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Ecophysiological consequences of differences in plant size: abscisic acid relationships in the epiphytic orchid *Dimerandra emarginata*

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Abstract Changes in abscisic acid (ABA) content and several water relations parameters were studied in the epiphytic orchid *Dimerandra emarginata* in the tropical lowlands of Barro Colorado Island, Panama. Similar to previous observations of size dependency of physiological parameters in vascular epiphytes, we found significant differences in ABA accumulation (both in terms of temporal patterns and amount) in small and large plants under drought stress. The highest values of ABA content in leaves, stems, and roots were found in the smaller specimens, reaching the levels of poikilohydric resurrection plants. Substantial accumulation of ABA occurred after stomatal closure, indicating other functions of ABA than stomatal regulation in this bark epiphyte.

Keywords Barro Colorado Island · Leaf gas exchange · Vascular epiphyte · Plant hormone · Plant water relations · Size dependence

Introduction

Water availability is arguably the most important environmental factor limiting growth and survival of vascular epiphytes (Lüttge 1989; Benzing 1990). While the response of both CO₂ and H₂O exchange to water shortage has been documented rather extensively (e.g. Adams and

Martin 1986; Griffiths et al. 1986; Zotz and Tyree 1996; Nowak and Martin 1997), little attention has been paid to possible, simultaneous changes at the level of plant hormones such as abscisic acid (ABA). This lack of information seems surprising, considering the well-established role of ABA in stomatal functioning (MacRobbie 1991). Clearly, for a more mechanistic understanding of plant water relations in this plant group, such information is indispensable. The few published studies on ABA in vascular epiphytes have all used greenhouse material and were limited in scope: Martin and Peters (1984) applied ABA to show that *Tillandsia usneoides* has functional stomata, while Zhang et al. (1995) investigated whether roots differ in their capacity to produce ABA and other hormones in two orchid species.

Clearly, the role of ABA in plant water relations is not restricted to direct or indirect effects on stomatal aperture. This hormone is also involved in the induction of synthesis of a number of water-deficit-stress-responsive polypeptides such as, e.g. dehydrins (Ingram and Bartels 1996). This class of polypeptides, which is at least partly responsible for the extreme desiccation tolerance of resurrection plants such as *Craterostigma plantagineum*, may be of similar importance for homoiohydric plants under prolonged drought. Rainless periods of several weeks are not uncommon in tropical moist forests during the dry season (Windsor 1990), and are often combined with strong winds and intense radiation, which in combination amplify the severity of drought stress in the epiphytic habitat. Finally, ABA may also play a significant role in the regulation of hydraulic conductivity of roots (Freundl et al. 2000). To include all these aspects of ABA action, we did not restrict our study to short-term changes in ABA contents in roots, stems, and leaves, i.e. until stomatal closure, but were also interested in long-term changes, i.e. changes in ABA content and leaf gas exchange parameters over several weeks without watering.

The present report is the first to address the role of ABA in a vascular epiphyte in situ. We chose the orchid *Dimerandra emarginata* for study because the morpholo-

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gy, physiology and demography of this species are already well characterised (e.g. Zotz and Tyree 1996; Zotz 1998, 1999), and it is rather common at our study site, the moist tropical lowland forest of Barro Colorado Island (BCI). Particular emphasis was paid to the importance of plant size. The need to do so has become clear only recently, when consistent size-related differences in gas exchange parameters were found in *D. emarginata* and a number of other epiphyte species (Zotz 1997; Schmidt et al. 2001). Considering the usual close link between ABA relations and plant gas exchange, we expected changes in ABA to depend similarly upon plant size, and thus included almost the entire size range of this species in our study, excluding only the smallest specimens for methodological reasons.

Specifically, we asked the following questions. (1) Which organs are the main sites of ABA production and accumulation? (2) How do ABA contents correlate with stomatal closure? (3) How do ABA contents change after stomatal closure under prolonged drought? (4) Are these findings dependent on plant size?

Materials and methods

Study site and plant material

Field work was conducted during the 1998 and 1999 dry seasons on BCI (9°10' N, 79°51' W) in the Republic of Panama. The forest is a biological reserve and classified as a tropical moist forest (Holdridge et al. 1971) with dry seasons lasting from late December to late April (see also Croat 1978; Windsor 1990).

Our study species, *Dimerandra emarginata* (G. Meyer) Hoehne, occurs from Mexico through Venezuela. On BCI, this C₃ species (see Zotz and Ziegler 1997) is found high in the canopy and on exposed branches along the shore of Lake Gatun (for detailed descriptions of its biology see Zotz and Tyree 1996; Zotz 1998). We chose the average length of the two most recent stems (stem length, SL) as a measure for plant size, and defined four size classes (SC): I (4.5–5.5 cm), II (7.0–13.0 cm), III (15.0–21.0 cm) and IV (23.0–33.0 cm).

In the early dry season, we collected specimens by cutting entire branches of the tree *Annona glabra* L. with populations of *D. emarginata* and transferred them to a greenhouse. The branches were put on metal racks, which were covered with transparent plastic to protect the epiphytes against occasional precipitation. Plants received about 50% of full irradiation, which is comparable to the conditions in *Annona* tree crowns (unpublished data). The average relative humidity was about 70% (minimum about 55%), average air temperatures were about 30°C. Prior to all experiments, orchids were watered intensively for at least 2 weeks.

Experiment 1: short-term drought and rewatering (one size class)

Forty-eight plants of SC II were kept dry until stomatal conductance approached zero. Then, plants were rewatered for several days (twice daily for about 10 min with a garden hose). During the entire experiment, stomatal conductance was measured daily as described below. Simultaneously, the leaves of the most recent stems and roots were sampled every morning for ABA analysis.

Experiment 2: long-term drought cycle (all size classes)

Twenty plants of each of the four size classes were kept dry for 45 days. Leaves, roots, and the stem of the most recent shoot were

sampled for ABA analysis on the following days: day 0, day after stomatal closure, day 15, day 30 and day 45. On the same days, we also determined both leaf water potential (Ψ) and the relative water contents (RWC). RWC (% dry mass) for different plant parts was defined as $100 \times (\text{fresh mass} - \text{dry mass}) / \text{dry mass}$.

Experiment 3: 50% water loss experiment (continuous size range)

Fourteen orchids varying in size from 1 to 25 cm were carefully detached from their natural substrate and all dead plant material was removed. For 6 weeks, plants were watered twice daily. They were kept on plastic sheets to prevent newly emerging roots adhering to the substrate. Watering was then stopped, and the orchids were weighed daily until they had lost 50% of their initial fresh weight. Leaves, roots, and stems were collected for ABA analysis.

Experiment 4: 3-week drought and rewatering (all size classes)

Fifteen plants of the four size classes were kept dry for 3 weeks. Then, plants were watered twice daily for about 10 min until complete recovery. Stomatal conductance was measured during the rewatering period. Leaf, stem and root samples for ABA determinations were taken every other day.

Leaf water potential

Additional plants not used for ABA analysis but treated according to the above experimental conditions were used to determine Ψ . Briefly, small discs were punched out of the centre of the youngest fully expanded leaf before dawn, and Ψ was determined psychrometrically. Thermally stabilised sample chambers (Stem Hygrometer; Plant Water Status Instruments, Ontario, Canada) and a Wescor HR-33T dew point microvoltmeter (Wescor, Logan, Utah) were used.

Stomatal conductance

Stomatal conductance (g_w) was determined with a Li-1600 steady-state porometer (Licor, Lincoln, Neb.). Measurements were taken at hourly intervals in the morning starting shortly after dawn on three to four leaves per plant. Individual measurements were performed fast enough to prevent the cuvette temperature from rising higher than 3°C above ambient. The highest reading of each plant was used for analysis.

Extraction and analysis of free and conjugated ABA

Pre-treatment, the removal of the velamen radicum was necessary in the case of roots to ensure that only living root tissue was used for analysis: only green roots or roots with new tips were processed. Leaves, stems and pre-treated roots were freeze-dried on BCI (Freeze-Dryer, Labconco Freeze Dry System, Freezone 6; Labconco, Kansas City, Mo.). For the ABA analysis at the University of Würzburg, dried leaves and roots were homogenised and extracted with 80% aqueous methanol. After centrifugation, the supernatant was purified by passing through SEP-PAK C₁₈-Cartridges (Milipore, Milford, USA) and eluted with 70% aqueous methanol. The methanol was evaporated, and the residue was re-suspended in water and partitioned three times against an equal volume of ethyl acetate at pH 3. The organic fraction was reduced to dryness, taken up with 300 μ l of Tris-buffered saline buffer (Tris HCl: 50 mM Tris, 150 mM NaCl, 1 mM MgCl₂, pH 7.8) and analysed immunologically by enzyme-linked immunosorbent assay (ELISA) as described by Weiler (1986). Xylem exudates from detopped stems inserted in a pressure chamber could only be obtained consistently from well-watered and slightly stressed plants. Therefore, ABA determinations of xylem sap are only shown for experiment 1. The processing of xylem sap was slightly different

from that of whole stems: freeze-dried sap from BCI was directly resuspended in 300 μl of Tris-buffered saline buffer and analysed by ELISA as described above. For analysis of conjugated ABA, which consists mainly of ABA-glucose ester (ABA-GE), the aqueous residue from partitioning with ethyl acetate was hydrolysed with NaOH (final concentration 1 M) for 1 h. Released ABA was measured as described above.

Statistical analysis

Data were analysed with STATISTICA software (STATISTICA 5.1; StatSoft, Tulsa, USA). If not stated otherwise, the error term represents the standard error.

Results

Experiment 1: short-term changes in leaf conductance and leaf, stem and root ABA content

The short-term responses of leaf stomatal conductance to changes in water availability are shown in Fig. 1 for plants of SC II. Stomatal closure was reached after about 5 days without external water supply; irrigation starting in the evening of the 5th day of the experiment led to an almost complete recovery of stomatal conductance (g_w) on day 8. Changes in the ABA contents of roots, leaves

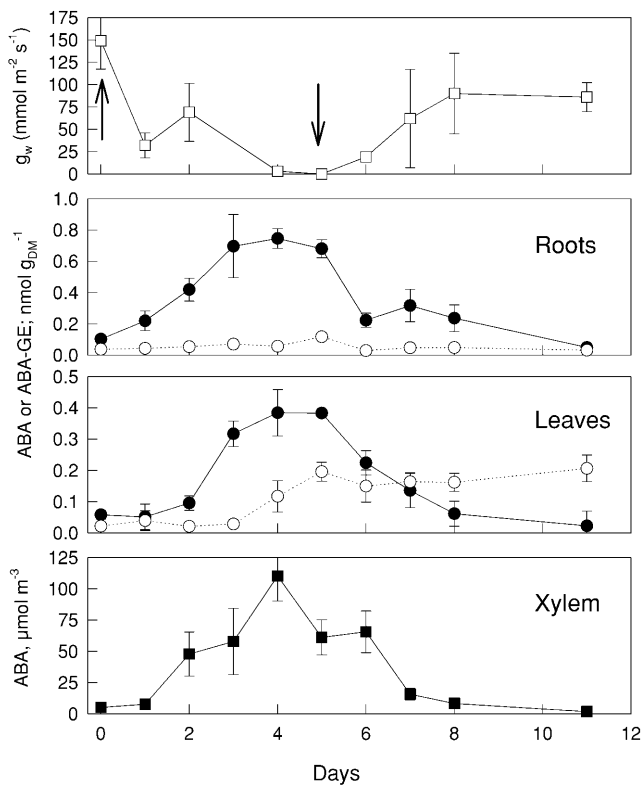


Fig. 1 Changes in stomatal conductance (g_w , open squares), ABA ($\text{nmol g}_{\text{DM}}^{-1}$, closed circles), and ABA-glucose ester ($\text{nmol g}_{\text{DM}}^{-1}$, ABA-GE, open circles) contents of leaves and roots, and ABA contents of xylem sap ($\mu\text{mol m}^{-3}$, closed squares) during a drought cycle in *Dimerandra emarginata*. Given are means \pm SE, $n=3-4$. The arrows indicate the beginning of drought and rewatering

and xylem were inversely related to changes in g_w (Fig. 1). However, while ABA started to rise immediately after the onset of drought in roots, there was some delay in xylem ABA content and an even stronger delay of about 2 days in leaves. Maxima of up to $0.75 \text{ nmol g}_{\text{DM}}^{-1}$ in roots and $0.4 \text{ nmol g}_{\text{DM}}^{-1}$ in leaves were observed during times of stomatal closure. Conspicuous differences between plant organs were found for ABA-GE. Here, hardly any changes occurred in roots throughout the entire experiment, but there was a substantial increase in leaves from about $0.03 \text{ nmol g}_{\text{DM}}^{-1}$ until stomatal closure to a rather stable level of about $0.16-0.2 \text{ nmol g}_{\text{DM}}^{-1}$ thereafter.

Experiments 2 and 3: changes in ABA content during prolonged drought

The ABA contents of well-watered plants (day 0) were low in all organs (ranges in $\text{nmol g}_{\text{DM}}^{-1}$: leaves $0.02-0.05$, stems $0.003-0.008$, roots $0.05-0.1$) and did not differ with respect to plant size (Fig. 2). Without irrigation, the ABA contents of leaves, stems and roots increased significantly, both as a function of the duration of drought and plant size (Fig. 2, Table 1): with few exceptions, the highest ABA contents in stems and roots were

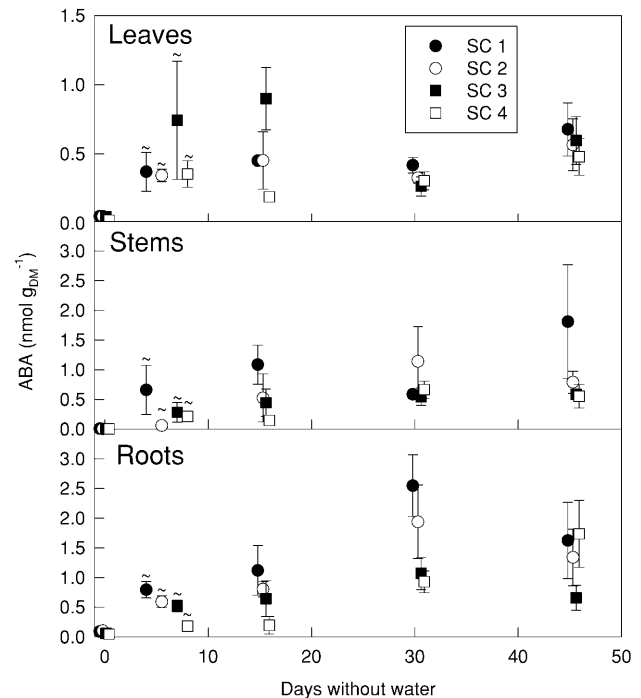


Fig. 2 Changes in ABA content in leaves, stems and roots during a 45-day drought experiment. Data are means \pm SE, $n=3-4$. Different symbols represent different size classes (SC). All four SCs were included on each sampling date. The data are shown slightly staggered for clarity. The symbol \sim indicates exceptions. These values were determined the day after stomatal closure was reached, i.e. on day 3 (SC I), day 5 (SC II), day 7 (SC III), and day 8 (SC IV)

Table 1 ANOVAs of the differences in abscisic acid (ABA) and relative water content (RWC) as a function of plant organ and duration of drought (see Fig. 2). Significant differences are indicated by asterisks

Dependent variable	Duration of drought			Plant size		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Leaf ABA	4,51	4.86**	0.003	3,51	2.10	0.11
Stem ABA	4,61	4.82**	0.004	3,61	2.88*	0.04
Root ABA	4,52	12.89***	<0.001	2,52	3.86*	0.01
Leaf RWC	4,67	69.81***	<0.001	3,67	6.01***	<0.001
Root RWC	4,67	28.71***	<0.001	3,67	0.65	0.58

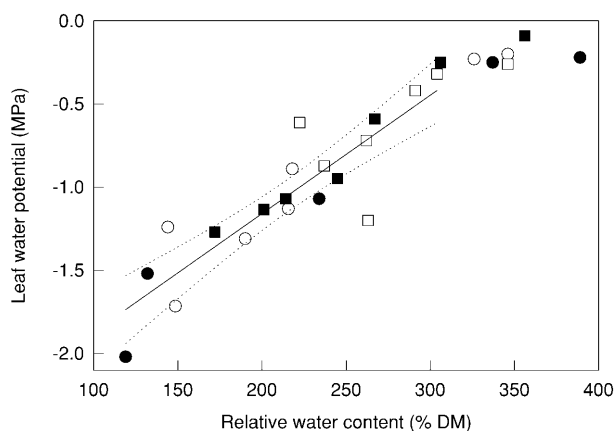


Fig. 3 Relationship of leaf relative water content (RWC) and leaf water potential (Ψ) during a 45-day drought experiment. The *solid line* is the regression of RWC and Ψ for all RWC < 300% ($r^2=0.78$, $P<0.01$); the *dotted lines* are 95% confidence intervals. Each data point is the average of three to five determinations. Different symbols represent different size classes (SCs; see Fig. 2)

found in small plants, while the lowest were observed in the largest plants. In leaves, the effect of size was not significant (ANOVA, $P=0.11$). The highest levels of ABA in leaves were already reached after about 1 week (Scheffé post hoc test, $P<0.05$), when stomatal conductance approached zero (determination of stomatal conductance during the 1st week of the experiment showed that stomatal closure was attained after 3 days in the smallest SC, 5 days in SC II, 7 days in SC III and 8 days in the largest SC), while the ABA contents in stems and roots continued to increase substantially during continued drought. The highest average ABA contents in small plants (SC I) were $1.8 \text{ nmol g}_{\text{DM}}^{-1}$ (stems, day 45) and $2.5 \text{ nmol g}_{\text{DM}}^{-1}$ (roots, day 30). This represented a 200-fold and 28-fold increase, respectively, compared to pre-stress levels.

In both roots and leaves, RWCs decreased significantly during drought (Table 1), from $360 \pm 8\%$ (day 0) to $171 \pm 14\%$ (day 45) in leaves and from $180 \pm 15\%$ (day 0) to $60 \pm 7\%$ (day 45) in roots. This decrease was inversely related to changes in ABA content (Pearson product moment correlation: leaves, $r=-0.39$, $P<0.001$; roots: $r=-0.47$, $P<0.001$). Changes in leaf RWC were size dependent (Table 1). Moreover, the interaction between plant size and time was significant (ANOVA, $P<0.001$): leaves of smaller plants had a higher RWC when well watered, but a significantly lower RWC than larger

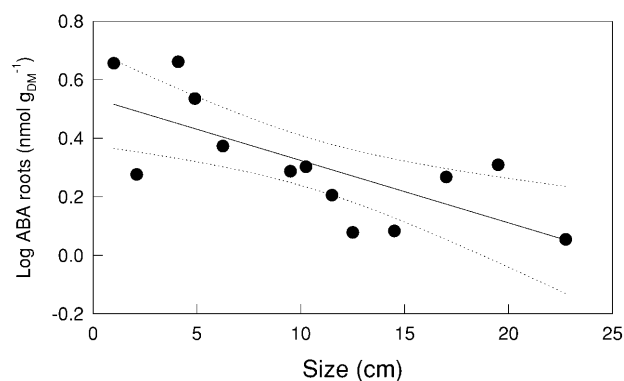


Fig. 4 Relationship of root ABA contents and plant size after reaching a plant water deficit of 50% compared to well-watered conditions. Each *circle* represents a different plant. The regression (*solid line*) is $\log \text{ABA} = 3.54 - (0.021 \times \text{plant size})$; $r^2=0.51$, $P<0.01$). *Dotted lines* are 95% confidence intervals

conspecifics during drought. The relationship between these changes in leaf RWC and changes in Ψ is shown in Fig. 3. Well-watered plants of all sizes had a Ψ of about -0.2 MPa . Once RWC was reduced to less than about 300%, Ψ decreased linearly with RWC ($r^2=0.78$, $P<0.01$). The lowest Ψ of -2.0 MPa was observed in SC 1.

The last finding raised the question whether the observed size-related differences in ABA contents were simply the consequence of lower RWCs in smaller plants at any given time of the drought experiment. This was addressed directly by studying the effect of an identical water deficit of 50% initial water content on plants varying in size. As shown in Fig. 4, there was a significant negative relationship between size and ABA content in roots, although larger plants had experienced a much longer period of drought to reach this level of water deficit. The correlations between plant size and stem ABA content ($P=0.18$) and leaf ABA content ($P=0.56$) proved to be non-significant.

Experiment 4: recovery after prolonged drought

Recovery after a drought period of 3 weeks was fast (Fig. 5). Stomatal conductance of the largest plants (SC 4) reached the level of well-watered controls after

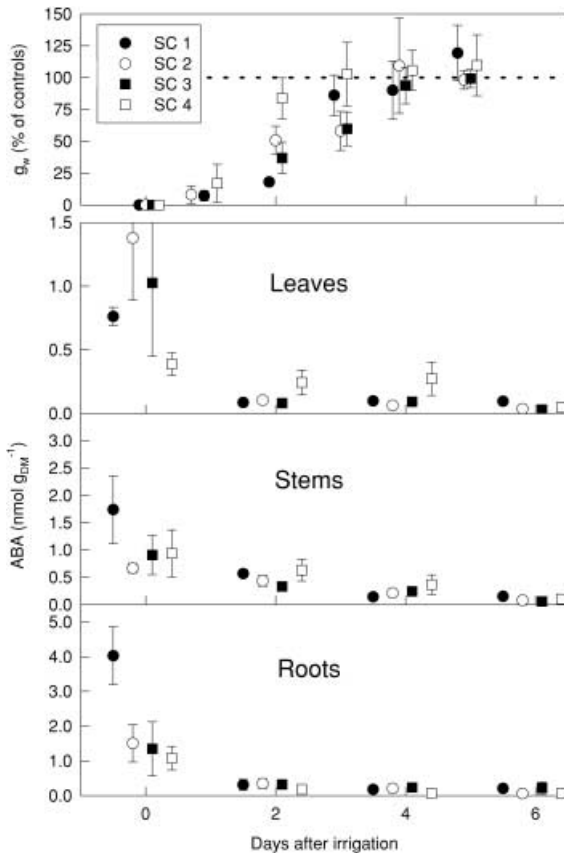


Fig. 5 The effect of rewatering after a drought of 3 weeks on stomatal conductance (g_w , dashed line=100%) and ABA contents in leaves, stems and roots. Data are means \pm SE, $n=3-4$. Different symbols represent different size classes (SC). All four SC were included on each sampling day. The data are shown staggered for clarity

only 2 days and smaller specimens had fully recovered after 4 days. Maximal g_w did not differ with size (ANOVA, $P>0.05$) and ranged from 160 to 225 $\text{mmol m}^{-2} \text{s}^{-1}$. ABA levels in leaves were reduced to pre-stress levels earlier than g_w . Similar kinetics in the reductions of ABA content were found in stems and roots. RWCs of all three organs were at pre-stress levels after 2 days. Again, RWCs were lowest in small plants during drought and highest after rewatering in all three organs (data not shown).

Discussion

This report is part of a series of ecological studies with the epiphytic orchid, *D. emarginata* (e.g. Zotz and Tyree 1996; Zotz 1997, 1998), addressing the implications of frequent drought in the epiphytic habitat from the level of plant organs (e.g. Zotz and Tyree 1996) to that of populations (Zotz 1998). A major finding of previous work was that ecophysiological characteristics of this and many other epiphyte species change substantially with plant size (Zotz 2000b). Size dependency has been dem-

onstrated for leaf gas exchange parameters such as photosynthetic capacity (Zotz 1997; Schmidt et al. 2001) and stomatal regulation (Schmidt and Zotz 2001), for whole-plant water relations (Schmidt and Zotz 2001) and nutrient allocation to reproduction (Zotz 2000a). We can now add ABA relations to this list. We were able to show both quantitative and qualitative differences in the drought-induced accumulation of ABA in conspecifics varying in size. On the one hand, the numerical results of smaller and larger individuals showed systematic variation (Figs. 2, 5, Table 1). On the other hand, we also observed differences between sizes even when water deficits were standardised (Fig. 4). This suggests that our observations cannot simply be explained by the fact that the same environmental conditions impose different levels of stress on plants of different size, i.e. that smaller plants experience a proportionately higher water loss after stomatal closure due to higher surface-to-volume ratios (Zotz 1997).

Zotz (2000b) discussed how the size dependency of physiological parameters complicates the ecological interpretation of previous studies with vascular epiphytes, most of which ignored plant size. For example, if we are to understand the physiological basis of spatial distributions, studying individuals of one particular size and simply assuming similar compartment for smaller or larger conspecifics may easily cause misleading conclusions (see e.g. Zotz and Andrade 1998). However, our results not only demonstrate again that plant size has to be treated as an important variable in ecophysiological studies. This is also the first time that in situ changes of leaf gas exchange and ABA accumulation in different plant tissues of any vascular epiphyte have been investigated. The production and accumulation of endogenous ABA reached surprisingly high levels for a homoiohydric plant. In leaves, ABA levels rose more than tenfold (to about $0.5 \text{ nmol g}_{\text{DM}}^{-1}$) before stomatal closure, without any further increase during a long-lasting drought experiment (Fig. 2). In roots and stems, on the other hand, ABA continued to increase considerably after stomatal closure (Fig. 2). For example, between the day of stomatal closure (approximately day 5) and day 30 without irrigation, ABA contents of roots approximately tripled, with ABA levels exceeding $2 \text{ nmol g}_{\text{DM}}^{-1}$ in SC I on day 30. These extraordinarily high values are comparable to those found in desiccated tissues of resurrection plants such as *Chamaejasme intrepidus* or *Craterostigma plantagineum* (Schiller et al. 1997), and exceed those of severely drought stressed root tissue of most homoiohydric plants by about an order of magnitude (Hartung et al. 1998; Hose et al. 2001; W. Hartung, unpublished data).

High levels of ABA in desiccating roots after stomatal closure may be important functionally in relation to the synthesis of dehydrins and other polypeptides (Bray 1991; Ingram and Bartels 1996), but also with respect to possible effects on the hydraulic conductivity of roots (L_{pr}). In maize, Freundl et al. (2000) showed that under transpiring conditions, ABA increases L_{pr} substantially.

This is the result of direct ABA action on the plasma membranes of root cortical cells (Hose et al. 2000), and is most pronounced when an exodermal layer prevents ABA loss to the surrounding medium (Freundl et al. 2000). The possibility of increased hydraulic conductivity in orchid roots after drought is particularly interesting considering the earlier suggestion by Zotz and Tyree (1996) that efficient water uptake during and immediately after sporadic rainfalls should be essential for the survival of this species during the long dry season in central Panama. To date, however, neither of these effects has been documented for this or any other epiphyte, but it is intriguing to speculate that stronger ABA accumulation in smaller plants may lead to increased drought tolerance and higher root hydraulic conductivity compared to larger conspecifics. Both may confer a considerable advantage on smaller plants with their high surface-to-volume ratios, which make them particularly vulnerable to desiccation: these physiological differences may partly mitigate the morphological disadvantage.

The observations that roots are the first to react to drought with a sharp increase in ABA (Fig. 1) and that the increase in ABA in the xylem is slightly delayed (Fig. 1) indicate at least some transfer of this hormone from roots to leaves, although our data do not allow us to quantify the proportion of leaf ABA derived from roots. Consistent with the notion that roots can be primarily sites of ABA production while leaves also accumulate and metabolise ABA originating from other organs is the finding that the conjugate ABA-GE accumulated in leaves but not in roots. Roots of bark epiphytes such as *D. emarginata* have to cope with a complete absence of soil. They start desiccating immediately after the end of a rain event and experience much stronger fluctuations in water content compared to leaves. Hence, with the possible exception of tank bromeliads, the role of epiphyte roots as the prime sensors of water availability may be at least as important as that of roots in terrestrial plants (Zhang et al. 1987; Lambers et al. 1998; Hose et al. 2001).

The recovery after stress release was quite fast, irrespective of the duration of the preceding drought (Figs. 1, 5). ABA returned much faster than stomatal conductance to pre-stress levels (Fig. 5), which is a common observation, and has been explained by localised differences in ABA concentrations within a leaf (Hartung and Slovik 1991). Lack of comparable studies does not allow a comparison with other epiphytes, but we may at least relate our results to other gas exchange studies under natural conditions. A recent field study on BCI with an epiphytic tank bromeliad indicates a fast response to improved water availability: *Vriesea sanguinolenta* reached pre-stress levels of net CO₂ uptake just one day after its tank had been refilled (Schmidt and Zotz 2001). However, this difference may be at least in part attributable to the watering regime. While the water supply of *D. emarginata* was intermittent (plants were watered twice daily), the possession of a tank allowed uninterrupted water uptake and hence faster recovery.

In conclusion, we report the first study on ABA relations in a vascular epiphyte under natural conditions in a lowland tropical forest. We demonstrate that both the temporal pattern and the magnitude of ABA accumulation in the bark epiphyte *D. emarginata* are size dependent. Small plants, in particular, show an extraordinarily high accumulation of ABA in all plant organs, similar to poikilohydric resurrection plants. Much of this accumulation occurs after stomatal closure, indicating a probable role of ABA in the induction of drought tolerance.

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