

## BRIEF COMMUNICATION

## The effect of drought on photosynthesis in two epiphytic and two terrestrial tropical fern species

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### Abstract

Water-withholding for 5 to 7 weeks and subsequent re-watering were made on potted plants of two epiphytic (E) and two terrestrial (T) fern species, which were collected from a seasonal tropical rainforest and had been grown in a screen-house with 5 % irradiance for 4 months. During the water stress, the two E species completely closed stomata when frond relative water content (RWC) reached about 70 % with fairly constant maximum photochemistry efficiency ( $F_v/F_m$ ), while the two T species kept partial stomata opening until RWC reached 45 % and reduction in  $F_v/F_m$  at the late stage. Also, chlorophyll content as indicated by a spectral reflectance index was gradually reduced in three species. Physiological recovery was completed after 3-d re-watering for the E species, which was more rapid than for the T species. The gas exchange measurements and regression analyses indicated higher photosynthetic water use efficiency in the E species than in the T species.

*Additional key words:* *Asplenium*; chlorophyll fluorescence; *Microsorium*; *Neottopteris*; net photosynthetic rate; *Paraleptochilus*; physiological recovery; relative water content; stomatal conductance; water conservation strategy.

Epiphytes are important components and contribute significantly to the biodiversity of tropical rainforests (cf. Hsu *et al.* 2006). Water availability is one of the most important environmental factors limiting growth and survival of epiphytes (Lüttge 1989, Benzing 1990). In humid tropical forests, short periods of drought can occur (Windsor 1990).

Two major types of response to drought in plants have been reported: drought-avoidance and drought-tolerance. Drought-avoidance plants rapidly close stomata with decrease in leaf water potential, while drought-tolerance plants are able to keep stomata open at low water potential (Blake and Jordan 1993, Calatayud *et al.* 2000, Costa Franca *et al.* 2000, White *et al.* 2000). Drought limits photosynthetic CO<sub>2</sub> assimilation through stomatal closure (Sharkey 1990, Chaves 1991, Cornic and Massacci 1996, Ramanjulu *et al.* 1998, Flexas *et al.* 2004) and metabolic impairment (Lawlor 1995, Tezara *et al.* 1999, Flexas and Medrano 2002, Lawson *et al.* 2003). Permanent photo-inhibition occurs under severe drought (Flexas and Medrano 2002), resulting in photooxidation and thus

degradation of chlorophyll (Chl). Water deficit also leads to reduction in synthesis of Chl; therefore, Chl content generally decreases during water stress (Calatayud *et al.* 1996, Ramanjulu *et al.* 1998, Singer *et al.* 2003).

Ferns usually proliferate in conditions of shade and high humidity (Andrade and Noble 1997); however, there are many fern species which grow in drought-prone epiphytic (E) habitats. E ferns use simply branched and occasionally reticulated vascular networks to irrigate fronds (Carlquist and Schneider 2001). Studies on water relations of epiphytes have focused primarily on few groups of epiphytes, particularly bromeliads, the genus *Clusia* and, to a lesser extent, orchids (Zotz and Hietz 2001), while little attention has been paid to the water relations of E ferns. Most researches on responses of E to drought have focused on CAM epiphytes (e.g. Winter and Smith 1996, Haslam *et al.* 2003); however, little attention had been paid to C<sub>3</sub> epiphytes.

Two E species, *Neottopteris nidus* (L.) J. Sm. and *Microsorium punctatum* (L.) Cop. and two terrestrial (T) ones, *Asplenium finlaysonianum* Wall. ex Hook and

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*Paraleptochilus decurrens* Bl. are widely distributed in the tropical rainforests in Xishuangbanna, southern Yunnan. Sixty mature plants per species were collected from the ravine forest of the nature reserve nearby Xishuangbanna Tropical Botanical Garden (XTBG; 21°41'N, 101°25'E, 600 m altitude), in southern Yunnan, China, transplanted into plastic pots (30×30 cm) with one plant per pot and placed in a screenhouse with 5 % full-sunlight in XTBG. Mean annual temperature is 21.7 °C. Mean annual precipitation is 1 560 mm; about 83 % of the annual rainfall is concentrated from May to October. The pots contained soil obtained from the vicinity of the host trees in the forest. These potted plants were watered every 2–3 d. After four months of growth in the screen-

house, between October and November 2006, a simulated-drought experiment was conducted by withholding water for 5–7 weeks, while keeping the control plants watered regularly. When stomata of the water-stressed plants almost completely closed, the plants were re-watered. During water-withholding and re-watering experiment, water status, gas exchange, Chl fluorescence, and frond spectral reflectance were monitored.

In order to avoid damage by frequent sampling, fronds used for measurement of relative water content (RWC) were collected from other set of leaves than used for photosynthetic measurements. Six fresh fronds or portions of fronds from six plants selected randomly from thirty plants per species per treatment were collected

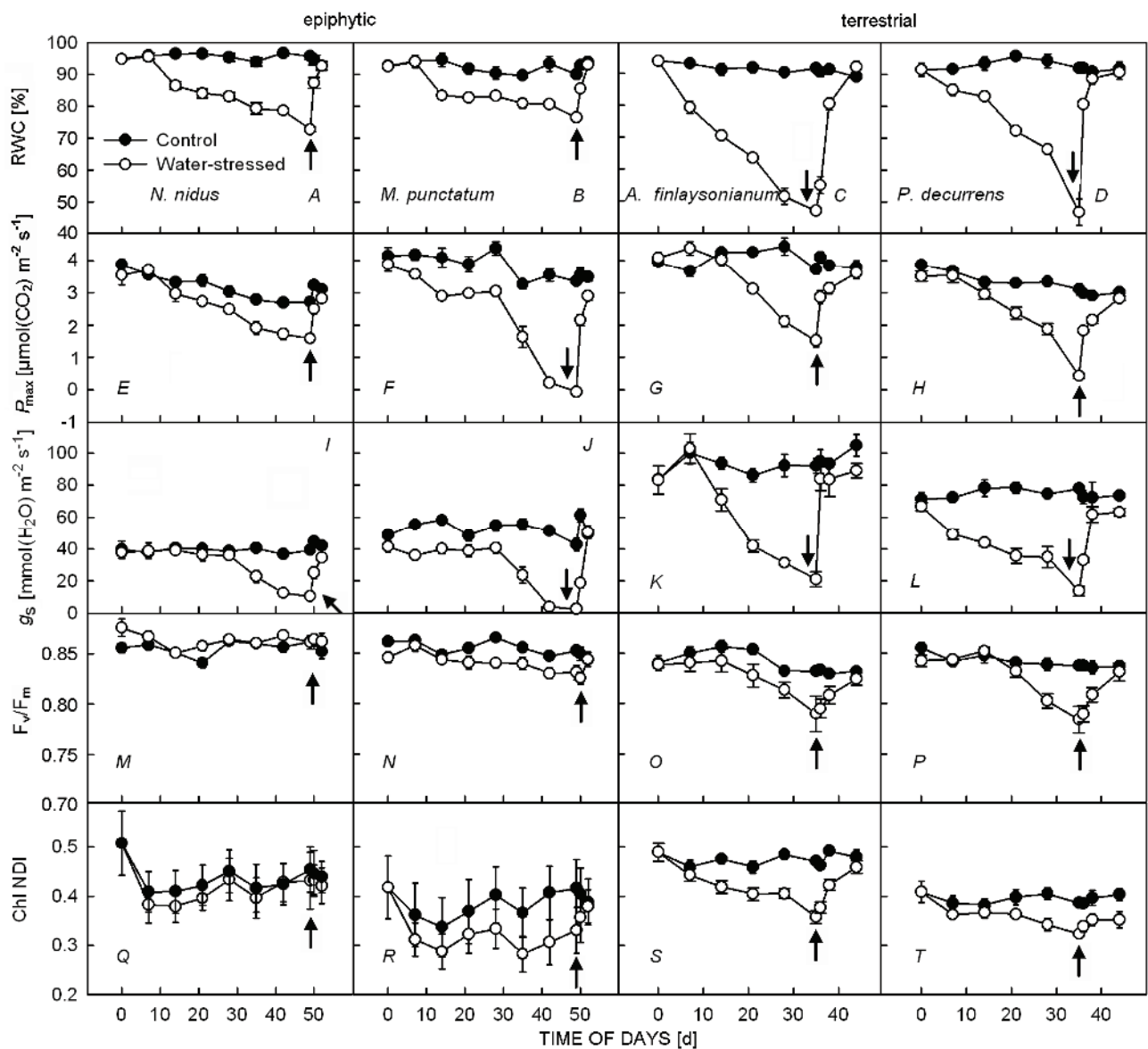


Fig. 1. The changes in leaf relative water content (RWC), net photosynthetic rate ( $P_{\max}$ ), stomatal conductance ( $g_s$ ), and  $F_v/F_m$  at predawn and chlorophyll normalized difference index (Chl NDI) in the four fern species during the water stress and recovery periods. Arrow indicates re-watering. Means  $\pm$  SE of six plants of each species.

Table 1. Summary of the linear regressions between net photosynthetic rate ( $P_{\max}$ ), stomatal conductance ( $g_s$ ), and relative water content (RWC) measured during the water stress and re-watering from the four fern species.

| Variable             | Life form   | Species                  | Regression equation  | $r^2$ | $p$ value |
|----------------------|-------------|--------------------------|----------------------|-------|-----------|
| $P_{\max}$ vs. $g_s$ | Epiphytic   | <i>N. nidus</i>          | $y = 0.06x + 0.90$   | 0.78  | <0.01     |
|                      |             | <i>M. punctatum</i>      | $y = 0.07x + 0.15$   | 0.83  | <0.01     |
|                      | Terrestrial | <i>A. finlaysonianum</i> | $y = 0.03x + 1.37$   | 0.68  | <0.01     |
|                      |             | <i>P. decurrens</i>      | $y = 0.04x + 0.42$   | 0.62  | <0.01     |
| $P_{\max}$ vs. RWC   | Epiphytic   | <i>N. nidus</i>          | $y = 0.09x - 5.12$   | 0.88  | <0.01     |
|                      |             | <i>M. punctatum</i>      | $y = 0.17x - 12.54$  | 0.61  | <0.01     |
|                      | Terrestrial | <i>A. finlaysonianum</i> | $y = 0.04x + 0.09$   | 0.65  | <0.01     |
|                      |             | <i>P. decurrens</i>      | $y = 0.06x - 2.03$   | 0.71  | <0.01     |
| $g_s$ vs. RWC        | Epiphytic   | <i>N. nidus</i>          | $y = 1.17x - 70.55$  | 0.63  | <0.01     |
|                      |             | <i>M. punctatum</i>      | $y = 1.92x - 133.92$ | 0.48  | <0.05     |
|                      | Terrestrial | <i>A. finlaysonianum</i> | $y = 1.25x - 20.99$  | 0.57  | <0.05     |
|                      |             | <i>P. decurrens</i>      | $y = 1.10x - 41.10$  | 0.84  | <0.01     |

early in the morning and used to determine RWC following Slama *et al.* (2006). Between 08:30 and 11:30, photon-saturated gas exchange was measured on six fully-developed fronds from six plants per species per treatment with a portable infrared gas analyzer (LI-6400, LI-COR, USA). Photosynthetic photon flux density of  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  on the cuvette surface was provided by a LED source, which was photosynthetically saturating for all species.  $\text{CO}_2$  concentration inside the leaf chamber was maintained at  $380 \text{ cm}^3 \text{ m}^{-3}$  through the  $\text{CO}_2$  controlling system of the LI-6400 attached to a tiny  $\text{CO}_2$  cylinder. During measurements, the relative air humidity was about 80 % and ambient temperature was about 25–27 °C. The maximum net photosynthetic rates ( $P_{\max}$ ) and stomatal conductance ( $g_s$ ) were obtained from these measurements. Predawn maximum quantum yield of photosystem 2, PS2 ( $F_v/F_m$ ) was detected in six fully-developed fronds from the same six plants per species per treatment with a portable fluorescence monitor system (FMS, Hansatech, Norwich, U.K.).

Three to five spectral reflectance values were determined for each frond (expressed as a mean) of six plants per species per treatment using a spectral analysis system (UniSpec, PP Systems, USA). The Chl normalized difference index (Chl NDI), an indication of Chl content, was calculated as  $(R_{750} - R_{705}) / (R_{750} + R_{705})$  according to Richardson *et al.* (2002). Standard reflectance was scanned periodically for the spectral calibration of the detector.

We found that the two E fern species employed the drought-avoidance strategy. The two E ferns closed their stomata after 49 d of water withholding and the two T ones after 35 d. The RWC of the two E species declined from 94.8 and 92.5 % to 72.7 and 76.4 %, respectively, after 49-d water-withholding, but that of the two T ones declined from 94.2 and 91.4 % to 47.0 and 46.6 %, respectively, after 35-d water-withholding. The RWC values of the former returned to the control level after 3-d re-hydration, while that of the latter resumed after 9 d of

re-hydration (Fig. 1A–D). Under sufficient water supply the E ferns had lower  $g_s$  but similar  $P_{\max}$ , thus higher photosynthetic water use efficiency than the T ferns. Moreover, during water stress the E ferns completely closed stomata before the frond RWC dropped to a detrimental level, while the T ferns still kept partial stomata opening until RWC decreased to around 45 %. These are important adaptations of the E ferns to the frequent water deficit in the E habitats (Winter 1985, Benzing 1990).

With the drought treatment, all of the four fern species displayed reduction in  $P_{\max}$  paralleled by reduction in  $g_s$ . The  $P_{\max}$  and  $g_s$  started to decrease significantly from the 28<sup>th</sup> and 7<sup>th</sup> d of water withholding, respectively, for the two E species, and from 21<sup>st</sup> and 7<sup>th</sup> d, respectively, for the two T species. After 49 d of water withholding,  $P_{\max}$  of *M. punctatum* approached to zero, while  $P_{\max}$  of *N. nidus* dropped to 50 % of the pre-treatment level (Fig. 1E,F). However, after 35-d water withholding,  $P_{\max}$  of the T ferns *A. finlaysonianum* and *P. decurrens* already declined from  $4.08$  and  $3.51 \mu\text{mol m}^{-2} \text{s}^{-1}$  to  $1.51$  and  $0.43 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Fig. 1G,H). Upon re-watering, the recovery in gas exchange was completed after 3- and 9-d re-hydration for the E and T fern species, respectively. The reductions in  $P_{\max}$  in the four fern species during the water stress experiment were largely caused by stomatal closure (Table 1). Irreversible damage to photosynthetic apparatus was not observed in all the four fern species as indicated by fairly stable predawn  $F_v/F_m$  during the water stress experiment even when the frond RWC dropped to about 45 % in the T ferns. Other studies have shown that photosynthesis is irreversibly impaired when leaf RWC is lower than 70 % (e.g. Lawlor and Cornic 2002). However, predawn  $F_v/F_m$  in all the four fern species indicated that no permanent photoinhibition occurred during the water stress treatment. The absence of irreversible photoinhibition in these fern species could largely be due to the shading of our plants studied, because the shading could prevent excess photon energy and photooxidation during

the drought stress (Quero *et al.* 2006). Photosynthesis in the two E ferns recovered more quickly than in the two T species. The possible reasons for this could be: (1) the E ferns suffered less water deficit than the T ferns during the water stress treatment; (2) the water stress might cause some degree of biochemical impairment in the terrestrial ferns (cf. Flexas *et al.* 2004); (3) it was possible that photorespiration might effectively protect the photosynthetic apparatus from photo-damage under drought, assists in maintaining a relatively high  $\Phi_{PS2}$ , and helps  $P_{max}$  to be rapidly recovered after re-watering (Guan *et al.* 2004).

Predawn recordings of fluorescence parameters showed that the maximum quantum yield of PS2 in the two E species, as revealed by  $F_v/F_m$  ratio, was not affected by drought stress (Fig. 1M,N). However, that in the two T species was only slightly affected after 21- or 28-d water withholding, respectively, and returned back to the control level after 9-d re-watering (Fig. 1O,P). Drought stress had a significant negative effect on Chl NDI in the fern species studied except for *N. nidus* (Fig. 1Q–T). Chl NDI value after water withholding in

E fern *M. punctatum* was lower in relation to the control and returned back to the control level after 3-d re-watering. Chl NDI in the two T species decreased after 14 or 21 d of water withholding, respectively, and returned to the control level after 9-d re-watering, while the Chl recovery in *P. decurrens* took more than 9 d (Fig. 1S,T).

Pooling the data measured during the water-withholding and re-watering periods, there were significant positive correlations between  $P_{max}$  and  $g_s$ , between  $P_{max}$  and RWC, and between  $g_s$  and RWC in all of the four species (Table 1). The linear slopes of the regressions in the two E fern species were steeper than in the two T ferns, indicating more efficient photosynthetic water use and more quick change in gas exchange in response to water deficit in the E ferns.

In summary, the two E fern species adopted the drought-avoidance strategy; their stomata sensitively responded to water loss, and completely closed before detrimental leaf water content reached. Keeping positive water balance during a long drought and possessing more rapid photosynthetic recovery after re-watering give advantage for the E ferns to survive in the E habitats.

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