 concentration ratio (C_i/C_a) were calculated using the values of CO_2 and humidity variation inside the chamber (~50%), both measured by the infrared gas analyzer of the *LI-6400*, when PPFD $\geq$ 600 μmol(photon) m^{-2} s^{-1} (saturating, but not inhibitory irradiance observed). The following equation was used for the construction of P_N *vs.* PPFD curves (Iqbal *et al.* 1997): $P_N = P_{max} [1 - \exp(-\alpha \text{ PPFD}/P_{max})] - R_D$, where P_{max} is the light-saturated photosynthetic rate, α is the apparent quantum yield, and R_D is the dark respiration rate, corresponding to the value of P_N, when PPFD = 0 μmol(photon) m^{-2} s^{-1}.

The experiment was conducted in a completely randomized design with five treatments (species) and five replications, using one pot per experimental unit. The results were submitted to a variance (*ANOVA*) and *Pearson*'s correlations analyses, followed by *Tukey*'s mean comparison test ($p<0.05$). All statistical procedures were performed using the *Statistica 6.0* software (*Statsoft Inc.*, College Station, TX, USA).

The terrestrial orchids showed higher values of photosynthetic parameters in comparison to the epiphytic ones, such as P_N (~79% higher), g_s (~92% higher), and E (~78% higher). The highest value of P_N (Fig. 1*A*), found for the terrestrial species, *Sobralia* sp., was similar to that of the terrestrial orchid, *Spiranthes cernua* [9.15 μmol(CO_2) m^{-2} s^{-1}]. The P_N values of the epiphytic orchids, *E. fragrans* and *M. flavescens*, were near to that of *Cymbidium sinense* [2.6 μmol(CO_2) m^{-2} s^{-1}], while the value found for *G. quinquenervis* was similar to those observed in *Paphiopedilum barbatum* [1.0 μmol(CO_2) m^{-2} s^{-1}] and *Angraecum sesquipedale* [0.8 μmol(CO_2) m^{-2} s^{-1}] (Goh and Kluge 1989, Hew *et al.* 1989, Antlfinger and Wendel 1997, Kluge *et al.* 1997). The maximal values of g_s (Fig. 1*B*) were observed for *Sobralia* sp. [0.36 mol m^{-2} s^{-1}] and *C. polyphyllum* [0.16 mol m^{-2} s^{-1}]; in addition, significantly positive correlations between P_N and g_s were also found (Table 1). *E. fragrans*, *M. flavescens*, and *G. quinquenervis* had lower g_s values (Fig. 1*B*). Highly significant, positive correlations between P_N and g_s (Table 1) were found for all species, showing that g_s accounted for more than two thirds of the variation in P_N. A significant correlation between E and g_s was also found for all species (Table 1). Furthermore, the highest values of C_i (Fig. 1*D*), C_i/C_a (Fig. 1*E*), and R_D (Fig. 1*F*) were also observed in the terrestrial orchids. The C_i/C_a ratio is a good indicator of stomatal limitation of photosynthesis, and its values change according to the habitat, being higher in mesomorphic than in xeromorphic species, such as vascular epiphytes (Lloyd and Farquhar 1994).

Table 1. *Pearson* correlations coefficients (r) between the net photosynthetic rate (P_N) and the stomatal conductance (g_s), the intercellular CO_2 concentration (C_i), and the intercellular to ambient CO_2 concentration ratio (C_i/C_a), and between the transpiration rate (E) and g_s of five orchid species. Significant interactions between parameters are: ns – not significant; $^{**}p<0.05$; $^{***}p<0.01$. ^{1} – Terrestrial orchids; ^{2} – Epiphytic orchids.

Species	P_N *vs.* g_s	P_N *vs.* C_i	P_N *vs.* C_i/C_a	E *vs.* g_s
Sobralia sp.^{1}	0.69^{**}	0.51^{ns}	0.52^{ns}	0.59^{**}
Cyrtopodim polyphyllum^1	0.75^{**}	0.53^{ns}	0.45^{ns}	0.55^{**}
Encyclia fragrans^2	0.90^{***}	0.04^{ns}	0.40^{ns}	0.95^{***}
Miltonia flavescens^2	0.94^{***}	0.37^{ns}	0.35^{ns}	0.81^{**}
Gongora quinquenervis^2	0.83^{***}	0.19^{ns}	-0.09^{ns}	0.95^{***}

P_N values of most epiphytes are often lower than those of terrestrial orchids (Stuntz and Zotz 2001), which could be limited by both low leaf nutrient contents (Field and Mooney 1986) and frequent water shortage periods (Zotz and Hietz 2001). The potentially low photosynthetic capacity is related to a low g_s resulting from low stomata densities of the leaf epidermis, a common feature of vascular epiphytes (Holbrook and Putz 1996). Thus, as a consequence, the low E rates found for the epiphytic orchids (Fig. 1*C*) ensured that tissue desiccation proceeds slowly and, therefore, that a cell turgor is preserved in situations of water deficit (Martin *et al.* 2004). However, the current knowledge in this field is often contradictory. The physiological behavior of epiphytes is commonly influenced by several factors, such as plant size, since the photosynthetic capacity is a function of the plant size (Schmidt *et al.* 2001), and environmental conditions (Pires *et al.* 2012). The current data indicate that as a group, epiphytes show low photosynthetic capacities (Stuntz and Zotz 2001, Zotz and Hietz 2001); however, these major studies have focused on different groups of epiphytes, with different plant sizes and growth conditions. Thus, a high variability (Zotz and Hietz 2001) is expected in the photosynthetic capacity of epiphytes, due to the greater diversity of environments to which those plants are subjected.

Most orchids have remarkable storage organs, such as corms, rhizomes, and tuberoids in terrestrial orchids, as well as enlarged stems, called pseudo-bulbs, in epiphytic orchids (Arditti 1992, Ng and Hew 2000). Pseudo-bulbs can be seen as an adjustment mechanism in situations of reduced water availability, since they can store water, nutrients, and carbohydrates, and thus, help in the maintenance of water balance and metabolism. However, the low P_N, shown by the epiphytic orchids in this study, might be due to increased investment in pseudo-bulbs and therefore carbohydrate storage. Furthermore, the presence of a thicker cuticle layer in the leaves of the epiphytic orchids can be associated with the reduced rates of P_N, g_s, and E observed. Since the environment of most vascular epiphytes is often subject to temporal and spatial variations in a light intensity and water supply (Maxwell *et al*. 1994), low cuticular permeability to water is a feature commonly observed in epiphytic orchids (Helbsing *et al.* 2000). Our results demonstrate that the photosynthetic parameters were related to the specific growth habits of the orchids under study. The net photosynthetic rates of the epiphytic orchids were more dependent on g_s and E, probably due to their growth habit and morpho-physiological adaptations, while the terrestrial orchids exhibited a behavior typical for mesomorphic species.

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