# Plant size and intraspecific variability in vascular epiphytes

Dissertation zur Erlangung des
naturwissenschaftlichen Doktorgrades der
Bayerischen Julius-Maximilians-Universität Würzburg

vorgelegt von

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aus Lindenberg im Allgäu

Würzburg, September 2000

Eingereicht am: ........................................................................................

Mitglieder der Prüfungskommission:

Vorsitzender: ............................................................................................

Gutachter: .................................................................................................

Gutachter: .................................................................................................

Tag des Promotionskolloquiums: ..................................................................

Dokumentурkunde ausgehändigt am: ...........................................................

# ACKNOWLEDGEMENT

First, and this is not for formal reasons, I want to thank my advisor Dr. Gerhard Zotz. As a source of constant inspiration and motivation he contributed profoundly to this thesis. Neither the enormous distance from continent to continent nor the vicinity from door to door was an obstacle for an excellent advisorship. I am also grateful for his patient way of introducing me into the 'art of writing papers'.

And then there are all the helping hands that assisted (sometimes for money, sometimes for chocolate) during the field work in Panama. These hands belong Edgardo Garrido, Jonathan Gonzales, Malte Jordy, Jaxelis Mendoza, Sabine Stuntz, Vera Thomas, and Christian Ziegler. – Thanks guys! Thanks as well to all the reliable co-workers in Würzburg, like Susanne Fischer and Ephraim Hoffmann, who assisted in experiments, while Astrid Boots and Frida Reisberg conducted the nutrient analyses.

A prerequisite for effective (field-)work during my time in Panama was the infrastructure provided by the Smithsonian Tropical Research Institute. Thanks, in particular, to 'Cajar' (boat mechanic), Oris Acevedo (scientific coordinator of BCI) and Steve Paton, who provided all climate data. Financially, this thesis was supported by a stipend of the 'Graduierten Kolleg' and the Deutsche Forschungsgemeinschaft' (SFB 251, Universität Würzburg).

Natural sciences are interesting, thrilling and fascinating. This is even more so if science is not the only topic to focus on. Therefore a special 'Thank You' to - as representatives – some of the distracters: Hubbi (the banana vine-dresser), Sabine (the big-tree conquest buddy), Jonathan (...wenn es dunkel ist, möchte ich leuchten...), Paul (for being Poll), Malte (the crater companion), and ... Vera (my love).

The final thanx go out to my parents for the everlasting certainty of providing support whenever support is needed.

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# INTRODUCTORY OVERVIEW

*The King of Propdimnak to Grildrik (Gulliver): "My beloved little Grildrik, how can one be that afraid of a monkey". Gulliver replied: "Majesty, do not forget that the monkey was a huge monster – as big as an elephant. If I were bigger or the monkey was of normal size I would be able to cope with a dozen of them – if they would dare to attack me at all".*

Gulliver in the land of the giants
from Gullivers Journeys, Jonathan Swift

Gullivers argument shows in a figurative way that being smaller confers extraordinary limitations and being larger confers extraordinary powers. 'Alice in wonderland' was able to swim in her own lake of tears when she was small and she got stuck in a room, not able to move, because she grew tremendously. Contrasting Gullivers experience being small offered new possibilities and being large posed profound problems. As exemplified in numerous fairytales and literary opuses absolute and relative changes in size have fascinated and still fascinate mankind. It is thus an ancient subject to deal with such imaginative effects of changes in size. Although it is hardly possible to forget about dwarfs and giants entirely when addressing the subject, there was and still is a scientific interest in the measurable effects of changes in size. In our western history recording Archimedes was the first to argue from the view point of elementary mathematics that in similar geometric figures of different sizes, the surface increases as the square of the linear dimensions and the volume increases as the cube. But it was Galilei (1638) who first laid down the general principle of similitude (the surface area changes proportionally with the 2/3 power of volume for objects of increasing size) and drew the first, fundamental conclusions from it: neither mankind nor nature may be able to construct buildings or organisms beyond a certain size without changing their form. Using this theoretical basis allowed the quantitative analysis of size-related properties of various functional units, be it an airplane, a boat, or a bridge. By the beginning of the last century dimensional analysis and the question of magnitude had become a major component in modern engineering. Presently, engineers increasingly take advantage of the "extraordinary powers" which are associated with extremely small object sizes (e.g. semiconductor technology).

Referring to biology it was again Galilei (1638) who hypothesized that if the rate of a metabolic process, which is associated with the volume of the organism, is dependent on the surface area the rate of this process must also scale to the 2/3 power of volume. Although mathematically correct this hypothesis does not hold for living organisms, because they undoubtedly exhibit size-related variations in form and process. In other words: they do not follow Galilei's presumptions of identical shape, bulk density and geometry. This yields the principal frame of the thesis: *What is the relation between the size of a living organism and its performance in the environment?*

## The size of an organism

The size of an organism can be measured and expressed in a multitude of ways. Generally it is referred to as a substantial variable (sensu Ipson 1960), e.g. mass, length, surface area or volume. Confusion sometimes occurs when different measures for size are used without additional information to express one by the other. However, looking for the most adequate measure for the size of living organisms we have to find a variable that is independent of changes in form and shape during growth. Units of in- and output of organisms (e.g. ions and molecules) are primarily mass defined. The mode of unit assemblage and disassemblage may be a combination of physical necessity, a genetically fixed program and an integrated response of the organism to the environment. Due to the latter case quantities like surface area, volume and length may vary although the same number of input units were incorporated into the organism the quantity mass will not. Thus the mass of an organism seems to be the most rigorous measure for size.

## Organism size and performance in the animal kingdom

Zoologists have long acknowledged body size as determinant of numerous physiological and ecological traits (e.g. Thompson 1917; McMahon 1983; Schmidt-Nielsen 1984). For example, as early as at the beginning of the last century physiologists realized that an animal looses heat through the surface of its body while the capacity for heat production is related to its volume (Sarrus & Rameaux 1838). Since then this concept has been elaborated and confirmed by a large set of experimental data: within and between species the metabolic rate (measured as oxygen consumption, food consumption, or heat production) scales with the body mass of the animal to the power of 0.75 (Kleibers law; Kleiber 1932). Another branch of zoology exclusively dealing with growth and the concomitant changes in size is the field of developmental biology (e.g. Bonner 1965). Finally, extensive effort was directed as well towards understanding, modeling and applying the relation of organism size and biomechanical aspects of zoology, e.g. supporting structure (e.g. Hertl 1963) and animal locomotion (e.g. Pedley 1977).

In short, the role of the size of an animal has long been acknowledged and subsequently treated as an important variable in ecophysiological zoology.

## Organism size and performance in the plant kingdom

Compared to the animal kingdom, effects of organism size on the physiology and comportment in the field are almost entirely neglected in the plant kingdom. Although the entire field of plant demography deals with plant size (or age), it is mostly referred to as a categorization criteria to assess plant population structure and model population dynamics (e.g. Solbrig 1980; Silvertown & Doust 1993). Plant demography does not deal with the effects of changes in size concerning plant functioning. A second field in which plant size is of elementary importance is the study of mechanical designs of plants and the obligations that are imposed on plant construction by the absolute size of the organism (e.g. Niklas 1994). Studying theses physical properties of plants is undoubtedly a valuable contribution to assessing the relationship of plants and their environment, but it is not meant to elucidate ecophysiological responses of plants to environmental constraints. This is, by definition, the task of plant physiological ecology. Although the topic of intraspecific variability of ecophysiological parameters has drawn the attention of numerous researchers during the last decades, plant ecophysiologists almost entirely ignored plant size as a potential source of intraspecific variability. The few studies conducted on size-related differences in physiological properties of vascular plants either yielded contrasting results (e.g. Donovan & Ehleringer 1991; De Soyza et al. 1996) or size-dependent morphological differences were recognized but not linked to potential physiological adaptations (e.g. Jordan & Nobel 1979).

There are two potential explanations for this conceivable lack of information: firstly, changes in size, shape and structure of multicellular plants (metaphytes) in most cases reflect an increase in the number of additional organs, e.g. leaves, or functional groups of organs, e.g. shoots. Plants are therefore considered to exhibit a modular construction mode. Clearly, each module surpasses morphological and anatomical changes before reaching a functionally mature size. But once fully differentiated each module is characterized by specific morphological, anatomical and physiological properties and serves as an expendable component in a plants construction plan which can be replaced in case of damage or malfunction. Secondly, methodological difficulties might as well have contributed to this lack of scientific curiosity. The attempt of examining solely size-related physiological phenomena by comparing e.g. small and large individuals of a tree species in the laboratory will bring about spatial and resolution problems. Field investigations, on the other hand, will have to cope with a multitude of other, constantly changing factors that potentially influence the outcome of the experiment. For example, studying effects of plant size on water relations of terrestrial plants in situ obliges various problems concerning the rooting media, e.g. differences in the water vailability of different soils, water holding capacity and drying kinetics of soils, differences in rooting depth of differently sized plants).

## Vascular epiphytes as study organisms

Vascular epiphytes feature some characteristics that make them the appropriate study organisms for answering questions about plant size and ecophysiological consequences:

(1) Many epiphytic species are highly abundant (Gentry & Dodson 1987) and their size range is small enough to find large and small plants growing side by side in the same host tree under almost identical microclimate conditions, which facilitates comparative measurements in situ. Due to the small size of even fully grown plants (compared to woody species) measurements can also be conducted on whole-plant level for the entire size range of a species.

(2) By definition, vascular epiphytes do not use the ground as an external water and nutrient reservoir. For plants growing on the same host tree and rooting mainly on the bark surface problems of different rooting depths and consequences thereof can thus be neglected in comparative studies. This is specifically true for many Bromeliaceae, in which water and nutrient uptake is supposed to function not via the root system but via trichomes on the leaf blades (e.g. Benzing 2000).

(3) Although vascular epiphytes occur mostly in moist subtropical and tropical regions a general feature of their habitat is the relative scarcity of water (e.g. Lüttge 1989; Benzing 1990). The hypothesis that the highly variable water availability shall help to investigate size-dependency phenomena arises from the following argument: Galilei predicted a size-dependency for metabolic processes that are concurrently related to the surface area and to the volume of an organism. In plants there are water relations and carbon budgets that are

> closely related to both surface and volume. Carbon balances, however, are strongly influenced by the water status of a plant, which makes plant water relations the presumable primary target, being influenced by a change in the ratio of (transpiring) surface and (water storing) volume of the organism. Changes in this ratio might be without any effect if the water status is constantly optimal over the entire size range of the species and throughout the year. Conversely, plants from drought-prone habitats should be influenced profoundly.

Studying a phenomenon in a group as important as vascular epiphytes ensures that research does not focus on an exceptional case and concurrently it offers the possibility that results might be extendable to other plant groups. The importance of the coherent ecological category of vascular epiphytes is illustrated by its taxonomic heterogeneity. Epiphytes constitute about 10% of all vascular plant species (Benzing 1990), comprising 876 genera from 84 families (Kress 1986). Moreover, they are a particularly important component of tropical and subtropical forests concerning both diversity and biomass. Typically about a third but sometimes up to 50% of all plant taxa in an evergreen tropical forest are vascular epiphytes (Gentry & Dodson 1987). Epiphyte biomass in relation to tree leaf biomass of the host tree also ranges from 35% to about 50% (Edwards & Grubb 1977; Tanner 1977).

## Scope of the dissertation

Progress in plant physiological ecology requires repeated observations of a phenomenon including a clear description followed by data analysis and the construction of hypotheses explaining the behavior of the plant(s) in the environment. A model may aid to combine different levels of ecophysiological information on the study object and may thus be able to confirm or refute the initial hypotheses.

Following these general guidelines I initially examined if the first observation of a size-dependent physiological parameter (photosynthetic capacity; Zotz 1997a), found in the epiphytic orchid *Dimerandra emarginata*, is a general phenomenon among vascular epiphytes or if this observation was an exceptional one. Given a general applicability, further investigations on one or only few focal species have to clarify if the size-dependency of one physiological parameter also the performance of epiphytes under field conditions. This part of the research shall include the relation of epiphytes with their abiotic and biotic environment on leaf as well as on whole-plant level. Modeling long-term carbon balance of entire plants covering the size range of a species shall test the hypothesis that plant size strongly influences ecophysiological properties of leaves as well as whole-plant performance in situ. Finally, the question as to the driving force behind any size-dependent physiological and ecophysiological changes shall be explored.

# PHOTOSYNTHETIC CAPACITY INCREASES WITH PLANT SIZE IN AN EPIPHYTIC ORCHID: AN EXCEPTIONAL CASE OR A WIDESPREAD PHENOMENON AMONG TROPICAL VASCULAR EPIPHYTES?

## Chapter abstract

A central objective of many ecophysiological investigations is the establishment of mechanistic explanations for plant distributions in time and space. The important, albeit mostly ignored, question arises as to the nature of the organisms that should be used as representative in pertinent experiments. It is suggested to use a "demographic approach" in physiological ecology, because physiological parameters such as photosynthetic capacity (PC, determined under non-limiting conditions with the oxygen electrode) may change considerably with plant size. A survey of ten vascular epiphyte species from central Panama revealed an up to fivefold continuous increase in PC from small to large conspecifics. Moreover, the *intraspecific* variation in PC was almost always higher than the *interspecific* variation when comparing only large individuals. Theoretical considerations and additional evidence from other ecophysiological experiments corroborate the conclusion that size must be taken into account in the design of ecophysiological experiments with vascular epiphytes. In the past, most studies have ignored plant size, which may now lead to complications in the hitherto unambiguous interpretation of their results.

## Introduction

Most vascular epiphytes show clear habitat preferences both between ecosystems or within a given forest (Benzing 1990). Numerous authors have tried to correlate physiological properties of epiphytic species with their habitat preferences (e.g. Benzing & Dahle 1971; Benzing & Renfrow 1971; Griffiths et al. 1984; Earnshaw et al. 1987; Zotz & Ziegler 1997; Hietz & Briones 1998). However, reviewing all available publications on the ecophysiology of vascular epiphytes of the past 80 years reveals that 75% of the 153 articles did not specify the size of the study organisms at all (Appendix 2.1). Sixteen percent gave vague descriptions like 'adult' and 'mature' or noted the age of the plants they used. Only 13 studies, i.e. about eight percent, provided a clear description of the actual size of the study organisms, e.g. plant height, length of the longest leaf or plant dry mass. In other words, most authors unwittingly treated individuals of unspecified size as representative for a given species.

Recent studies by Zotz (1997a) and Zotz & Ziegler (1999) with the epiphytic orchid, *Dimerandra emarginata*, question this tacit assumption by showing that important physiological parameters such as photosynthetic capacity (PC, as determined in an oxygen electrode under non-limiting conditions) or carbon isotope discrimination change with plant size. Both studies, however, were conducted on only one species. To allow a generalization of their conclusions, I repeated one of these studies with a range of species: PC was determined on individuals of nine additional species. Within a given species various specimens were considered, covering the entire size range of the species. Although the studied organisms comprised species from a variety of habitats, e.g. species typically found in the outer canopy, at mid-level growing sites, or in the understory, I expected to find the same pattern of size-related changes in PC. This expectation arises from the following argument. Assuming a decrease of surface/volume-ratios with increasing plant size and similar transpiration rates irrespective of size, internal water reservoirs of smaller plants will be depleted faster than those of larger conspecifics, resulting in a less favorable water status in smaller plants under otherwise identical conditions. The optimal partitioning theory (Bloom et al. 1985) predicts that plants respond to a limiting environmental resource in such a way as to make all resources equally limiting. If smaller plants experience water as a limiting factor earlier and more often than larger conspecifics, they should invest less of other important resources such as nitrogen (Chapin  1980) in comparatively short-lived photosynthetic tissues than larger conspecifics. Given the well documented correlation between leaf nitrogen content and PC (e.g. Field & Mooney 1986; Evans 1989), a size-related change in PC should be the consequence. Key parameters of these theoretical considerations are plant surface/volume ratio, leaf nitrogen content and PC. Determining these parameters for individuals that represent the entire size range of a species will either support or contradict the proposed theory.

## Methods

### *Field site and study organism*

The study was conducted on Barro Colorado Island (BCI; 9°10´N, 79°51´W), a 1500 ha reserve within the Barro Colorado Nature Monument, Republic of Panama. Mean annual rainfall at this tropical moist forest site (Holdridge et al. 1971) is approximately 2600 kg $m^{-2}$ with a pronounced dry season from late December to late April (Leigh & Wright 1990).

Epiphytes and hemiepiphytes comprise about 16% of the BCI flora, totaling 207 species (Croat 1978; Zotz & Ziegler 1997). The ten species used for this study (nine taxa plus *Dimerandra emarginata*, Zotz 1997a) were chosen from all strata of the forest and represent all major epiphytic taxa, e.g. orchids, bromeliads, aroids and ferns. Information on photosynthetic pathway and preferred habitat type is given in Table 2.1. Species names of flowering plants and ferns follow the Flora of Panama Checklist and Index (D'Arcy 1987) and the Flora of Barro Colorado Island (Croat 1978), respectively.

Within each species 9 - 18 individuals of different size were studied , trying to include the entire size range of the taxon from the smallest to the largest specimen encountered in the natural habitat. Maximal leaf length (non-orchids) or shoot length (orchids) was used as a measure for size. In the four species tested, these parameters were highly correlated (i.e. $r^2 >$ 0.95) with a more rigorous measure for size, i.e. plant dry mass (data not shown). Adopting this non-destructive measure for size allowed the collection of a large number of conspecifics from the same host tree without destroying the population. Within a given species I avoided to collect samples of conspecifics growing under obviously differing microclimate conditions. Furthermore, whenever it proved impossible to find all individuals, e.g. in the outer canopy on a single tree, specimens from exposed sites on several host trees (phorophytes) were collected

**Table 2.1** Characterization of study species. Given are photosynthetic pathway (compare Griffiths & Smith 1983; Zotz & Ziegler 1997) and habitat preferences (sensu Zotz & Ziegler 1997): canopy sites in the outer crown of trees (c), mid-level growing sites (m), and the understory (u).

| **Species** | **Family** | **Pathway** | **Habitat** |
|---|---|---|---|
| *Caularthron bilamellatum* (Reichb.f.) Schult. | Orchidaceae | CAM | c |
| *Dimerandra emarginata* (G. Meyer) Hoehne | Orchidaceae | $C_3$ | c |
| *Polypodium crassifolium* L. | Polypodiaceae | $C_3$ | c |
| *Vriesea sanguinolenta* Cogn. & Marchal | Bromeliaceae | $C_3$ | c |
| *Anthurium brownii* Mast. | Araceae | $C_3$ | m |
| *Epidendrum nocturnum* Jacq. | Orchidaceae | $C_3$ | m |
| *Guzmania monostachya* (L.) Rusby ex Mez | Bromeliaceae | $C_3$-CAM | m |
| *Polystachya foliosa* (Lindl.) Reichb.f. | Orchidaceae | $C_3$ | m |
| *Anthurium salviniae* Hemsl. | Araceae | $C_3$ | u |
| *Aspasia principissa* Reichb.f. | Orchidaceae | $C_3$ | u |

### *Gas exchange and leaf nitrogen content*

Oxygen exchange was measured in the laboratory using a LD2 Leaf Disc Electrode (Hansatech Ltd., King's Lynn, UK). From each selected study plant, the youngest fully expanded leaf was collected and brought to the laboratory in a moist zip-lock bag. Within three hours after collection, I started the measurements by enclosing the freshly punched, weighed leaf disc in the cuvette of the leaf disc electrode unit (kept at 30°C using a circulating water bath). Subsequently, the cuvette was charged with c. 5% $CO_2$. After calibration, the leaf discs were induced in six light-dark cycles of 5 min (c. 300 µmol $m^{-2}$ $s^{-1}$) – 5 min (0 µmol $m^{-2}$ $s^{-1}$). To determine saturating but not inhibiting light conditions for each species I first studied the response of $O_2$ evolution to PPFDs of up to 1800 µmol $m^{-2}$ $s^{-1}$ with specimens of intermediate size. Using a fixed light intensity for each species (range: 950 - 1500 µmol $m^{-2}$ $s^{-1}$) the photosynthetic capacity of leaf discs was determined. Samples were subsequently dried at 60°C for 4-7 days, weighed and nitrogen content was determined at the University of Würzburg using a CHN-O Element Analyzer (Heraeus, Hanau, Germany).

### *Shoot water content and plant leaf area*

Shoot water content was calculated as the difference of fresh and dry mass of individuals, which were similar to the plants used for leaf gas exchange measurements. Roots, however, were discarded because of their strongly varying water contents, which depended on the preceding weather conditions. Transpiring plant leaf surface was determined using a LI-3100, Li-Cor leaf area meter (Lincoln, Nebraska, USA). Since none of the study organisms is amphistomatal, only one side of the leaf was considered.

## Results

A basic assumption of the argument outlined above is a size-related change in the ratio of shoot water content (SWC) to transpiring leaf surface of the plant (PLA). Table 2.2 shows that in all six species for which data were available, this ratio scaled with plant size. The linear increase of SWC/PLA from smallest to largest individual was as low as c. 60% in *P. crassifolium* to almost 300% in *D. emarginata*. Thus, changes in plant size correspond to substantial changes in surface/volume-ratios.

**Table 2.2** Ratio of shoot water content and plant leaf area (SWC/PLA, in mg $H_2O$ $m^{-2}$) as a function of plant size for different species: x = maximal leaf length (non-orchids) or maximal shoot length (orchids), in cm. Regression equations (y = SWC/PLA), as determined by Pearson product-moment correlation, coefficients of determination ($r^2$), sample size (n), and significance levels (p) are given.

| **Species** | **Linear regression** | **$r^2$** | **n** | **p** |
|---|---|---|---|---|
| *Dimerandra emarginata* | y = 60.7 + 2.97x | 0.70 | 34 | < 0.001 |
| *Epidendrum nocturnum* | y = 53.2 + 2.67x | 0.55 | 14 | < 0.01 |
| *Guzmania monostachya* | y = 22.3 + 1.36x | 0.84 | 15 | < 0.001 |
| *Polypodium crassifolium* | y = 42.1 + 0.33x | 0.38 | 14 | < 0.05 |
| *Polystachya foliosa* | y = 30.3 + 3.02x | 0.56 | 11 | < 0.01 |
| *Vriesea sanguinolenta* | y = 60.3 + 0.93x | 0.65 | 13 | < 0.001 |

**Table 2.3** Leaf nitrogen content (N, in g N $m^{-2}$) as a function of plant size for different species: x = maximal leaf length (non-orchids) or maximal shoot length (orchids), in cm. Regression equations (y = N), as determined by Pearson product-moment correlation, coefficients of determination ($r^2$), sample size (n), and significance levels (p) are given.

| **Species** | **Linear regression** | **$r^2$** | **n** | **p** |
|---|---|---|---|---|
| *Anthurium brownii* | y = 0.69 + 0.012x | 0.39 | 12 | < 0.05 |
| *Anthurium salviniae* | y = 0.61 + 0.009x | 0.43 | 9 | 0.056 |
| *Aspasia principissa* | y = 0.43 + 0.015x | 0.58 | 12 | < 0.01 |
| *Caularthron bilamellatum* | y = 0.94 + 0.052x | 0.52 | 17 | < 0.01 |
| *Dimerandra emarginata* | y = 0.59 + 0.014x | 0.61 | 15 | < 0.001 |
| *Epidendrum nocturnum* | | | 14 | n.s. |
| *Guzmania monostachya* | y = 0.13 + 0.008x | 0.57 | 18 | < 0.001 |
| *Polypodium crassifolium* | y = 0.41 + 0.005x | 0.49 | 15 | < 0.01 |
| *Polystachya foliosa* | y = 0.35 + 0.028x | 0.44 | 12 | < 0.05 |
| *Vriesea sanguinolenta* | y = 0.28 + 0.003x | 0.66 | 14 | < 0.001 |

Leaf nitrogen content (leaf N) changed with plant size in nine of ten taxa (Table 2.3). Likewise the well-established positive relationship between leaf N and photosynthetic capacity (PC; e.g. Field & Mooney 1986) applied to these taxa as well (data not shown). Not surprisingly, I also found size-related changes in area-based PC in nine species (Figure 2.1). The increase in *E. nocturnum* was non-significant though marginally ($p = 0.055$). Changes in PC from smallest to largest individuals ranged from c. 110% (*P. crassifolium*) up to almost 500% (*G. monostachya*). The finding of a size-related increase of PC is not merely a consequence of changes in leaf anatomy, e.g. an increase in leaf thickness. Dry mass based PC showed clear increases in seven of the investigated species (data not shown), although in two cases (*G. monostachya*, *A. salviniae*) trends were marginally non-significant though ($p = 0.056$ and $p = 0.058$ respectively).

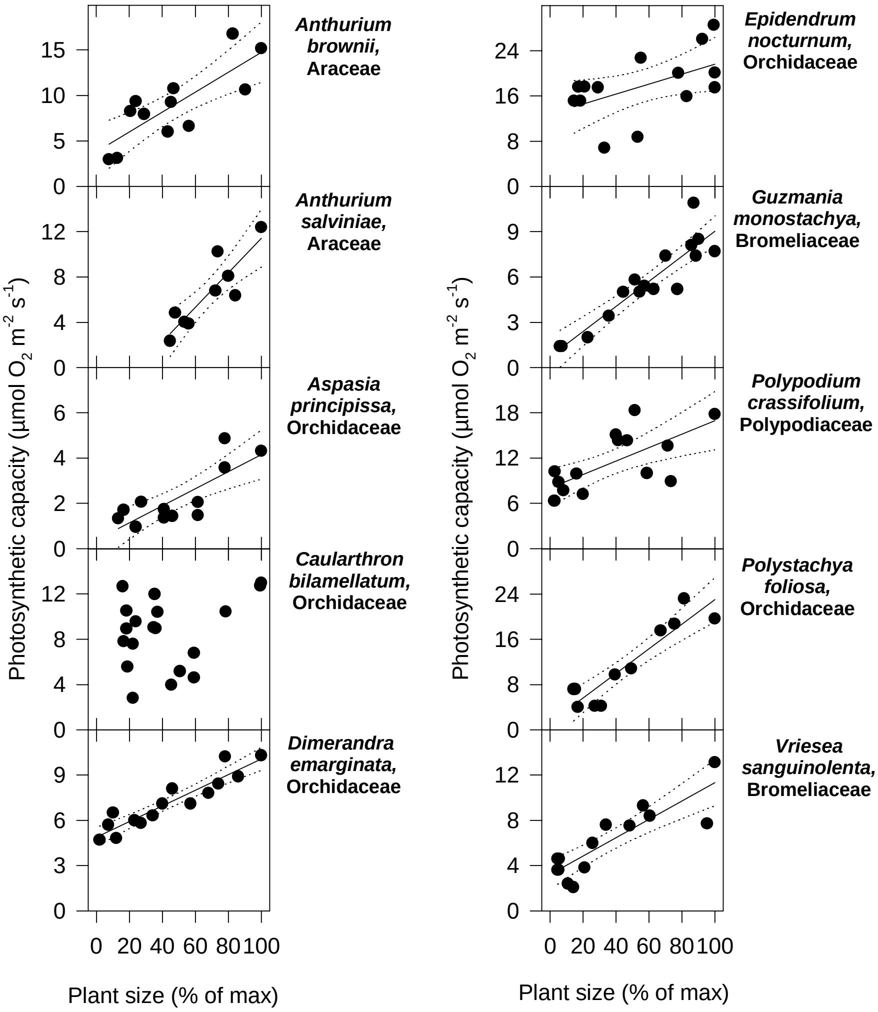

**Figure 2.1** Relationship of photosynthetic capacity (PC, in µmol $O_2$ $m^{-2}$ $s^{-1}$) and plant size (in % of maximal of the species) in ten epiphyte species. Each circle symbolizes an individual plant. Coefficients of determination range from 0.47 to 0.86 (Pearson product-moment correlation), regression lines (solid) and 95% confidence intervals (dotted lines) are given for species with $p \leq 0.05$. Data for *D. emarginata* are from Zotz (1997a).

The magnitude of the interspecific and intraspecific variation in PC is illustrated in Figure 2.2. Comparing the variability in PC of individuals within a species (expressed as the deviation of the extreme values from the mean in percent), with the variability between the larger individuals of different species yields a surprising result: intraspecific variability equals or even exceeds interspecific variability in most cases.

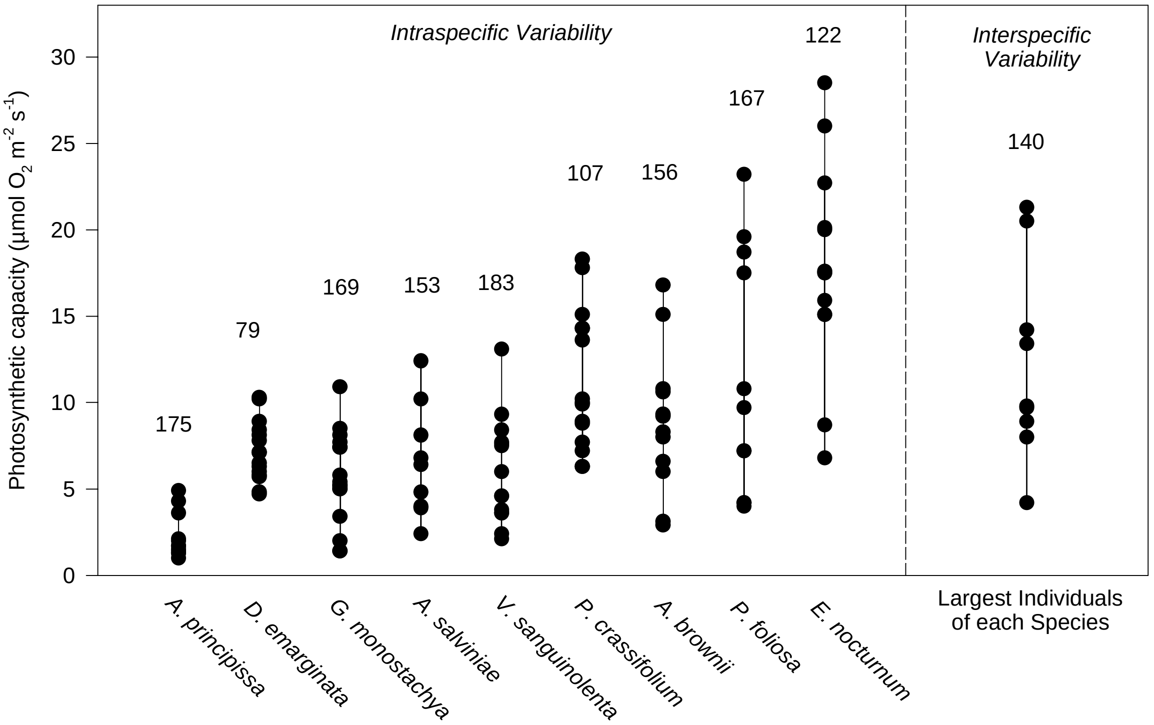

**Figure 2.2** Comparison of inter- and intraspecific variability of photosynthetic capacity (PC). Numbers above bars are relative deviations (in %) of the extremes from the mean. Data for *D. emarginata* are from Zotz (1997a). **Left panel** *intraspecific* variability of (PC, in µmol $O_2$ $m^{-2}$ $s^{-1}$). Only those species were considered in which PC scales with plant size (compare Figure 2.1). Given are ranges of PC (solid line) with each circle symbolizing one plant. **Right panel** *interspecific* variability of PC with each point representing one taxon. Species values were calculated as the mean PC of the largest individuals (80 – 100 % of maximal size) of each species.

## Discussion

This study strongly suggests that the finding of a size-related change in PC is not limited to an exceptional case, i.e. *Dimerandra emarginata* (Zotz 1997a), but is observed in almost all taxa investigated to date (Figure 2.1). Physiological results, i.e. PC and leaf N values, from larger individuals are clearly not representative for smaller conspecifics, and vice versa. Hence, the concept of a species-specific PC for the members of a given species growing under similar climate and nutritional conditions does not necessarily apply to epiphytes. The tightness of the correlation between plant size and PC is surprising considering that samples were collected in the field: in almost all cases, plant size alone explained more variation in PC than all other sources such as possible small scale differences in microclimate or differences in genotype (i.e. $r^2 > 0.5$; Figure 2.1). Moreover, the intraspecific variability may even exceed interspecific differences (Figure 2.2). As a consequence, it is suggested to use a “demographic” approach in ecophysiological studies with vascular epiphytes. When seeking physiological explanations for plant distributions, it is not sufficient to exclusively study larger individuals or individuals of unspecified size, and interpret correlations of ecophysiological comportment and habitat preference as meaningful for “the” species. Although numerous studies in the past have yielded apparently reasonable relationships (e.g. Benzing & Dahle 1971; Medina 1974; Griffiths et al. 1984; Earnshaw et al. 1987; Zotz & Ziegler 1997; Hietz & Briones 1998), the fact that smaller individuals may function quite differently from larger conspecifics now calls for caution in the interpretation of these studies in an ecological context. The key to finding an ecophysiological basis of epiphyte distribution patterns is certainly the study of physiological properties of different individuals representing the entire size range of a species.

As yet, this statement is based mostly on oxygen electrode measurements (Zotz 1997a; this study). To put measurements of $O_2$ exchange in an ecologically more meaningful framework, we should relate them to in situ $CO_2$ exchange. Zotz et al. (1997) - in a study on tropical hemiepiphytes - reported a close correlation of PC (as determined under non-limiting conditions in the oxygen electrode) and the maximum rate of net photosynthesis under favorable field conditions ($NP_{max}$; determined with an infrared gas analyzer, IRGA, at ambient $CO_2$): $NP_{max}$ was consistently lower by about 20 - 40%. I extended this analysis to $C_3$ epiphytes by using unpublished data or by including values available in the literature (Table 2.4). Although parameters in the field while determining $NP_{max}$ can not be assumed to be uniform and although the exact definitions of $NP_{max}$ differed in the cited studies (Table 2.4), the correlation of PC and $NP_{max}$ is surprisingly robust (Figure 2.3). This suggests that size-dependent changes in PC do correspond to similar changes in the maximal rates of in situ $CO_2$ exchange. Moreover, given the correlation of $NP_{max}$ and diel carbon gain (Zotz & Winter 1993), the results can even be used as an indicator of potential 24 h leaf carbon budgets.

**Table 2.4** $NP_{max}$ (IRGA; in µmol $CO_2$ $m^{-2}$ $s^{-1}$, ) and PC (oxygen electrode; in µmol $O_2$ $m^{-2}$ $s^{-1}$) for epiphytic and hemiepiphytic $C_3$-species. “Dev” is the deviation of $NP_{max}$ from PC (in %). If available, specifications on plant size are provided. Grouped in size categories (SC) maximal leaf length ($LL_{max}$) served as a measure of plant size.

| Species | Family | Size specification | $NP_{max}$ | PC | Dev | References |
|---|---|---|---|---|---|---|
| *Aspasia principissa* | Orchidaceae | $LL_{max}$=5 - 10cm | $1.1^1$ | 1.4 | 21 | Schmidt unpubl. |
| *Aspasia principissa* | Orchidaceae | $LL_{max}$=15 - 20cm | $1.5^1$ | 2.4 | 37 | Schmidt unpubl. |
| *Aspasia principissa* | Orchidaceae | $LL_{max}$ > 28cm | $2.2^1$ | 3.4 | 35 | Schmidt unpubl. |
| *Catasetum viridiflavum* | Orchidaceae | none | $6.4^1$ | | 52 | Zotz & Winter 1994 |
| | | max. size | | 13.2 | | Stuntz & Zotz 2001 |
| *Cosmibuena macrocarpa* | Rubiaceae | none | $16.1^1$ | 24.4 | 34 | Zotz et al. 1997 |
| *Coussapoa magnifolia* | Moraceae | none | $23.2^1$ | 30.1 | 23 | Zotz et al. 1997 |
| *Coussapoa panamensis* | Moraceae | none | $14.8^1$ | 19.7 | 25 | Zotz et al. 1997 |
| *Dimerandra emarginata* | Orchidaceae | max. size | $7.0^1$ | | 30 | Zotz & Tyree 1996 |
| | | max. size | | 10.0 | | Zotz 1997a |
| *Ficus citrifolia* | Moraceae | none | $10.6^1$ | 16.5 | 36 | Zotz et al. 1997 |
| *Guzmania lingulata* | Bromeliaceae | adult | $1.9^2$ | | 21 | Benzing & Friedman 1981 |
| | | max. size | | 2.4 | | Stuntz & Zotz 2001 |
| *Havetiopsis flexilis* | Clusiaceae | none | $8.1^1$ | 10.1 | 20 | Zotz et al. 1997 |
| *Polypodium crassifolium* | Polypodiaceae | none | $7.8^1$ | | 11 | Zotz & Winter 1994 |
| | | max. size | | 8.8 | | Stuntz & Zotz 2001 |
| *Souroubea sympetala* | Marcgraviaceae | none | $10.1^1$ | 20.2 | 50 | Zotz et al. 1997 |
| *Vriesea sanguinolenta* | Bromeliaceae | $LL_{max}$=5 - 10cm | $2.8^1$ | 4.2 | 33 | Schmidt unpubl. |
| *Vriesea sanguinolenta* | Bromeliaceae | $LL_{max}$=20 - 25cm | $3.3^1$ | 7.7 | 57 | Schmidt unpubl. |
| *Vriesea sanguinolenta* | Bromeliaceae | $LL_{max}$ > 70cm | $5.3^1$ | 13.9 | 62 | Schmidt unpubl. |

[1] maximal values from diel courses in the field.

[2] values from light response curves at saturating light intensities

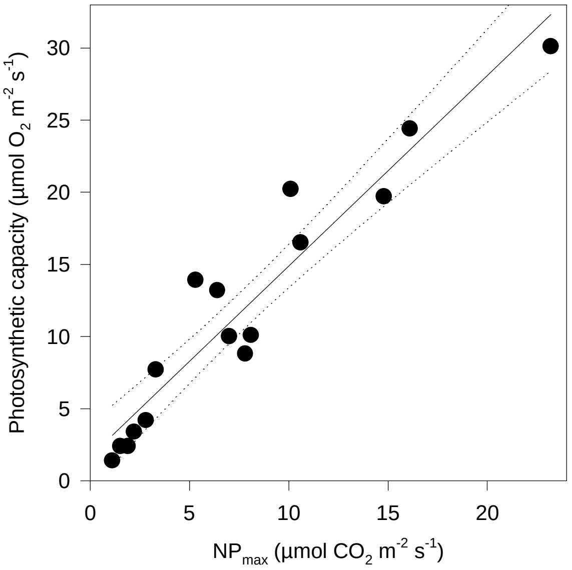

**Figure 2.3** Relationship of maximal photosynthesis rates ($NP_{max}$; IRGA; in µmol $CO_2$ $m^{-2}$ $s^{-1}$) and photosynthetic capacity (PC; oxygen electrode, in µmol $O_2$ $m^{-2}$ $s^{-1}$). Each circle symbolizes a taxon (or a size category of a taxon) for which both parameters were available. Given are regression line (solid) and 95% confidence intervals (dotted lines). The linear regression is: $PC = 1.32\ NP_{max} + 1.69$, $r^2 = 0.91$, $n = 16$, $p < 0.001$ (Pearson product-moment correlation). Data for individual species are from Table 2.4

However, the maximum rate of $CO_2$ uptake is dependent on plant water status (Larcher 1994). Here, smaller plants should strongly differ from larger conspecifics (Table 2.2; Zotz & Andrade 1998). Plant size may not only affect tissue water content, but also the efficiency of external water storage as in water impounding tanks of many bromeliads (Benzing 1980). Using a model approach, Zotz & Thomas (1999) showed for two epiphytic bromeliads, *Guzmania monostachya* and *Tillandsia fasciculata*, that smaller individuals experienced empty tanks more often than larger conspecifics. Thus, a smaller plant is not only expected to show a lower PC (and therefore also a lower $NP_{max}$; Figure 2.3) but the frequency with which maximal assimilation rates can be performed should also be lower. The integrated long-term carbon gain under field conditions should therefore be even more reduced in smaller plants than indicated by the differences in PC. Strictly speaking, this argument is only valid for the leaf type measured, i.e. the youngest fully expanded leaf. Considering that this developmental stage of a leaf is usually the most productive (Tichá 1982) and that water availability should affect the investment strategies of the entire organism, I suggest that similar size-related changes could be seen in older leaves as well. A whole-plant model for predicting long-term carbon gain for the $C_3$ tank bromeliad, *V. sanguinolenta* (Chapter 5) will incorporate all the different aspects mentioned above, and also address the problem of size-related architectural differences, which might change the relative importance of self-shading.

We do not yet have a conclusive, experimental proof of the actual mechanism behind the results of this study. Thus, other explanations cannot be ruled out. For example, given the nutrient-poor habitat, smaller and hence normally younger plants may simply have not had the time to take up sufficient amounts of, e.g. nitrogen. Until experiments prove or disprove the proposed scheme, we have to rely on circumstantial evidence and the consistency of the data with theoretical considerations, i.e. the optimal partitioning theory (Bloom et al. 1985). Irrespective of the actual mechanism, the basic conclusion retains its validity: size-related changes in ecophysiological parameters do exist and have to be adequately addressed in future studies.

## Appendix

**Appendix 2.1** Literature survey: information about the size (or age) of vascular (hemi-)epiphytes in ecophysiological studies during the last 80 years. Numbers in brackets are the sums of articles per category.

*Plant size as an ecophysiological variable (6)*

Putz & Holbrook 1989
Zimmerman 1990
Zotz & Andrade 1998
Zotz & Thomas 1999
Zotz & Ziegler 1999
Zotz 1997a

*Specific information on plant size (7)*

Borland et al. 1996
Clifford et al. 1995
Winter et al. 1992
Zimmerman 1991
Zotz & Tyree 1996
Zotz 1999
Zotz et al. 1999

*Specific information on plant age (3)*

Benzing 1970
Kluge et al. 1997
Savé et al. 1985

*Information on ontogenetic stage (22)*

Ball et al. 1991a
Ball et al. 1991b
Benzing & Dahle 1971
Benzing & Davidson 1979
Benzing & Renfrow 1971a
Benzing & Renfrow 1971b
Benzing & Renfrow 1971c
Benzing 1978
Bergstrom & Tweedie 1998
Bermudes & Benzing 1991
Fu & Hew 1982
Gay 1993
Goh et al. 1977
Griffiths 1988
Hew et al. 1989
Holbrook & Putz 1996
Mercier et al. 1997
Ng & Hew 1996
Roberts et al. 1996
Wadasinghe & Hew 1995
Yong & Hew 1995c
Zotz & Ziegler 1997

*No information (115)*

Andrade & Nobel 1996
Barthlott et al. 1997
Benzing & Burt 1970
Benzing & Friedman 1981
Benzing & Pockman 1989
Benzing & Renfrow 1974a
Benzing & Renfrow 1974b
Benzing et al. 1982
Beßler et al. 1998
Biebl 1964
Bonates 1993
Borland & Griffiths 1990
Borland et al. 1992
Boyer 1964
Burt & Benzing 1969
Carter & Martin 1994
Clarkson et al. 1986
Cockburn et al. 1985
Coutinho 1965
Coutinho 1969
Diaz et al. 1996
Donovan et al. 1984
Dycus & Knudson 1957
Earnshaw et al. 1987
Erickson 1957
Feild et al. 1997
Fisher et al. 1990
Goh 1983
Goh et al. 1983
Gregg 1982
Griffiths & Smith 1983
Griffiths 1989
Griffiths et al. 1984
Griffiths et al. 1986
Griffiths et al. 1989
Guralnick et al. 1986
Harper 1919
Harris 1918
Hew & Lim 1989
Hew & Ng 1996
Hew 1984
Hew et al. 1984
Hew et al. 1991
Hew et al. 1993
Hew et al. 1995
Hew et al. 1996
Hew et al. 1998
Hietz & Briones 1998
Ho et al. 1983
Jordan 1984
Kluge et al. 1973
Kluge et al. 1989
Kluge et al. 1995
Lieske 1915
Loeschen et al. 1993
Lüttge et al. 1986a
Martin & Adams III 1987
Martin & Schmitt 1989
Martin & Siedow 1981
Martin et al. 1985
Martin et al. 1986
Maxwell et al. 1992
Maxwell et al. 1994
Maxwell et al. 1995
McWilliams 1970
Medina & Minchin 1980
Medina & Troughton 1974
Medina 1974
Medina et al. 1977
Medina et al. 1989a
Milburn et al. 1968
Mooney et al. 1989
Neales & Hew 1975
Nobel et al. 1984
Nowak & Martin 1997
Nyman et al. 1987
Ong et al. 1986
Pessin 1924
Pessin 1925
Popp et al. 1987
Rada & Jaimez 1992
Renner 1933
Rickson 1979
Schäfer & Lüttge 1986
Schlesinger & Marks 1977
Schmitt et al. 1988
Sengupta et al. 1981
Shreve 1908
Sinclair 1983a
Sinclair 1983b
Sinclair 1984
Sipes & Ting 1985
Smith et al. 1985
Smith et al. 1986
Starnecker & Winkler 1982
Stewart et al. 1995
Stiles & Martin 1996
Stuart 1968
Ting 1989
Ting et al. 1987
Treseder et al. 1995
Winter et al. 1983
Winter et al. 1985
Winter et al. 1986
Wong & Hew 1975
Wong & Hew 1976
Yong & Hew 1995a
Yong & Hew 1995b
Zimmerman & Ehleringer 1990
Zotz & Winter 1994a
Zotz & Winter 1994b
Zotz & Winter 1994c
Zotz & Winter 1996
Zotz et al. 1994
Zotz et al. 1997

# ECOPHYSIOLOGICAL CONSEQUENCES OF DIFFERENCES IN PLANT SIZE – IN SITU CARBON GAIN AND WATER RELATIONS FOR AN EPIPHYTIC BROMELIAD

## Chapter abstract

The present field study with the $C_3$ bromeliad *Vriesea sanguinolenta* was initiated to explore the importance of size-related ecophysiological changes in vascular epiphytes in a natural tropical setting. In this species a step change from atmospheric to tank-forming life form occurs during early ontogeny, followed by a continuous size increase of individuals with water-impounding tanks. Although the study focused on the water-impounding phase this growth pattern also allowed a comparison of ecophysiological consequences of a step change in life form with those associated with size increments among plants of identical life form.

The shift in life form was accompanied by relatively minor changes, e.g. in leaf morphology (decrease in leaf thickness and trichome density) or leaf physiology (decrease in photosynthetic capacity), while there were more substantial changes during the tank forming phase. A major trend was a decreasing dependence of larger plants on internally stored water due to a more efficient tank. It is suggested that the resulting, more reliable water supply in larger plants may be the proximate cause for the observed size-related differences in leaf anatomy (relative reduction of water storage tissue, and relative and absolute increase in chlorenchyma thickness), leaf morphology (increase in stomatal density, decrease in trichome density), and leaf physiology (increase in net rates of $CO_2$ uptake, more conservative stomatal behavior, higher residual transpiration). The results are compared with previous studies on heteroblasty in bromeliads, but are also discussed in the context of a gradual shift from a drought-tolerance to a drought-avoidance strategy.

## Introduction

In many epiphytic bromeliads there is a conspicuous shift between two ontogenetic phases (Benzing 2000): while small juveniles have morphological characteristics of atmospheric tillandsioids (i.e. possess non-impounding rosettes of small, linear leaves, which are completely covered by the shields of foliar trichomes), larger conspecifics may form tanks (i.e. feature broad leaves, which overlap basely forming water-filled chambers). This shift in life form was first analyzed functionally in *Tillandsia deppeana* (Adams III & Martin 1986a; Adams III & Martin 1986b). It could be shown, that there are a number of morphological and anatomical differences between “juvenile” atmospherics and “adult” tanks. Moreover, Adams & Martin (1986b) observed profound variation in a number of physiological characteristics. For example, tank forming individuals of *T. deppeana* showed systematically higher rates of $CO_2$ exchange and transpiration when well watered, but gas exchange was affected more severely by the onset of drought than in smaller, atmospheric conspecifics.

While these and other researchers (e.g. Reinert & Meirelles 1993) recognized significant physiological changes during ontogeny, they treated individuals within a phase as homogeneous groups. Recent evidence indicates, however, that physiological parameters in vascular epiphytes may scale continuously with size. For example, Schmidt et al. (2001) showed for a set of 10 species of epiphytes (mostly bromeliads and orchids) that intraspecific variation in photosynthetic capacity (PC) as determined in an oxygen electrode may be substantial: size-related differences in PC within a species frequently exceeded the interspecific variation in this parameter. Thus, lumping atmospheric “juveniles” and water-impounding “adults” in two groups may actually conceal morphoanatomical and physiological differences within the groups. Conversely, identical environmental conditions should affect plants of similar ontogenetic phase but varying size differentially, because a) smaller individuals have a higher ratio of transpiring surface to stored tissue water (Zotz 1997a; Schmidt et al. 2001) and b) smaller tanks are less efficient than large ones in bridging rainless periods (Zotz & Thomas 1999). Counteracting these continuously changing environmental constraints, physiological characteristics may also change continuously with plant size.

At present, our understanding of size-related changes in physiological parameters in vascular epiphytes is still in its infancy. Available information is restricted to measurements of PC with the oxygen electrode (Zotz 1997a; Schmidt et al. 2001) and determinations of carbon isotope ratios in leaves of the orchid, *Dimerandra emarginata*, as an indicator of long-term water use efficiency (Zotz & Ziegler 1999). Direct measurements of in situ gas exchange as a function of plant size are missing, as are investigations of the size dependency of other physiological parameters. However, the ecological importance of the published findings of size-related changes in physiological parameters can only be assessed with field data, which motivated the present study. *Vriesea sanguinolenta* was chosen, because it features a phase change between atmospheric and tank form and a wide range of sizes among tank forming individuals (due to continuous size increments over decades, unpubl. observ.). These characteristics along with already available information on its ecology (Zotz 1997b; Zotz et al. 1999a) made it an ideal species for our purposes.

Specifically, I asked the following questions: (1) how does leaf morphology and anatomy change with plant size? (2) under well watered conditions, is there a similar size-related increase in diel leaf carbon gain as there is in PC (Schmidt et al. 2001)? (3) how does leaf $CO_2$ exchange react to decreased water availability? 4) after stomatal closure, does residual transpiration of leaves scale with plant size?

## Methods

### *Field site and study organism*

The study was conducted on Barro Colorado Island (BCI, 9°10´N, 79°51´W), a 1500 ha reserve within the Barro Colorado Nature Monument, Republic of Panama and the adjacent peninsulas. Mean annual rainfall at this tropical moist forest site (Holdridge et al. 1971) is approximately 2600 kg $m^{-2}$ with a pronounced dry season from late December to late April

(Leigh & Wright 1990). Detailed descriptions of vegetation, climate and ecology are provided by Croat (1978) and Leigh et al. (1982).

The study species *Vriesea sanguinolenta* Cogn. & Marchal (Bromeliaceae; syn. *Werauhia sanguinolenta*; Grant 1995) occurs from Costa Rica to Colombia and on various Caribbean islands from lowland up to lower montane wet forests (Croat 1978). This epiphytic bromeliad can be found on a range of different host tree species, but on BCI it is particularly abundant on *Annona glabra* L. (Annonaceae; Zotz et al. 1999). Throughout the study I used the length of the longest leaf per plant ($LL_{max}$) as a measure of plant size. This parameter was highly correlated with a more rigorous measure for size, i.e. plant dry mass (DM; Table 3.2). Adopting this non-destructive measure of size allowed the collection of samples from a large number of conspecifics from the same host tree without destroying the population: in this study all plant material stemmed from the phorophyte *A. glabra*. The life cycle of *V. sanguinolenta* comprises three distinct life stages a) an atmospheric stage, b) a vegetative tank forming stage and c) a reproductive tank forming stage. Plants are polycarpic, but individual shoots flower only once. Reproductive plants were not included in the experiments.

### *Morphometry and anatomy*

Leaf area index (LAI) of individual plants was estimated by taking photographs from above with a digital camera along with a reference area. Images were analyzed with Corel Paint 7.0 (Corel Corp. Ltd., Ontario, Canada) to derive the projected plant leaf area (PPLA). Leaves were counted, and their cumulative area (plant leaf area, PLA) was determined using a LI-3100 leaf area meter (Li-Cor Inc., Lincoln, Nebraska, USA). The ratio of PLA and projected ground area, i.e. PPLA, was defined as LAI. Specific leaf length was defined as the ratio of leaf length and leaf breadth. Following Kluge & Ting (1978) succulence index (SI) was $SI = (FM_{sat} – DM) / LA$, with $FM_{sat}$ = fresh mass of fully hydrated leaves, DM = leaf dry mass, LA = leaf area (one sided).

Prior to all anatomical studies plants were well watered for at least three subsequent days. On hand-made cross sections of fresh leaf material I determined tissue thickness under a light microscope. Leaf thickness was calculated as the sum of all tissue layers of a given cross section. Random determinations of leaf thickness with a caliper prior to anatomical studies deviated by less than 5 % from these estimates. Leaves of *V. sanguinolenta* are hypostomatous, therefore stomatal densities refer only to the abaxial side. The number of trichomes, in contrast, was related to a bi-layered leaf surface. Trichome and stomatal densities were both determined in the center of the distal half of a leaf.

### *Physiological measurements during the drought experiment*

The drought experiment was conducted during the 1998 dry season. Plants were collected in the field and transferred to the lab clearing of BCI. In situ microclimate conditions were simulated with a large metal rack covered with shade cloth. Natural habitat conditions, i.e. upper crown of *Annona glabra* were: photosynthetic photon flux density (PPFD; as daily integrals; n = 23): 9 - 27 mol $m^{-2}$ $d^{-1}$, temperature (as daily means; n = 14): 25.6 – 30.7 °C, relative humidity (as daily minimum; n = 14): 65 – 78 %; experimental set-up: PPFD (n = 12): 5 - 28 mol $m^{-2}$ $d^{-1}$, temperature (n = 12): 24 – 31 °C, relative humidity (n = 12): 62 –

72%. Four size categories (SC) were defined: SC 1: 0.5 – 4 cm $LL_{max}$, atmospheric (20 plants), SC 2: 5 – 8 cm $LL_{max}$ (ten plants), SC 3: 28 - 35 cm $LL_{max}$ (eight plants), SC 4: > 73 cm $LL_{max}$ (four plants). For gas exchange studies four plants were chosen at random from SC 2 and SC 3, and two from SC 4. The remaining plants were used for the determination of bulk leaf water potentials and nocturnal acidification. For all measurements the youngest, fully expanded leaves were used, all samples being taken from the distal half of the respective leaf. Although the gas exchange of only one plant per SC was measured at a given time, the variability within a SC was included by switching to a different plant of that SC after each measurement. Psychrometric measurements and measurements of nocturnal acidification were made on a daily basis with three replicates per SC.

Prior to the experiment plants were irrigated for at least three days twice a day. At the onset of the experiment (day 0) plants were thus fully hydrated. After day 0 tanks of all individuals were emptied and plants were kept dry for 11 days. During the experiment I monitored in situ leaf gas exchange hourly using a $CO_2$/$H_2O$-porometer (CQP 130, Walz, Effeltrich, Germany) operating in a continuous open-flow mode, connected to an infrared gas analyzer (IRGA; BINOS 100, Rosemount, Hanau, Germany): for methodological details see Zotz et al. (1995). Gas exchange parameters were calculated following Von Caemmerer & Farquhar (1981). Photosynthetic photon flux density was monitored outside the cuvette, in close vicinity of the leaf. Ambient $CO_2$ concentrations ranged from 340 – 380 µl $l^{-1}$.

Every day, leaf discs were punched out of the distal half of study leaves before dawn. Leaf water potential ($\Psi$) was determined psychometrically using sample chambers (stem hygrometer, Plant Water Status Instruments, Ontario, Canada) that were thermally stabilized, and a Wescor HR-33T dew point microvoltmeter (Wescor, Logan, Utah, USA).

For the determinations of titratable acidity ($H^+$) samples from the distal half of leaves (c. 5 $cm^2$) were taken at dusk and the following dawn. Samples were frozen and then boiled in 40% (v/v) ethanol for about half an hour. Cold extracts were titrated to pH 7.0 with 2 mM NaOH. Nocturnal acidification ($\Delta H^+$) was estimated from $H^+_{dawn}$ - $H^+_{dusk}$.

*Other measurements*

Light response curves were determined using a similar set-up as described above, but with an artificial light source (Fiber Illuminator F-400 & FL-400, Walz, Effeltrich), which provided homogeneous PPFDs over the leaf area enclosed in the cuvette. Other parameters were: cuvette temperature: 28.5 – 30.5 °C, relative humidity inside the cuvette: 90 – 95%. Plants, which covered the entire size range of the non-reproductive tank category, were well watered for at least three days. Gas exchange of leaves was measured between 9:30 - 11:30 in their distal half. Leaves had already been induced by exposure to saturating ambient light. For light response curves I initially determined gas exchange rates in the dark, then increased PPFD to c. 5 µmol $m^{-2}$ $s^{-1}$ and thereafter roughly doubled light intensities up to 1600 µmol $m^{-2}$ $s^{-1}$ in ten steps. A spectral shift towards longer wavelengths at lower PPFD was avoided by using neutral Grey filters. Subsequently, cardinal points of the photosynthetic light response were calculated after Smith (1937) as modified by Tenhunen et al. (1976).

For the determination of photosynthetic capacity (PC) I used a LD2 Leaf Disc Electrode (Hansatech Ltd., King's Lynn, UK). From the same plants used for light response curves, I collected leaf discs of the two youngest fully expanded leaves, and brought them to the laboratory in a moist zip-lock bag. Immediately after collection (i.e. after 5 – 10 min), I started the measurements by enclosing leaf discs in the cuvette of the oxygen electrode unit (kept at 30°C with a circulating water bath). Subsequently, the cuvette was charged with c. 5% $CO_2$ (Walker 1992). After calibration, the leaf discs were induced in two light-dark cycles of 10 min – 5 min intervals with a PPFD of c. 400 µmol $m^{-2}$ $s^{-1}$ for the first and 800 µmol $m^{-2}$ $s^{-1}$ for the second cycle. Then PC was determined at saturating PPFD (1300 µmol $m^{-2}$ $s^{-1}$). Samples were subsequently dried at 60°C for seven days and weighed. The PC of a plant was defined as the highest value of at least three measurements for different leaves and/or different positions within a leaf.

Leaf drying curves were conducted with fully hydrated leaves, i.e. plants were well watered for several days prior to measurements. Then, in the early morning, leaves were detached with a razor blade, sealed immediately with silicon grease, and leaf surfaces dried with tissue paper. After an initial weighing leaves were placed on the lab bench with the following conditions: temperature: c. 25 °C, PPFD: c. 5 µmol $m^{-2}$ $s^{-1}$ (day); 0 (night), relative humidity: c. 70%. Leaves were weighed about hourly during the first day, later every eight hours. The weighing procedure was continued until a constant water loss rate was detected, i.e. for six days. Finally, samples were dried at 60°C for three days and DM was determined. For the estimate of the relative water deficit at stomatal closure ($RWD_{STC}$) I followed the procedure of Pisek & Berger (1938) by plotting RWD vs. the inverse transpiration rate ($T^{-1}$). Values were referred to the total leaf area of ad- and abaxial surface. The RWD was defined as 1 – (FM – DM) / ($FM_{SAT}$ – DM) and T = ΔFM / Δt LA, with FM = leaf fresh mass, $FM_{SAT}$ = fresh mass of a leaf when fully hydrated, ΔFM / Δt = change of fresh mass per time interval, and LA = leaf area. The break point of a piecewise linear regression of RWD and $T^{-1}$, determined graphically, was assumed to represent $RWD_{STC}$ (Figure 3.1). Although the exact time of stomatal closure is not well defined, this approach yields a useful index of the relative water content at which stomatal closure is complete (Cape & Percy 1996). The steady state water loss after the break point was interpreted as residual transpiration (RT). Finally, permeances (P) were calculated as P = RT / Δc, with Δc being the gradient of water vapor concentration inside and outside of the leaf.

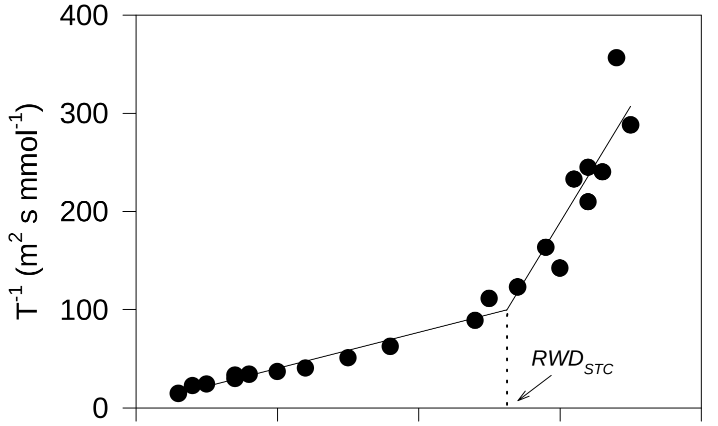

**Figure 3.1** Relationship of relative water deficit of a leaf (RWD) and inverse transpiration rate ($T^{-1}$). Values are from a leaf drying experiment using a leaf of 87 cm length. Solid lines represent a linear piecewise regression with $T^{-1}$ = 366.7 RWD + 3.48 and $T^{-1}$ = 2366.8 RWD – 521.1, $r^2$ = 0.98, n = 21. The RWD at the break point (RWD = 0.26), i.e. the intersection of the two regression lines, was assumed to reflect the approximate RWD, at which stomata close ($RWD_{STC}$).

For chlorophyll analysis, which followed the extraction procedure of Arnon (1949) and calculation after Röbbelen (1957), I took the same plants on which the light response curves were conducted, always using samples from the distal half of a leaf.

Tank water reservoir efficiency was the number of days before 90% of the water from completely filled tanks had evaporated or had been taken up by the plant. Water loss was determined gravimetrically during a rainless period of the 1997 dry season on plants covering the size range of the vegetative tank phase. Conditions were: PPFD: $32.4 \pm 9.4$ mol $m^{-2}$ $d^{-1}$, evaporation: $2.7 \pm 1.2$ kg $m^{-2}$ $d^{-1}$, temperature: $27.8 \pm 1.5$ °C (all values are means ± SD, $n = 11$).

### Data analysis

All results were statistically analyzed with STATISTICA software (STATISTICA 5.1, StatSoft Inc., Tulsa, Oklahoma, USA). The continuous size dependency of various parameters was mainly evaluated by regression analysis including all tank plants. Most data sets had to be log-transformed before analysis. The relationship between leaf conductance ($g_w$) and leaf water potential ($\Psi$) was explored using the software TableCurve for Windows (Jandel Scientific 1992, Corte Madera, California, USA). From about 30 fitted simple functions I chose the equation with the fewest parameters and the highest $r^2$, which was equally suited for all size categories, i.e. $\ln g_w = a + b\,\Psi^{-1}$.

## Results

### Size-related morphometrical and anatomical features

Atmospheric seedlings reached leaf lengths of up to 4 cm, with a maximum of 12 leaves arranged in non-impounding rosettes. Trichomes had large, overlapping shields and covered the entire ad- and abaxial leaf surface (Table 3.1). This atmospheric stage was followed by a transition to leaf rosettes forming a water-impounding tank: significantly fewer, but broader leaves (specific leaf length; Table 3.1) captured and stored water in their leaf bases. In addition, trichome densities in the distal portion of the leaves declined after the transition, while trichome size, stoma size and stomatal density remained unaffected. In addition, the thicknesses of all tissue types were reduced in the tank form, resulting in thinner leaves. In relative terms, however, hydrenchyma increased while chlorenchyma and epidermis proportions decreased (Table 3.1). Chlorophyll content did not differ but photosynthetic capacity was higher in the atmospheric life form (Table 3.1) compared to the smallest tank-forming individuals.

Initially, the 4 - 6 leaves of the small, water-impounding plants formed a single central tank. Subsequent growth was again associated with morphological (subtle increase of specific leaf length; Table 3.2) and architectural changes, e.g. the formation of numerous separate tanks in the axils of an increasing number of leaves (Table 3.2). Tank water capacity ranged from $5 \times 10^{-4}$ l in small plants ($LL_{max}$ = 5 cm) up to c. 3 l in large individuals ($LL_{max}$ = 85 cm), representing an increase of about four orders of magnitude. Plant leaf area (PLA) was between $1.4 \times 10^{-4}$ and 1.5 $m^2$, i.e. representing an increase of similar magnitude. Leaf area index (LAI) showed a three-fold increase (1.3 to 4.0 $m^2$ $m^{-2}$) for the same range of plant sizes (Table 3.2). Among tank forming plants, trichome density scaled negatively with plant size, while there was a significant increase in specific leaf weight, succulence index (Table 3.2) and leaf thickness; the latter increase was primarily due to substantial changes in chlorenchyma thickness (Figure 3.2A). The epidermis and the water storage tissue were also significantly thicker in larger plants, but increments were subtle, at least in absolute terms. Relative changes, in contrast, reflected a shift in proportions: the hydrenchyma declined as the chlorenchyma increased (Figure 3.2B). This shift in relative importance of functional tissue types was accompanied by a significant increase in area based chlorophyll content, while the chlorophyll a / b ratio and fresh mass based chlorophyll content remained unchanged (Table 3.2). Stomatal density in the distal half of the leaves also scaled with plant size (Table 3.2).

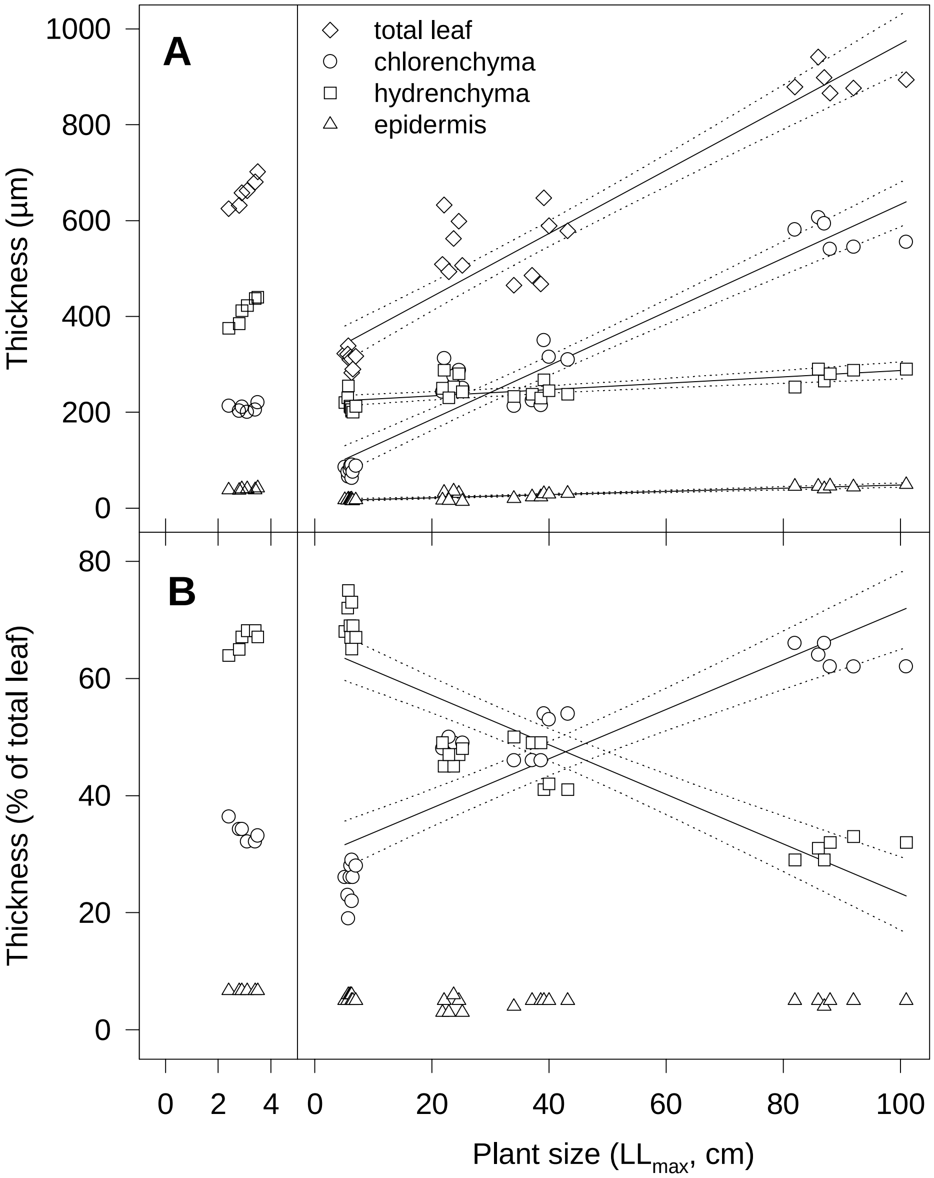

**Figure 3.2** Size dependency of anatomical features. Each symbol represents a measurement on one leaf of a separate plant. **Left panels** represent the atmospheric stage, **right panels** show plants of the vegetative, tank forming stage. For the tank phase linear regressions (solid lines) and 95% confidence intervals (dotted lines) are shown for significant trends. **Figure 3.2A** gives absolute leaf and tissue thickness (µm) as a function of plant size ($LL_{max}$). Coefficients of determination ($r^2$) of the linear regressions range from 0.53 – 0.94, $p < 0.001$. **Figure 3.2B** shows proportional tissue thickness (% of leaf thickness) as a function of plant size. The $r^2$ of both linear regressions are 0.81, $p < 0.001$.

**Table 3.1** Comparison of leaf characteristics of the atmospheric (SC 1, $LL_{max}$ = 2 – 3 cm) and the smallest tank forming stage (SC 2, $LL_{max}$ = 5 – 8 cm) of *V. sanguinolenta*. For each parameter, means ± SD of atmospherics and small tanks are given along with significance levels (p; t-test for independent samples) and degrees of freedom (df). n.s., not significant. Stomatal and trichome densities, chlorophyll content and photosynthetic capacity were determined in the distal half of a leaf.

| **Parameter** | **Atmospheric** | **Tank** | **p** | **df** |
|---|---|---|---|---|
| Leaves per plant | 8 ± 1.4 | 5 ± 0.8 | < 0.001 | 15 |
| Specific leaf length (cm $cm^{-1}$) | 16.8 ± 5.8 | 3.9 ± 0.4 | < 0.001 | 16 |
| Leaf thickness (µm) | 660 ± 29 | 312 ± 17 | < 0.001 | 13 |
| Thickness of | | | | |
| Chlorenchyma (µm) | 209 ± 7 | 79 ± 10 | < 0.001 | 13 |
| Hydrenchyma (µm) | 412 ± 27 | 216 ± 17 | < 0.001 | 13 |
| Epidermis (µm) | 39 ± 2 | 17 ± 1 | < 0.001 | 13 |
| Relative thickness of | | | | |
| Chlorenchyma (%) | 31.6 ± 1.4 | 25.2 ± 3.1 | < 0.001 | 13 |
| Hydrenchyma (%) | 62.4 ± 1.6 | 69.3 ± 3.2 | < 0.001 | 13 |
| Epidermis (%) | 5.9 ± 0.2 | 5.5 ± 0.3 | = 0.003 | 13 |
| Succulence Index (g $m^{-2}$) | 37 ± 11.4 | 334 ± 18.7 | < 0.001 | 14 |
| Relative water content (%) | 39.1 ± 7.9 | 89.3± 0.9 | < 0.001 | 14 |
| Trichome density ($mm^{-2}$) | 61 ± 4 | 48 ± 5 | < 0.001 | 13 |
| Diameter of dome cell (µm) | 52 ± 5 | 53 ± 2 | n.s. | 13 |
| Diameter of shield (µm) | 256 ± 45 | 260 ± 14 | n.s. | 13 |
| Stomatal density ($mm^{-2}$) | 12.7 ± 3.9 | 15.3 ± 3.6 | n.s. | 13 |
| Length of stomatal opening (µm) | 52 ± 2.9 | 54 ± 2.3 | n.s. | 13 |
| Chlorophyll content (mg $m^{-2}$) | 158 ± 59 | 147 ± 46 | n.s. | 6 |
| Chlorophyll content (µg $g_{FM}^{-1}$) | 418 ± 225 | 514 ± 150 | n.s. | 6 |
| Chlorophyll a / b ratio | 2.0 ± 0.9 | 2.1 ± 0.5 | n.s. | 6 |
| Photosynthetic capacity (nmol $O_2$ $g^{-1}$ $s^{-1}$) | 81 ± 13.9 | 47 ± 2.1 | = 0.030 | 4 |

**Table 3.2** Morphometrical, anatomical and physiological parameters as a function of plant size ($LL_{max}$) in *V. sanguinolenta*. For parameters, which significantly correlated with plant size, regression equations, correlation coefficients (r), sample size (n), and significance levels (p) are provided along with non-logged data (Min / max) as estimated by the regression for a small (SC 2, $LL_{max}$ = 5 cm) and a large individual (SC 4, $LL_{max}$ = 85 cm). For parameters not correlating with plant size means ± SD and sample size (n) are given. Stomatal and trichome densities as well as chlorophyll content were determined in the distal half of the youngest, fully developed leaf.

| **Parameter** | **Equation/mean ± SD** | **Min/max** | **r** | **n** | **p** |
|---|---|---|---|---|---|
| Plant dry mass (g) | $10^{2.87 \log LLmax – 2.84}$ | 0.147 / 503 | 0.99 | 38 | < 0.001 |
| Number of leaves | $10^{0.64 \log LLmax + 0.13}$ | 4 / 23 | 0.94 | 55 | < 0.001 |
| Plant leaf area ($m^2$) | $10^{2.45 \log LLmax - 0.55}$ | 1.4 $10^{-4}$ / 1.5 | 0.99 | 55 | < 0.001 |
| Leaf area index ($m^2$ $m^{-2}$) | $10^{0.40 \log LLmax – 0.17}$ | 1.3 / 4.0 | 0.90 | 10 | < 0.001 |
| Tank water capacity (l) | $10^{3.04 \log LLmax – 5.39}$ | 5 $10^{-4}$ / 3.0 | 0.99 | 11 | < 0.001 |
| Days until tank is empty | $10^{0.61 \log LLmax - 0.26}$ | 1 / 8 | 0.93 | 8 | = 0.002 |
| Specific leaf length (cm $cm^{-1}$) | $10^{0.19 \log LLmax + 0.43}$ | 3.7 / 6.3 | 0.82 | 25 | < 0.001 |
| Specific leaf weight (g $m^{-2}$) | $10^{0.50 \log LLmax + 1.20}$ | 35.4 / 146 | 0.98 | 12 | < 0.001 |
| Succulence index (g $m^{-2}$) | 170 log $LL_{max}$ + 180 | 299 / 509 | 0.96 | 20 | < 0.001 |
| Relative water content (%) | -0.16 $LL_{max}$ + 89.6 | 89 / 76 | -0.95 | 20 | < 0.001 |
| Stomatal density ($mm^{-2}$) | $10^{0.35 \log LLmax + 0.93}$ | 15 / 40 | 0.88 | 27 | < 0.001 |
| Trichome density ($mm^{-2}$) | $10^{– 0.38 \log LLmax + 2.03}$ | 58 / 20 | -0.84 | 27 | < 0.001 |
| Diameter of dome cell (µm) | $10^{-0.052 \log LLmax + 1.76}$ | 53 / 46 | -0.41 | 48 | = 0.004 |
| Diameter of shield (µm) | $10^{-0.15 \log LLmax + 2.53}$ | 266 / 174 | -0.46 | 48 | = 0.001 |
| Chlorophyll content (mg $m^{-2}$) | $10^{0.20 \log LLmax + 2.04}$ | 151 / 267 | 0.76 | 8 | = 0.027 |
| Chlorophyll content (µg $g_{FM}^{-1}$) | 654 ± 153 | | | 8 | n.s. |
| Photosynthetic capacity (nmol $O_2$ $g^{-1}$ $s^{-1}$) | $10^{0.30 \log LLmax + 1.57}$ | 60 / 140 | 0.41 | 16 | = 0.008 |
| Ratio Chlorophyll a / b | 2.1 ± 0.5 | | | 8 | n.s. |

### *Size-related changes in leaf physiological parameters during drought*

During a drought experiment I measured 24 h courses of in situ leaf $CO_2$ exchange using three size categories (SC 2, smallest to SC 4, largest). A typical diel pattern of net $CO_2$ exchange (= net photosynthesis, NP) of *Vriesea sanguinolenta* at the onset of the experiment, i.e. under well watered conditions is depicted in Figure 3.3. Irrespective of size, the highest rates of net $CO_2$ uptake were observed late in the morning. Area related rates of net photosynthesis (maximal rates per day, $NP_{max}$; diel integrals, $NP_{24h}$) and transpiration (T) were always highest in the largest plants (Figures 3.3 & 3.4). When NP was calculated on a dry mass basis size-dependent trends disappeared (Kruskal-Wallis ANOVA, dry mass based daily carbon gain under well watered conditions for the three size categories, df = 9, p = 0.09). Dark respiration showed no size effect (ANOVA, area based dark respiration under well watered conditions for three SCs, df = 57, p = 0.91). During the day, light conditions were rarely saturating (compare Figures 3.3 & 3.5). However, size dependency was also observed in the results of light response curves. As shown in Figure 3.6, the maximum rate of net $CO_2$ uptake ($NP_{max}$) at saturating PPFD increased linearly with plant size. Proportionally, however, the light response of NP did not scale with size: the pooled and standardized light response curves were similar for individuals of varying size (Figure 3.5). For six leaves used in these $CO_2$ exchange measurements, I also determined photosynthetic capacity (PC) under non-limiting conditions with the oxygen electrode. Similar to $NP_{max}$, PC increased continuously with plant size (Figure 3.6), but with a steeper slope. The correlation of $NP_{max}$ and PC was: $NP_{max} = 10^{1.32 \log PC + 0.21}$, $r^2 = 0.86$, $p = 0.008$, $n = 6$. In contrast to the results of the $CO_2$ exchange measurements, mass based PC scaled significantly with plant size ($PC_{DM} = 10^{15.3 \log LLmax - 9.4}$, $r^2 = 0.81$, $p = 0.014$, $n = 6$).

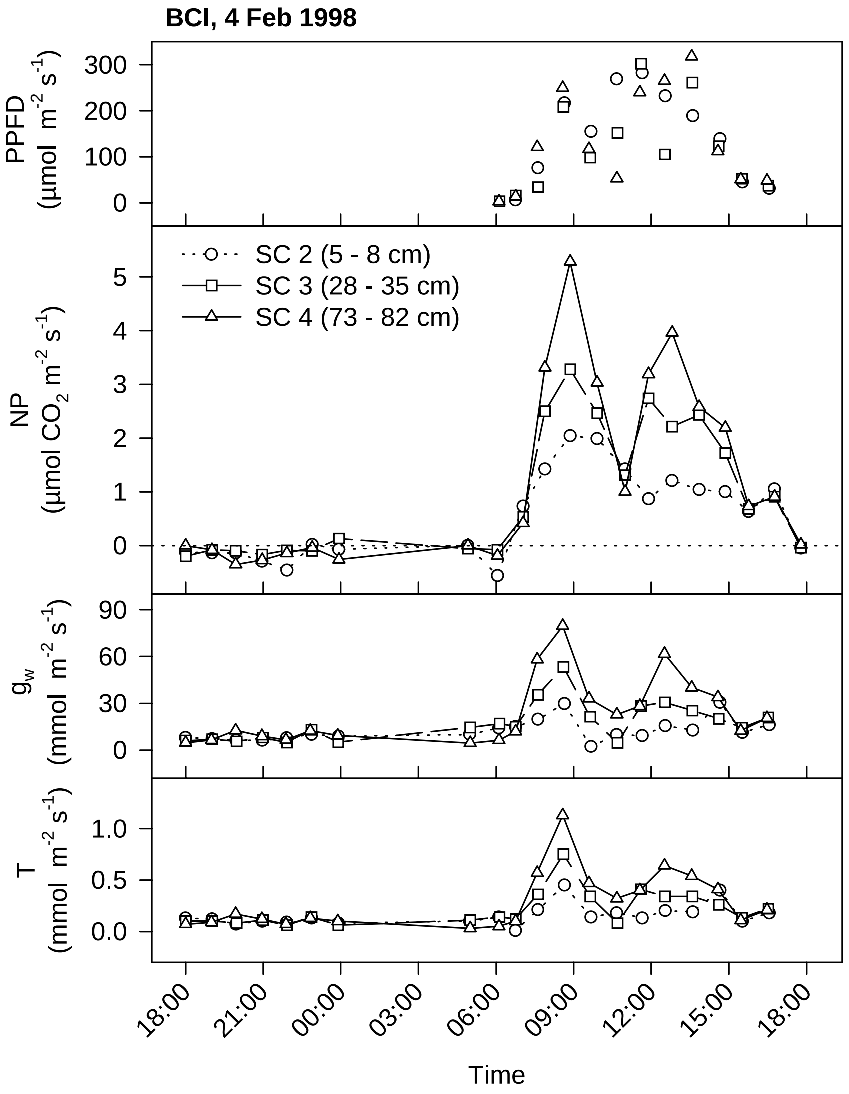

**Figure 3.3** Diel courses of selected gas exchange parameters typical for well watered individuals of *V. sanguinolenta* belonging to different size categories (SC). Depicted are incident photon flux density (PPFD, µmol $m^{-2}$ $s^{-1}$), net rate of $CO_2$ exchange (NP, µmol $CO_2$ $m^{-2}$ $s^{-1}$), leaf conductance to water vapor transfer ($g_w$, mmol $H_2O$ $m^{-2}$ $s^{-1}$), and transpiration rate (T, mmol $H_2O$ $m^{-2}$ $s^{-1}$).

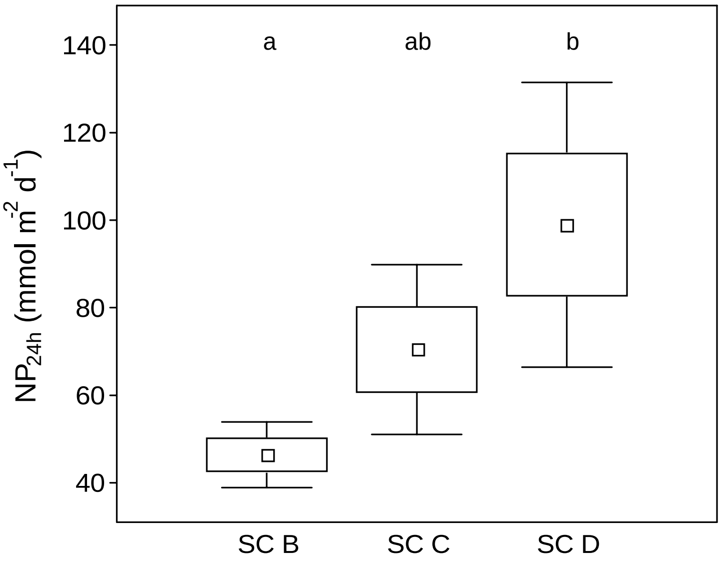

**Figure 3.4** Daily carbon gain ($NP_{24h}$) under well watered conditions for three different size categories (SC). Shown are means (small squares), standard errors (boxes) and standard deviations (error bars). Significant differences between size categories are indicated by different letters ($p < 0.05$, categories = 3, repetition per category = 4, Kruskal-Wallis-ANOVA, Nemenyi post hoc test).

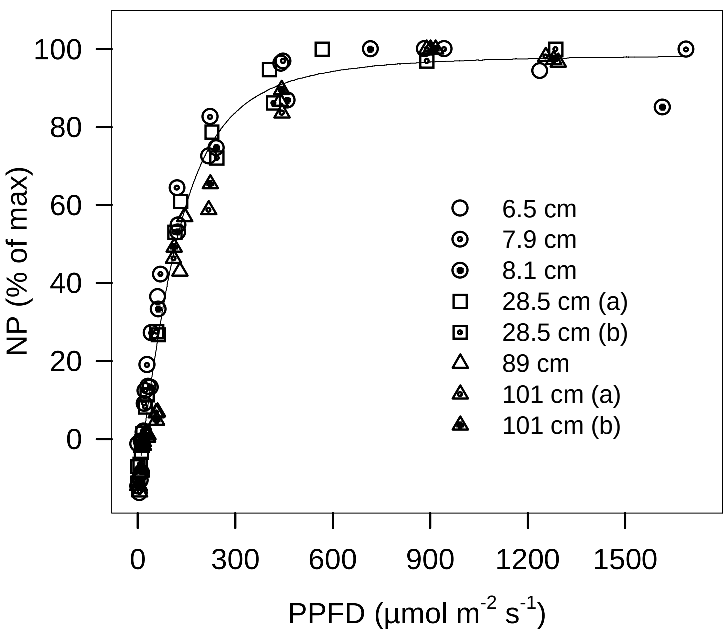

**Figure 3.5** Light response curve of net $CO_2$ exchange for leaves of *V. sanguinolenta* of different size ($LL_{max}$). Different symbols represent different plants. In the case of two plants two different positions within the leaf were measured (a: close to the tip of the leaf, b: in the center of the leaf). Data were analyzed after Smith (1937): $NP = (0.62\ PPFD / \sqrt{(1 + (0.62\ PPFD)^2 / (98.7 + 10.4)^2)}) - 10.4$, $r^2 = 0.98$, $n = 81$, saturating light intensity (PPFD at which 90% of $NP_{max}$ were reached) = 385 µmol $m^{-2}$ $s^{-1}$, light compensation point = 17 µmol $m^{-2}$ $s^{-1}$

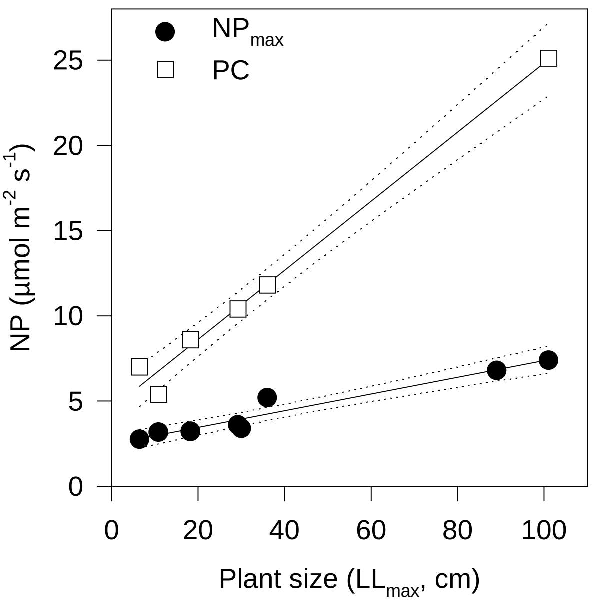

**Figure 3.6** Maximal photosynthetic rates of tank-forming specimens under field conditions ($NP_{max}$) and photosynthetic capacities determined under non-limiting conditions in the lab (PC) as a function of plant size ($LL_{max}$). Depicted are linear regressions (solid lines) and 95% confidence intervals (dotted lines). Regressions are $NP_{max} = 10^{0.37 \log LL_{max} + 0.09}$, $r^2 = 0.86$, $p < 0.001$, $n = 8$; $PC = 10^{0.51 \log LL_{max} + 0.30}$, $r^2 = 0.88$, $p = 0.006$, $n = 6$.

All preceding results were obtained from well watered plants with filled tanks. After tanks were emptied (at the end of day 0), carbon gain (expressed as area based diel integrals, $NP_{24h}$) declined steadily for about one week, irrespective of size (Figure 3.7). Rewatering after 11 days of drought resulted in an almost immediate recovery of net $CO_2$ exchange, although a somewhat lower leaf conductance ($g_w$) compared to day 0 indicated an hysteresis in stomatal response (Figure 3.7). In the course of the experiment the maximum rates of NP on a given day ($NP_{max}$) proved to be a reliable predictor of the respective diel integral ($NP_{24h} = 16.96\ NP_{max} - 5.34$, $r^2 = 0.86$, $p < 0.001$, $n = 116$). During the entire experiment, I also determined leaf water potentials (Ψ) and nocturnal acidification ($\Delta H^+$). Although nocturnal acidification was consistently detectable, it was low and there were no differences between SCs (ANCOVA; $F_{2,32} = 3.19$, $p > 0.05$, covariate: Ψ); $\Delta H^+$ averaged 12.7 ± 4.7 µeq $H^+$ $g_{FM}^{-1}$

(mean ± SD, n = 36), predawn titratable acidity was 23.0 ± 6.9 µeq $H^+$ $g_{FM}^{-1}$ (n = 36). Analyzed for all plants, nocturnal acidification increased slightly with decreasing leaf water potential ($\Delta H^+ = -0.55 + 0.014 \log(-\Psi)$, $r^2 = 0.16$, $p = 0.015$, n = 36). The leaf water potential of well watered plants did not vary with size. Shortly after the onset of drought, however, $\Psi$ began to decrease in the smallest plants, while it remained rather constant in larger individuals (Figure 3.7). Surprisingly, while smaller plants had a lower diel carbon budget when well watered (Figure 3.4; day 0 and day 12 in Figure 3.7), $NP_{24h}$ was quite similar between size categories during the entire drought experiment (Figure 3.7). This resulted from differences in stomatal behavior: the relative decrease of leaf conductance for water vapor ($g_w$) with decreasing leaf water potential was qualitatively quite different for individuals of varying size (Figure 3.8). For example, while in SC 4 a small decrease in $\Psi$ of 0.05 MPa was associated with a substantial decrease in $g_w$ of c. 75 %, the same change in $\Psi$ only led to a decrease in $g_w$ of about 30% in smaller plants (SC 2). Even at - 0.8 MPa, i.e. after a decrease in $\Psi$ of 0.6 MPa compared to favorable conditions, $g_w$ in SC 2 was still about 25% of the maximum $g_w$. Applying the same mathematical model (compare legend of Figure 3.8) to the absolute data of $\Psi$ and $g_w$ showed that a leaf conductance approaching values at stomatal closure ($g_w$ = 10 mmol $m^{-2}$ $s^{-1}$) was reached at a $\Psi$ of - 0.35 MPa in large, - 0.4 MPa in intermediate and at - 0.5 MPa in small individuals. Further evidence for size-related differences in stomatal behavior was obtained independently from leaf drying curves (Figure 3.9A). Consistent with the above results from gas exchange measurements, the relative water deficit at which stomata close ($RWD_{STC}$) correlated negatively with plant size. In contrast, changes in intercellular $CO_2$ concentration ($c_i$) did not show any clear size-related trends; $c_i$ invariably increased after stomatal closure (Figure 3.7). Other environmental factors, which might potentially influence stomatal behavior did not vary with plant size either: no systematic size-related differences in leaf temperature or incident radiation were observed during the experiment (data not shown).

Once stomata attain minimal aperture, cuticular transpiration may contribute considerably to the rate at which water is lost. Again, a significant size dependency was observed, but the trend was reversed: the residual transpiration was lower in smaller plants (Figure 3.9B). Permeances (at 25°C), which were calculated from these residual transpiration data, increased from $7.23 \times 10^{-6}$ m $s^{-1}$ for the smallest to $13.0 \times 10^{-6}$ m $s^{-1}$ for the largest individuals. These values are in the range of the few data available for other epiphytic bromeliads (Kerstiens 1996; after Benzing & Burt 1970).

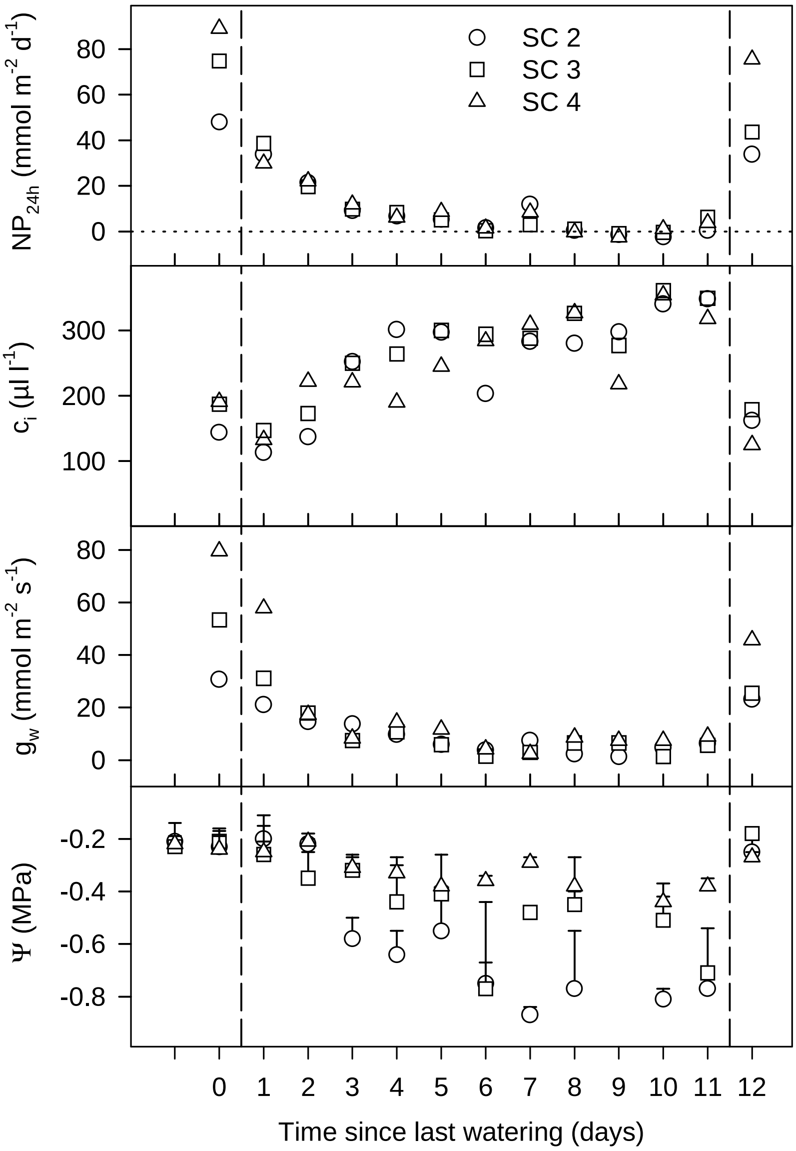

**Figure 3.7** Changes in selected leaf physiological parameters during a drought cycle in three size categories (SC) of *V. sanguinolenta*. Depicted are diel carbon budgets ($NP_{24h}$, mmol $CO_2$ m$^{-2}$ d$^{-1}$), minimal internal $CO_2$ concentration ($c_i$, µl l$^{-1}$), maximal leaf conductance to water vapor ($g_w$, mmol $H_2O$ m$^{-2}$ s$^{-1}$), and predawn leaf water potential (Ψ, MPa). Values for Ψ are means (n = 3) with standard deviations (error bars). Tanks were emptied at 18:45 on day 0, and refilled at the same time on day 11.

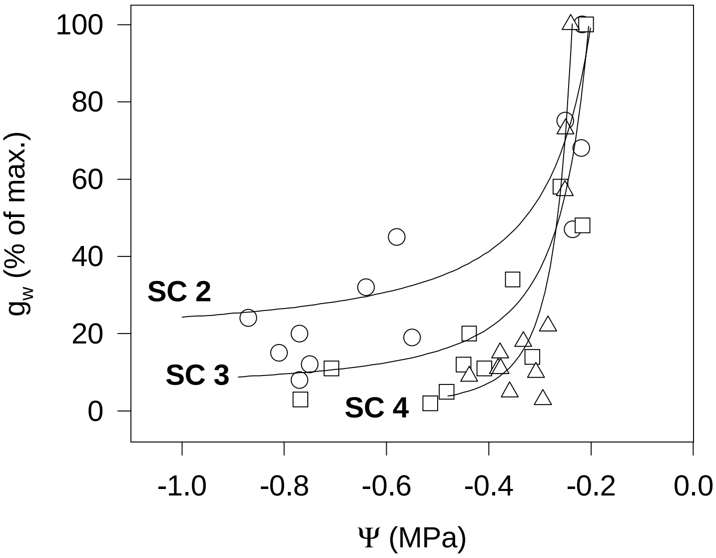

**Figure 3.8** Relative changes in leaf conductance to water vapor ($g_w$) at changing leaf water potentials (Ψ). Data originate from the experiment shown in Figure 3.3. Non-linear regressions ($\ln g_w = a + b\,\Psi^{-1}$, solid lines, $r^2$ between 0.79 and 0.90) are for size category 2 (circles), SC 3 (squares), and SC 4 (triangles).

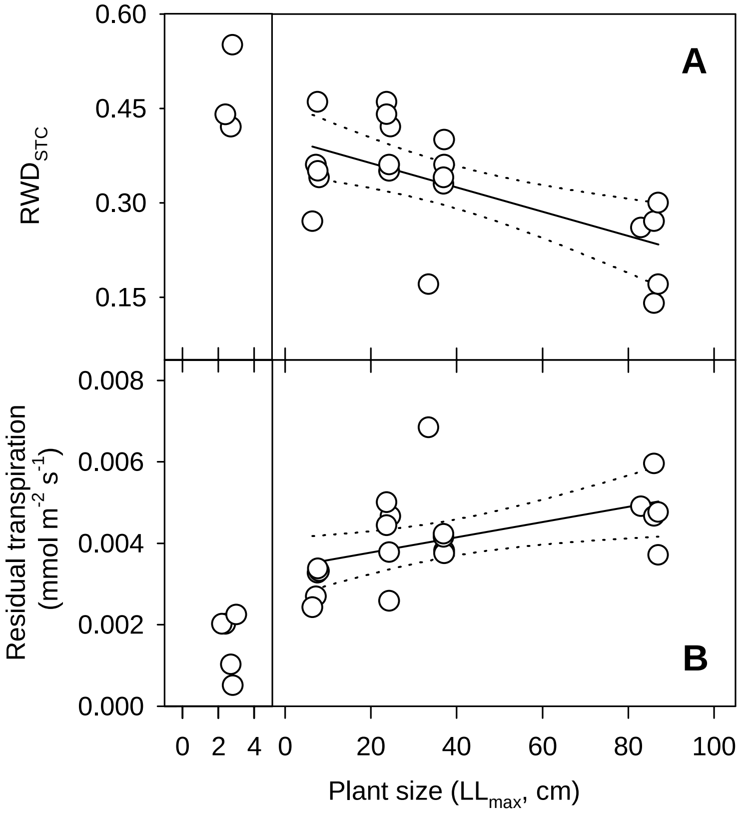

**Figure 3.9** Differences in leaf water relations parameters as a function of ontogenetic stage and plant size (data from leaf drying curves). **Left panels** represent the atmospheric stage, **right panels** show plants of the vegetative, tank forming stage. Given are linear regressions (solid lines) and 95 % confidence intervals (dotted lines) for data on tank individuals. **Figure 3.9A** Relationship of relative leaf water deficit at which stomata close ($RWD_{STC}$) and plant size ($LL_{max}$). The regression equation is $RWD_{STC} = -0.02 \log LL_{max} + 0.40$, $r^2 = 0.39$, $p = 0.003$, $n = 20$. **Figure 3.9B** Residual transpiration (RT) after stomatal closure as a function of plant size. The regression equation is $RT = 1.9 \times 10^{-5} LL_{max} + 0.0034$, $r^2 = 0.26$, $p = 0.02$, $n = 20$.

## Discussion

Laboratory measurements with a number of epiphyte species have indicated that important characteristics of leaf physiology vary continuously with size (Zotz 1997a; Schmidt et al. 2001). The present field study with the $C_3$ species *Vriesea sanguinolenta* corroborates and substantially extends these findings. It is demonstrated that after a step change in early ontogeny with a shift from atmospheric to tank form there are gradual changes in virtually all ecophysiological parameters investigated.

### *Shift between life forms*

The transition from atmospheric to water-impounding stage was associated with a variety of ecophysiological changes (Table 3.1; Figure 3.2). Small tanks had, e.g. thinner leaves due to a changed leaf anatomy, fewer leaves of different morphology with a less dense cover of trichomes, and a decreased photosynthetic capacity. But not all differences and similarities between ontogenetic phases in *V. sanguinolenta* were consistent with those found in other studies with related taxa, i.e. *Tillandsia deppeana* (Adams & Martin 1986a; 1986b) or in *Vriesea geniculata* (Reinert & Meirelles 1993). In part, these discrepancies are due to the fact that these authors compared atmospherics with tank individuals of unspecified size (in Reinert & Meirelles 1993: “large plants”), while I compared plants of approximately the same size. For example, comparing relative proportions of functional tissue types (Figure 3.2), stomatal densities, or the size of dome cells and shields (Tables 3.1 & 3.2) of atmospherics with those of large tank forms would have yielded qualitatively different results. Obviously, most changes in anatomy, morphology (Figure 3.2; Tables 3.1 & 3.2), and physiology (Table 3.1; Figures 3.7 – 3.9) were more pronounced within a life form, i.e. among tank individuals, than between life forms in *V. sanguinolenta*. Moreover, while size-related differences in morphology or physiology could falsely be attributed to a change in life form, other differences could be missed. For example, while there is a decrease in photosynthetic capacity after the transition to tank form (Table 3.1), PCs of larger tank individuals exceed those of atmospherics (Table 3.2). This implies that studying ecophysiological differences in relation to a step change in life form without attention to the subsequent increase in plant size can lead to erroneous conclusions.

### *Size differences in the water-impounding phase*

After the transition to the vegetative, tank-forming life stage almost all studied parameters varied with plant size (Table 3.2; Figures 3.2 – 3.4 & 3.6 – 3.9). One major trend was a decreasing dependence on internal water storage with plant size (Figure 3.2B). As a corollary, tanks of larger plants were more efficient in bridging rainless periods (Table 3.2), corroborating the observations made with two other tank bromeliads (Zotz & Thomas 1999). For example, while the water reservoir of small individuals was depleted within 24 h without refill in the dry season, larger conspecifics could draw on externally stored water for about eight days (Table 3.2). This low efficiency of small tanks may be the ultimate reason why tank bromeliads such as *V. sanguinolenta* or *T. deppeana* (Adams & Martin 1986a; 1986b) possess an atmospheric juvenile stage in the first place.

Differences between smaller and larger tank individuals may also be discussed in the context of stress theory, more specifically in the context of drought-avoidance and -tolerance (Jones 1992; Kramer 1982). Plants employing a drought-avoidance strategy prevent excessive water loss and tissue damage by early stomatal closure before substantial changes in leaf water potential occur, whereas drought-tolerant plants exhibit simultaneous decreases in stomatal conductance and water potential (Guehl et al. 1991; Picon et al. 1996). The results indicate that *V. sanguinolenta* performs a gradual shift between these strategies during ontogeny. By efficiently bridging rainless periods (Table 3.2) large specimens avoid water deficits, with relatively high stomatal conductance and net photosynthesis rates under well watered conditions (Figures 3.3 & 3.4). Smaller individuals, in contrast, showed lower values for NP and $g_w$ when well watered, and were rather inefficient in storing water externally (Table 3.2). Upon tank water depletion, the relative reduction in stomatal conductance was less pronounced in small plants (Figure 3.8), and stomatal closure was observed at higher water deficits (Figure 3.9), thus allowing larger fluctuations of tissue water content. The results for tank individuals of different size are similar to the findings of Adams & Martin (1986b) for plants belonging to distinct life forms. They are also consistent with the general observation that stomata of plants with higher maximal $g_w$ are more sensitive to drought conditions than those of plants with lower conductances (Schulze & Hall 1982).

The results of a study with two other tank bromeliads (Zotz & Thomas 1999) and own observations indicate that larger plants will less frequently experience an empty tank in their natural habitat. This difference in water availability with respect to plant size has both long-term and short-term consequences. Long-term effects are, for example, differences in morphology (Table 3.2; Figure 3.2) and anatomy (Figure 3.2), but also physiological differences, e.g. in PC (Figure 3.6), in leaf N content (Schmidt et al. 2001), or in stomatal behavior (Figures 3.8 & 3.9A). The notion that differences in water availability are the proximate cause for the present case of size-related ecophysiological changes has been suggested earlier (Zotz 1997a). However, water availability will also strongly affect plant performance in the short-term. Thus, smaller plants do not only exhibit lower area based rates of photosynthesis (Figures 3.3 & 3.4), but the duration of net $CO_2$ uptake is also expected to be shorter due to a faster depletion of external water reservoirs. By allowing larger water deficits after the external water supply is depleted (Figures 3.7, 3.8 & 3.9A), smaller plants partially compensate for this disadvantage by prolonging the photosynthetically active period during drought. Once stomata are closed, however, leaves of small plants are better protected against desiccation (Figure 3.9B). Whether this difference is due to variation in cuticular properties or the consequence of higher stomatal densities in larger plants (and hence higher peristomatal transpiration; compare Muchow & Sinclair 1989), remains to be tested.

Similar adaptations of stomatal control are known from other taxa, e.g. from cotton: in drought cycles in a controlled environment the water potential when stomata closed shifted from -2.8 MPa to -4.0 MPa with proceeding drought (Brown et al. 1976). The relationship of $\Psi$ and $g_w$ can be altered as a response to previous stress or the degree of desiccation in many other species (Jones 1992), which suggests that the size-related differences in stomatal response in *V. sanguinolenta* are probably the result of such a response.

Internal $CO_2$ ($c_i$) invariably increased with decreasing $g_w$ (Figure 3.7). Such an unusual observation was also made studying the midday depression of net $CO_2$ uptake in mediterranean shrubs (Tenhunen et al. 1984; Demmig-Adams et al. 1989). High $c_i$ at closed stomata was explained by a decrease in photosynthetic capacity, but also by a reduction in carboxylation efficiency and an increase in the $CO_2$ compensation point. Tenhunen et al. (1984) and Demmig-Adams et al. (1989) suggested that high internal $CO_2$ levels may aid in minimizing photoinhibition during stomatal closure. Unfortunately, no measurements of chlorophyll fluorescence are available for *V. sanguinolenta* to test this notion. Although the possibility that $c_i$ may also be influenced by the slight CAM activity in *V. sanguinolenta* cannot be completely discarded , it seems to be an unlikely explanation for the elevated $c_i$. The highest nocturnal acidification was just c. 5 mmol µeq $m^{-2}$. Assuming a stochioimetry of 2 mol $H^+$: 1 mol $CO_2$, this would correspond to 2.5 mmol $CO_2$ $m^{-2}$ or a moderate rate of $CO_2$ fixation of 1 µmol $CO_2$ $m^{-2}$ $s^{-1}$ for less than one hour.

In conclusion, this first in situ study on the importance of size-related ecophysiological changes in vascular epiphytes corroborates and extends the results of earlier laboratory studies. In contrast to previous investigations with other bromeliads, which emphasized the importance of heteroblasty in some tank forming taxa, I show that the changes in anatomical, morphological, and physiological properties associated with a gradual increase in size over decades are at least as important as the step change in life form from juvenile atmospheric to tank.

# GROWTH IN TWO EPIPHYTES – ANALYSIS OF WHOLE-PLANT PERFORMANCE UNDER NATURAL AND CONTROLLED CONDITIONS WITH SPECIAL CONSIDERATION OF PLANT SIZE

## Chapter abstract

Due to the general notion of a drought prone and nutrient poor habitat vascular epiphytes are routinely referred to as inherently slow growing plants, although actual evidence, i.e. measurements of epiphyte growth, is rare. To test this notion I chose two study organisms as different as the understory orchid *Aspasia principissa* and the tank bromeliad *Vriesea sanguinolenta*, measuring their growth in situ and, for the latter species, also under favorable conditions in the greenhouse. Using a demographic approach to growth analysis it could be shown: (1) that in an intraspecific comparison small to intermediate individuals yield the highest relative growth rates in situ (RGR; *A. principissa*: 3.5 mg $g^{-1}$ $d^{-1}$; *V. sanguinolenta*: 7.3 mg $g^{-1}$ $d^{-1}$), (2) that the bromeliad reaches maximum size after about 10 years, while the orchid needs at least 5 years more and (3) in terms of RGR small *V. sanguinolenta* plants exhibit a highly plastic response to favorable conditions in the greenhouse, with RGRs (190 mg $g^{-1}$ $d^{-1}$) similar to those of annual plants. These results indicate that despite a substantial interspecific difference epiphytes are indeed growing slowly in situ. High growth rates under favorable conditions, however, prompt us to refine the notion of epiphytes as *inherently* slow growing organisms.

## Introduction

Vascular epiphytes represent a group of plants, highly diverse in regard to taxonomy, life forms and physiology (e.g. Lüttge 1989; Benzing 1990), reflecting at least in part the heterogeneity of their common habitat, the forest canopy. Growing sites are distributed in a three dimensional space and characterized by a high variability in microclimate conditions (Benzing 1990) and water- and nutrient supply (Lesica & Antibus 1991). Moreover, the substrate is more unstable than most terrestrial systems (Benzing 1981) and hamper long-term survival.

In spite of this complexity, the epiphytic habitat is mostly referred to as drought-prone and nutrient-poor. Epiphyte growth and development is thus generally considered to be very slow (e.g. Lüttge 1989; Benzing 1990), although only few studies provide a quantitative basis for this conclusion. For example, in a study on the population structure of *Tillandsia circinnata*, Benzing (1981) estimates that this bromeliad requires 8 – 10 years to reach reproductive size and Larson (1992) predicts first flowering of the orchid *Encyclia tampensis* at an age of about 15 years. For another epiphytic orchid, *Dimerandra emarginata*, Zotz (1995) modeled vegetative growth using annual size increments of specimens of varying sizes. According to these calculations it takes a model plant about 10 years to reach potentially reproductive and about 25 years to reach maximum size.

Using a similar demographic approach in this study growth parameters of two epiphyte species are compared, differing strongly in a number of physiological, morphological and life history characteristics. Growing in the exposed parts of the forest canopy, the tank bromeliad *Vriesea sanguinolenta* Cogn. & Marchal exhibits rather high rates of photosynthesis, more than doubling those of *Aspasia principissa* (Schmidt et al. 2001; Schmidt & Zotz 2001). During early ontogeny the bromeliad experiences a change in life form from an atmospheric to a water-impounding plant, which is able to draw on externally stored water, i.e. is “continuously supplied” (sensu Benzing 1990). In contrast, *A. principissa* Reich.f. is an epiphytic understory orchid featuring only low photosynthetic rates (Schmidt et al. 2001) and is “pulse supplied” (sensu Benzing 1990). Generally, species with high rates of photosynthesis from sites with better resource availability tend to grow faster than taxa with low photosynthetic performance from resource poor habitats (e.g. Evans 1989; Poorter et al. 1990). I thus expected pronounced differences in respect to in situ growth between the two study species, probably encompassing the possible range of different growth rates among vascular epiphytes in a seasonal tropical lowland forest of central Panama. Moreover, growth of *V. sanguinolenta* under favorable conditions in the greenhouse was studied to explore the plasticity of growth and possibly question the notion of generally slow inherent growth in this group.

## Methods

### *Habitat and study species*

The study was carried out in the Barro Colorado Nature Monument (9°10´N, 79°51´W), Republic of Panama. The forest of this biological reserve is classified as a tropical moist forest (Holdridge et al. 1971). Mean annual rainfall is c. 2600 kg $m^{-2}$ with a pronounced dry season from late December to late April (Windsor 1990). Detailed descriptions of vegetation, climate and ecology are reported by Croat (1978) and Leigh et al. (1982).

*Vriesea sanguinolenta* (Bromeliaceae) Cogn. & Marchal (syn. *Werauhia sanguinolenta* Cogn. & Marchal; Grant 1995) is found from Costa Rica to Colombia and on various Caribbean islands from lowland up to lower montane wet forests (Croat 1978). The species colonizes a number of host tree species high in the forest canopy, but is particularly abundant on *Annona glabra* L. (Annonaceae; Zotz et al. 1999). Throughout the study I used maximal leaf length ($LL_{max}$, in cm) as a measure of size. This parameter was highly correlated with a more rigorous measure for size, i.e. plant dry mass (DM), in situ: $DM = 10^{2.87 \times \log LLmax - 2.84}$, $r^2 = 0.99$, $n = 38$, $p < 0.001$ (Pearson-moment correlation) and under near optimal conditions in the greenhouse: $DM = 10^{2.83 \times \log LLmax - 3.09}$, $r^2 = 0.98$, $n = 6$, $p < 0.001$ (Pearson-moment correlation).

*Aspasia principissa* Reichb. f. (Orchidaceae) occurs in central and eastern Panama, preferring the wetter forests of the region (Croat 1978). Most plants are found in the understory, growing on trunks of various tree species but may be encountered occasionally in higher strata of the forest. Again I chose a non-destructive measure of plant size (length of the most recent pseudobulb, PsbL, in cm) that allowed repeated size determinations in the field and correlated closely with plant DM: DM = $10^{2.66 \times \log \text{PsbL} + 0.69}$, $r^2 = 0.95$, $n = 34$, $p < 0.001$ (Pearson-moment correlation).

### *Census data*

In 1997, 25 plots with *V. sanguinolenta* were set up in an area of about 1 ha. Each plot consisted of branch sections (1 - 2.5 m in length) within the crown of *A. glabra*, sometimes with more than one plot per tree. Due to its height of maximal 8 m, epiphytes on this flood-tolerant, evergreen tree were easily accessible in its sparse canopy. From 1997 to 1999, respectively, 313, 287 and 315 plants were monitored individually. All larger *V. sanguinolenta* were labeled with colored telephone wire or a colored thumb tag nearby. From the smaller individuals ($LL_{max} < 10$ cm) only a representative subset was labeled and monitored individually, while the size of unlabelled plants (1997: 2099 plants, 1998: 1443 plants, 1999: 1588 plants) was estimated and then assigned to size categories. Annually, at the beginning of the rainy season, I recorded $LL_{max}$, the total number of leaves, and fruiting events. To determine the annual leaf production the very tip of the youngest leaf was clipped as a marker for the next census. For plants that died between two observation intervals the cause of death was noted. Categories were: (1) individuals that fell off the substrate for unknown reasons (missing), (2) plants still in the plot but dried (drought), (3) plants that died because trees fell or branches broke (branch/tree fall), and 4) dead specimens with more than 70% of the plant leaf area consumed (herbivory). To compensate for the decreasing number of specimens due to mortality I added an appropriate number of plots on different *A. glabra* trees during each census.

Similarly, 90 plots with a total of 191 specimens of *A. principissa* were set up in 1997. Along 10.3 km of the trail system on Barro Colorado Island (2.5 m to the left and to the right) I recorded all individuals of *A. principissa*, thus covering a transect of 5.2 ha. Each plot was defined as the section of a tree stem from ground level up to 2 m in height. Orchids were individually labeled with a colored thumb tag nearby. I determined the length of all pseudobulbs, leaves and bracts and noted flowering and fruiting events. Missing or dead plants were classified similar to *V. sanguinolenta*. All plants were re-censused annually, at the beginning of the rainy season. Extending the observation area along the trail system, plots were added annually to maintain a more or less constant number of individuals.

### *Determination of size categories*

In the case of *V. sanguinolenta* size categories (SC) were distinguished corresponding to the ontogenetic stages: a) atmospheric, non-impounding, b) tank-forming, vegetative, c) tank-forming, reproductive. To subdivide the large category of non-reproducing tank bromeliads I applied the procedure suggested by Vandermeer (1978), which minimizes distribution and sampling errors, yielding a total of six size categories (Table 4.1). In the case of *A. principissa* limits of size categories (Table 4.1) were only computed after mathematical criteria (Vandermeer 1978).

### *Controlled environment*

*Vriesea sanguinolenta* plants were potted in “Blähton” and kept in the greenhouse in Würzburg, Germany. Climate conditions were: mean daily PPFD: c. 10 mol $m^{-2}$ $s^{-1}$, air temperature 24 to 26°C, relative humidity: 60 to 80 %. Tanks were filled twice daily with a fertilizer solution (N: 10 µg $g^{-1}$, K: 10 µg $g^{-1}$, P: 7 µg $g^{-1}$, POLY CRESCAL, Aglukon Spezialdünger GmbH, Düsseldorf, Germany).

### *Growth and data analysis*

For simulating growth from annual size increments in two subsequent years three regression types were employed : (1) a best-fit regression as calculated with the software TableCurve (Jandel Scientific, Corte Madera, California, USA). Processing data from the two study species for different observation periods I chose the equation that yielded the highest coefficient of determination ($r^2$) in most cases, which was a power function, (2) a linear regression model and (3) a typical growth function (as used e.g. by Zotz 1995): $\text{size}_{\text{year n + 1}} = a \times \text{size}_{\text{year n}} / (1 + b \times \text{size}_{\text{year n}})$. Calculating relative growth rate (RGR) required a conversion of the non-destructive size measurements ($LL_{max}$, PsbL) into DM via the regression equations given earlier. The RGR was then calculated as RGR = $(DM_{\text{year n+1}} - DM_{\text{year n}}) / DM_{\text{year n}}$. Whenever data were not normally distributed a log-transformation was performed prior to analysis.

## Results

### *Phenology*

The life cycle of *V. sanguinolenta* is comprised of three distinct life stages a) an atmospheric stage, b) a vegetative tank forming stage and c) a reproductive tank forming stage. Initial size after germination was c. 0.5 cm maximal leaf length ($LL_{max}$). Atmospheric seedlings in average reached a size of c. 2 cm (maximal 4 cm) $LL_{max}$ before undergoing a shift of life forms. During the subsequent tank forming stage each leaf base forms a water tight chamber resulting in a multi-tank rosette. Larger plants (> 60 cm $LL_{max}$) may produce one tall inflorescence (up to 2 m in height; Table 4.1) bearing 44 ± 29.1 (mean ± SD, n = 7) capsules, each containing 1008 ± 322.8 seed (mean ± SD, n = 6). Plants are polycarpic, but individual shoots flower only once.

*Aspasia principissa* grows sympodially. At the end of the dry season (March to April) a new shoot emerges from the base of the previous shoot, growing continuously during most of the rainy season. While the shoot morphology is almost identical in differently sized specimens (each pseudobulb has mostly two leaves at the apex and is sheathed by up to three leafy bracts), the absolute number of living shoots (or parts thereof) depends on plant size (Table 4.1). Large plants may hold up to eight pseudobulbs, while leaf longevity ranged from roughly one year in SC 1 to almost three years in SC 7 (Table 4.1). Although bracts were almost identical in morphology, anatomy, and physiology (unpubl. data), they were shed earlier than the apical leaves, i.e. their life spans ranged from one to two years (Table 4.1). Once reaching a pseudobulb length (PsbL) of about 7 cm plants were mature, i.e. they potentially produce flowers and fruits (Table 4.1).

**Table 4.1** Life history and morphological characteristics of *V. sanguinolenta* and *A. principissa*. For *V. sanguinolenta I* determined six size categories (SC), using maximal leaf length ($LL_{max}$) as a measure for plant size. Numbers of annual probability of fruiting are means of two observation periods. Proportions of leaves that were replaced annually are means ± SD for (n) individuals per SC. For *A. principissa* the limits of the seven SCs are given as the length of the most recent pseudobulb (PsbL). Numbers of pseudobulbs, leaf and bract longevity as well as annual probability of fruiting are means ± SD, n = three years.

| **Species / parameter** | | **SC 1** | **SC 2** | **SC 3** | **SC 4** | **SC 5** | **SC 6** | **SC 7** |
|---|---|---|---|---|---|---|---|---|
| *V. sanguinolenta* , $LL_{max}$ (cm) | | ▸ 2 | 2.1 – 7 | 7.1 - 13 | 13.1 - 25 | 25.1 – 60 | > 60 | – |
| Proportion of leaves | 1997/98 | n.d. | 82 ± 26.1 (63) | 89 ± 30.0 (22) | 79 ± 25.3 (31) | 68 ± 11.4 (19) | 45 ± 6.1 (3) | – |
| replaced annually (%) | 1998/99 | n.d. | 93 ± 38.6 (47) | 77 ± 36.5 (30) | 83 ± 35.1 (25) | 81 ± 31.4 (16) | 83 ± 3.5 (2) | – |
| Probability of fruiting (%) | | 0 | 0 | 0 | 0 | 4 | 58 | – |
| *A. principissa* , PsbL (cm) | | ▸ 1.5 | 1.6 – 2.5 | 2.6 – 4 | 4.1 – 6 | 6.1 – 8 | 8.1 – 11 | > 11 |
| Pseudobulbs per plant | | 1.5 ± 0.24 | 2.3 ± 0.19 | 2.9 ± 0.37 | 3.5 ± 0.27 | 3.5 ± 0.67 | 3.8 ± 0.41 | 4.1 ± 0.26 |
| Leaf longevity (a) | | 1.2 ± 0.07 | 1.5 ± 0.16 | 1.8 ± 0.18 | 2.0 ± 0.12 | 2.2 ± 0.10 | 2.5 ± 0.05 | 2.9 ± 0.05 |
| Bract longevity (a) | | 1.0 ± 0.01 | 1.2 ± 0.14 | 1.2 ± 0.12 | 1.4 ± 0.05 | 1.5 ± 0.02 | 1.7 ± 0.13 | 2.0 ± 0.10 |
| Probability of fruiting (%) | | 0 | 0 | 0 | 0 | 2 ± 2.9 | 10 ± 4.3 | 11 ± 6.4 |

*Plant size and vegetative growth*

The size category distribution of individual *V. sanguinolenta* plants (Figure 4.1) illustrates that the majority (> 65 %) of the population were atmospheric. While the smallest tank forming size category (SC 2) still represented roughly 20%, members of larger SC became increasingly rare: fewer than 1 % of all plants were assigned to the category of potentially reproductive individuals (SC 6). This pattern remained relatively constant during the study period (Figure 4.1). The SC distribution of *A. principissa* was similar over the three years, but trends were less pronounced: while the smallest SC represented about 20% of all individuals, the largest SC still accounted for c. 7% (Figure 4.1).

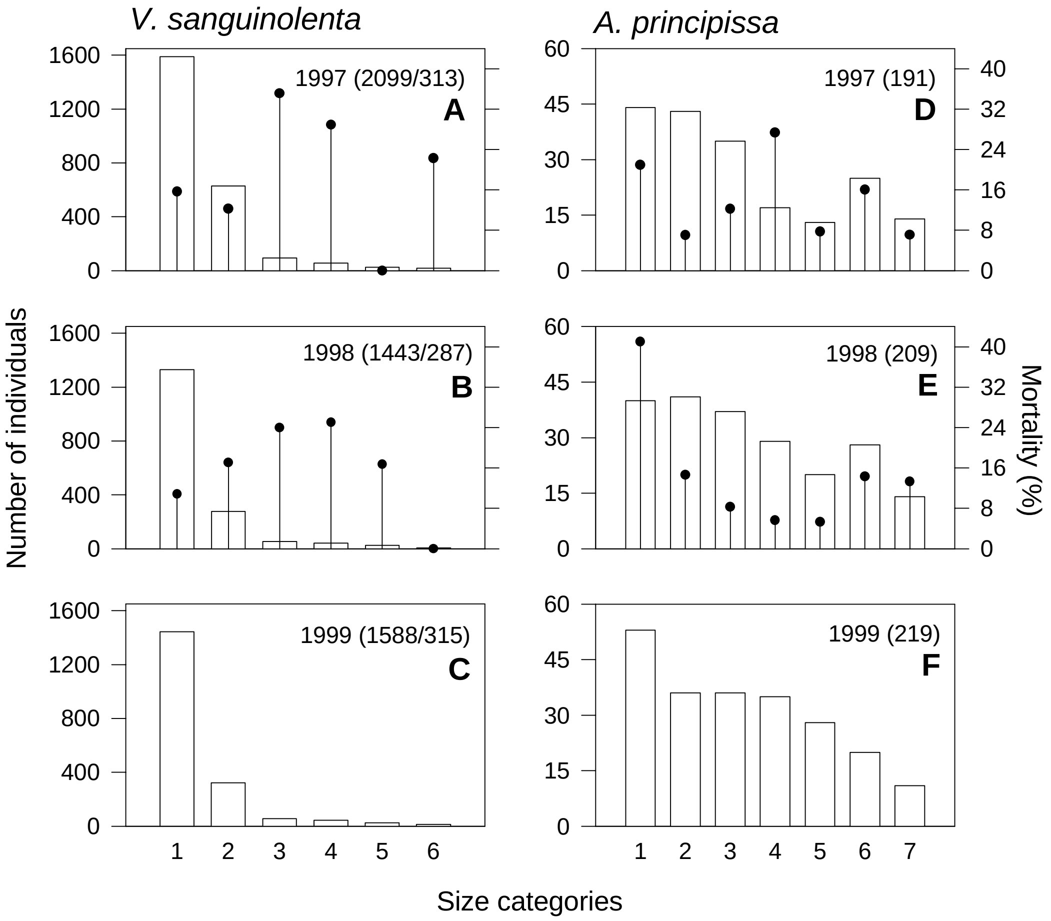

**Figure 4.1** Size category distribution (open bars) and subsequent annual mortality rates (closed circles) in *V. sanguinolenta* and *A. principissa* from 1997 to 1999. Numbers in brackets are total number of plants, at the beginning of each period. For *V. sanguinolenta* the majority of plants was assigned to SCs by estimation, represented by the first number in brackets. The second number in brackets refers to all labeled and measured plants, from which data on mortality were derived as well. *A. principissa* individuals were all labeled and measured.

Annual measurements of maximal leaf length ($LL_{max}$) in *V. sanguinolenta* revealed only subtle size increments from 1997 to 1998, while during the following year most plants increased considerably in size (Figures 4.2A & B), with about doubled relative growth rates compared to the preceding year (RGR; Figure 4.3A). Analyzed for all specimens mean RGR differed significantly between years: $p < 0.001$, df = 0 478 (t-test for independent samples) with 1997/98: 2.0 ± 3.8 (mean ± SD, n = 248), 1998/99: 6.5 ± 8.5 (mean ± SD, n = 232). Flowering shoots died, but plants produced a new, albeit smaller rosette (Figures 4.2A & B). Irrespective of plant size, almost the entire leaf set was replaced between two observations (Table 4.1). Growth was also studied on individuals under controlled greenhouse conditions: under high resource availability RGRs of small tank plants (SC 2) increased from 2.2 to 190 mg $g^{-1}$ $d^{-1}$, i.e. by almost two orders of magnitude compared to field conditions during 1997 (Figure 4.3A). Conversely, larger plants (SC 5 & 6) grew at similar rates under favorable greenhouse conditions and in situ (Figures 4.2A - C & 4.3A). In *A. principissa* annual size increments in situ were also subtle in the first observation period (1997; Figures 4.2D & 4.3B), while during 1998 growth was accelerated in small to medium sized individuals (Figures 4.2E & 4.3B).

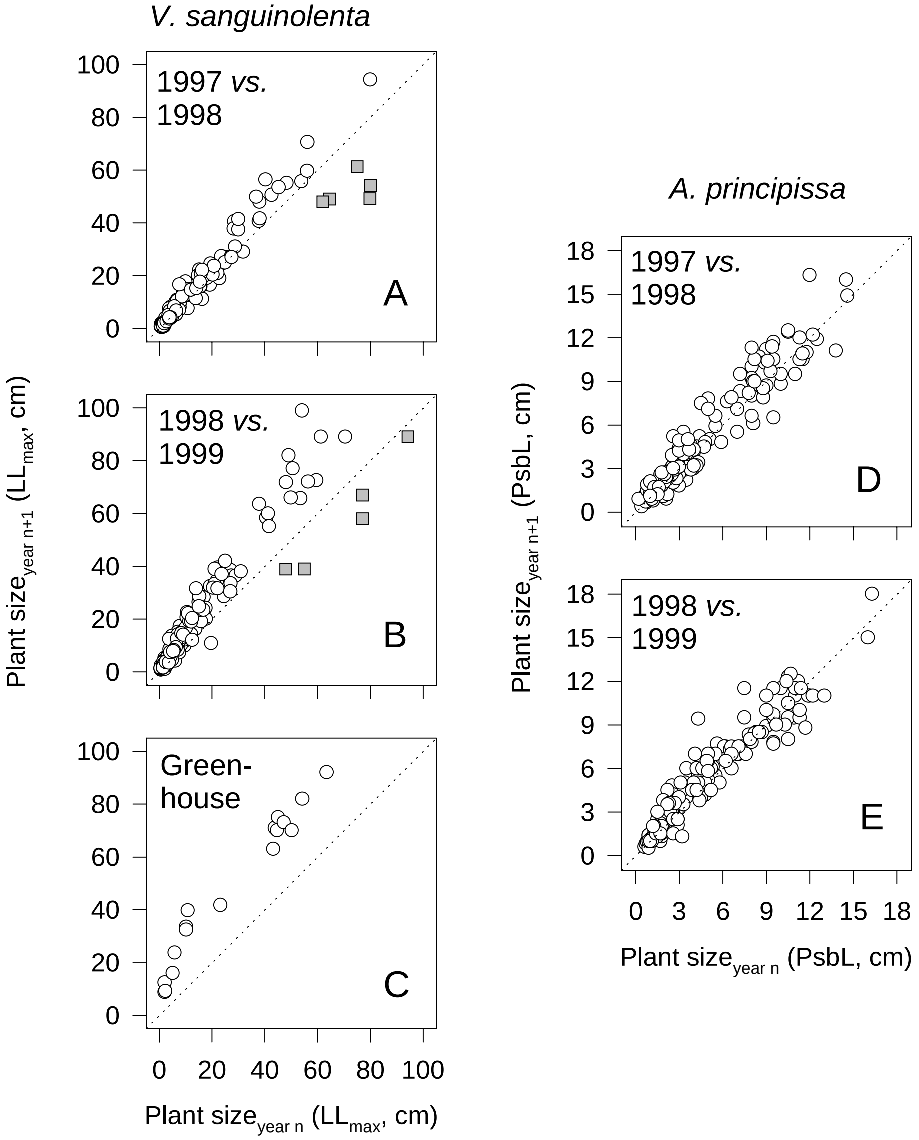

**Figure 4.2** Vegetative growth in the two epiphytes. Depicted are size increments of plants in two consecutive years in situ (**Figures 4.2A, B & D, E**) and in the greenhouse (**Figure 4.2C**). Vegetative (circles) and reproductive individuals (squares) were distinguished in *V. sanguinolenta*. Dotted lines indicate zero annual increment.

Vegetative growth was analyzed by using three different types of regressions to process the data given in Figure 4.2: a) the best fit regression as calculated by TableCurve, which was a power fit, b) a linear regression and c) a non-linear regression, frequently used to describe plant growth. By re-iteration of size-dependent annual size increments annual growth was simulated with these regressions, starting with the size of a seedling (*V. sanguinolenta*, $LL_{max}$ = 0.5 cm; *A. principissa*, PsbL = 0.5 cm) until the maximal size of the two species was reached ($LL_{max}$ > 90 cm; PsbL > 10 cm). As shown in Figure 4.4A a model bromeliad would need more than 30 years to reach maximal size assuming permanent growing conditions like in 1997. Given the conditions of 1998 the same plant would need only c. 10 years to attain a comparable size. In the greenhouse under high resource supply, development is even faster: plants need only 4 to 5 years from seedling to maximal size (Figure 4.4A). For *A. principissa* the model calculations yielded similar year-to-year differences. Subjected constantly to the growing conditions of 1997 a model plant would need about 40 years to complete ontogeny, while under growth conditions of 1998 comparable size would be reached after c. 15 years (Figure 4.4B). Although in both species the three regression models used yielded different quantitative results, variability due to differences in growing conditions between years exceeded by far the differences due to the particular regression (Figures 4A & B).

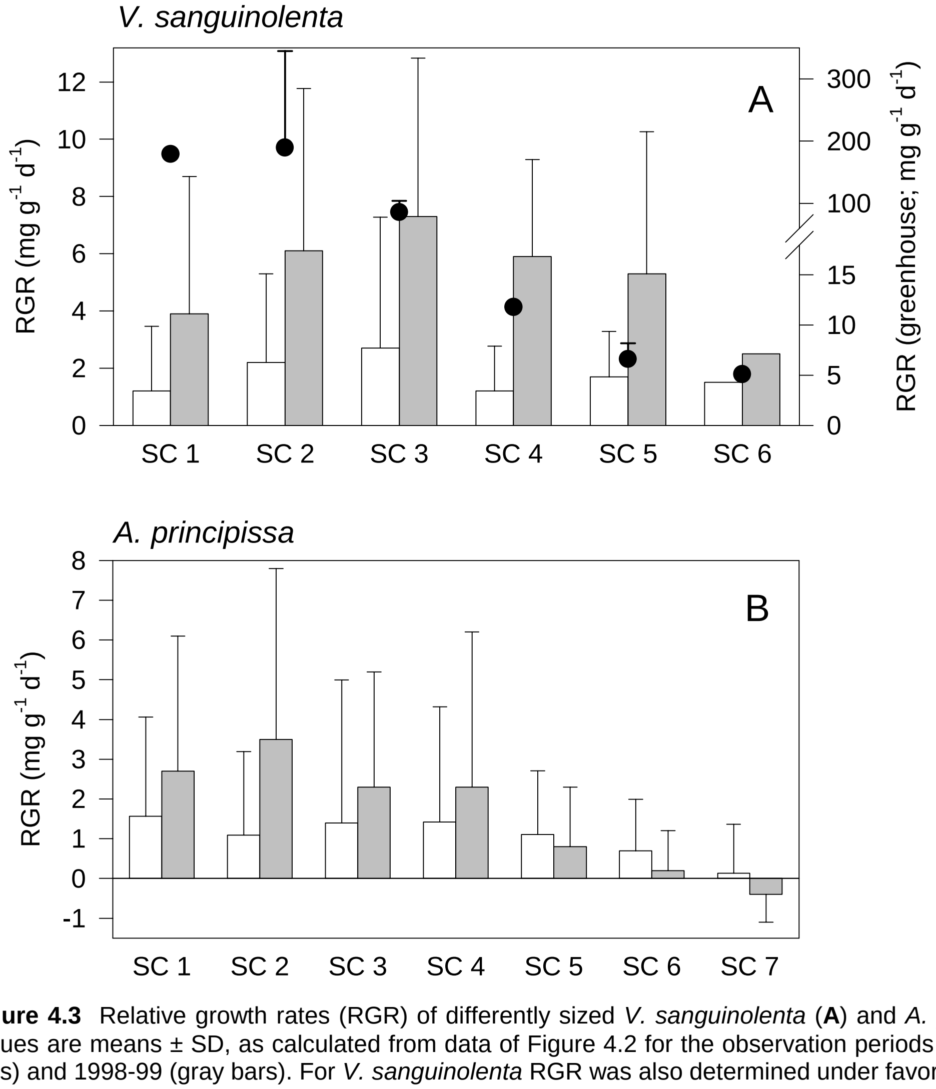

**Figure 4.3** Relative growth rates (RGR) of differently sized *V. sanguinolenta* (**A**) and *A. principissa* (**B**). Values are means ± SD, as calculated from data of Figure 4.2 for the observation periods 1997-98 (open bars) and 1998-99 (gray bars). For *V. sanguinolenta* RGR was also determined under favorable conditions in the greenhouse (circles; note different scale).

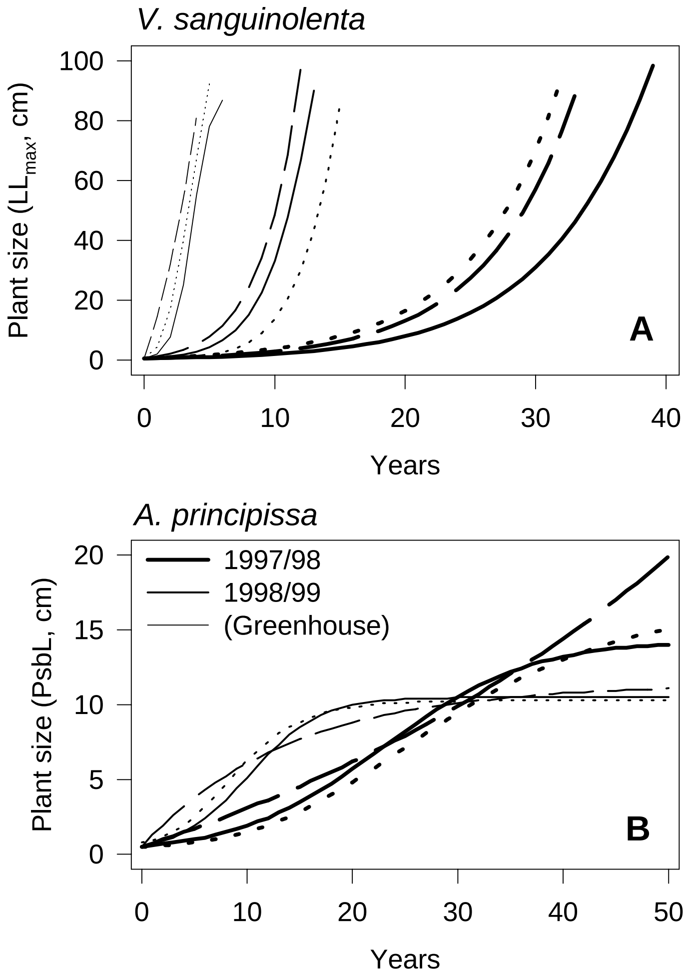

**Figure 4.4** Simulated growth for *V. sanguinolenta* and *A. principissa*, computed with different regression equations for data from Figure 4.2: best fit regression (power, dotted lines), linear regression (dashed lines) and a typical growth function ($size_{year\ n+1} = a \times size_{year\ n} / (1 + b \times size_{year\ n})$; solid lines). Thick lines: model calculations with size increments from 1997/98, intermediate lines: data from 1998/99 and, thin lines (*V. sanguinolenta* only): greenhouse conditions.

### *Mortality*

In *V. sanguinolenta* patterns of mortality were similar for the two observation periods: mortality rates ranged from 0 up to 35 % for the different size categories with highest rates in medium sized individuals, while smaller and larger specimens showed lower mortality (Figures 4.1A & B). The average mortality rate for all individuals during the entire observation period was 16.5%. A third of the bromeliads could not be relocated after one year, i.e. they either had lost contact to the substrate, fell off with peeling bark or were torn down by falling tree parts or animals. Calculated for the entire population 36% of plants that died between two observations were found dry (Table 4.2). However, drought related death was never encountered in SCs 5 and 6. About 17% died because of branch or tree fall (Table 4.2). Herbivory and other causes of death (i.e. plants squeezed between branches) each accounted for less than 10% of the overall mortality (Table 4.2).

**Table 4.2** Proportions of individuals that died for a specific reason between two observations. Numbers are means (in % of all death cases) during the entire observation period from 1997 to 1999 and the sample size (n).

| Species | Missing | Drought | Branch/tree fall | Herbivory | Other | n |
|---|---|---|---|---|---|---|
| *V. sanguinolenta* | 33.3 | 36.4 | 17.2 | 8.1 | 5.1 | 99 |
| *A. principissa* | 55.0 | 26.7 | 16.7 | 0 | 1.7 | 60 |

Average mortality in *A. principissa* was similarly high (c. 15%), but there was no trend towards increased mortality rates at a specific ontogenetic stage (Figures 4.1D & E). Again, the fate of a high proportion of individuals remains unclear: 55% were missing at the subsequent census (Table 4.2). Drought related death was somewhat less important in the understory orchid *A. principissa* (26%) compared to the bromeliad, but it was likewise restricted to small to intermediate SCs (data not shown). Branch or tree fall accounted for c. 17% of death cases (Table 4.2). Herbivory was encountered frequently, but never reached a lethal level, while pathogens caused irregular growth and finally death in one specimen (categorized as “other” causes of mortality in Table 4.2).

## Discussion

Population structure and dynamics differed greatly in the two epiphyte taxa. In *V. sanguinolenta* most of the specimens found in the plots were atmospheric seedlings (Figures 4.1A - C). Mortality, however, was comparatively low in this SC, indicating that the annual probability of atmospherics to conduct the phase shift to small tank plants is rather low in situ and plants remain atmospheric for several years. Small and medium sized tanks had higher growth and mortality rates (Figures 4.1A, 4.1B & 4.3A). In *A. principissa* small individuals were not as numerically dominant as they were in the bromeliad population (Figure 4.1). However, highest growth rates were encountered in small to medium sized plants, while rates declined in larger individuals of SC 5 to 7 (Figure 4.3B), coinciding with the potential to reproduce (Table 4.1; see also Zimmerman & Aide 1989).

Under natural conditions values of RGR in *V. sanguinolenta* were about twice those of *A. principissa* (Figure 4.3), which were consistent with similar differences in photosynthetic capacity (Schmidt et al. 2001). Nevertheless, in situ RGRs in both species were rather low compared to other life forms in similar forests (e.g. Poorter et al. 1990). In contrast to the field, RGR of small bromeliads under greenhouse conditions were surprisingly high: SC 1: 179 mg $g^{-1}$ $d^{-1}$ and SC 2: 190 mg $g^{-1}$ $d^{-1}$ (Figure 4.3A). Grime & Hunt (1975) determined RGR under near optimal conditions for seedlings of 22 annuals with a range of 120 to 299 mg $g^{-1}$ $d^{-1}$. The comparison of RGR of small *V. sanguinolenta* with these data does clearly not support the notion of generally slow inherent growth rates in vascular epiphytes.

The greenhouse experiment also illustrated the potential of small *V. sanguinolenta* to respond to differences in resource availability. Conversely, larger plants grew at similar rates in the greenhouse and in situ (Figure 4.3A). This prompted me to hypothesize that small bromeliads were strongly limited by resource availability in their natural environment, while an increase in size alleviated these external limitations. This notion is concordant with earlier findings: tanks of larger *V. sanguinolenta* are increasingly more effective in bridging rainless periods (Schmidt & Zotz 2001), i.e. they can draw on externally stored water when small plants are already water limited. However, even larger tank bromeliads are not completely independent of the natural variability in annual climate in the study area. As shown in Figure 4.3A, the RGRs of basically all SCs were reduced in 1997. This year was strongly influenced by an El Niño event during which annual precipitation and the number of rain events were reduced, while the evaporative demand and radiation intensity were elevated (Table 4.3). Conversely, climate conditions of 1998 were close to average conditions compared to preceding years (Table 4.3). This led to the assumption that the measured slow growth during the El Niño year (in both species) was the exception, which means that in situ growth rates and computed plant growth of the observation period 1998 are more appropriate for long-term growth scenarios. According to the growth simulations in Figure 4.4 both species would then need about ten years to reach potentially reproductive size (*V. sanguinolenta*: 60 cm $LL_{max}$; *A. principissa*: 7 cm PsbL), which matches estimations for other epiphytes (Benzing 1981; Larson 1992; Zotz 1995). However, these numbers are only valid for specimens in the study area, which is the southern distribution border of *V. sanguinolenta* across the isthmus of Panama. Across the isthmus annual precipitation increases towards the atlantic coast in the north (3500 kg $m^{-2}$, S. Paton, STRI, pers. comm.), where *V. sanguinolenta* is highly abundant (pers. obs.). If a decrease in annual precipitation during the El Niño year 1997 retarded growth in *V. sanguinolenta* in the study area an increase in precipitation towards the atlantic coast probably would accelerate growth and thus reduce the risk of dying before reaching reproductive size.

**Table 4.3** Comparison of climate data for different years in the study area. Average values are means ± SD, n = 7 years (excluding 1997). Original data are for Barro Colorado Island, provided from the Smithsonian Tropical Research Institute.

| **Year** | **Precipitation (kg $m^{-2}$)** | **Days with rain** | **Evaporation (kg $m^{-2}$)** | **PPFD (mol $m^{-2}$ $a^{-1}$)** |
|---|---|---|---|---|
| Average 1991 - 98 | 2685 ± 320 | 163 ± 11 | 1314 ± 120 | 11.8 ± 1.1 |
| 1997 | 1700 | 120 | 1454 | 15.2 |
| 1998 | 2684 | 154 | 1359 | 12.6 |

Simulating growth for model plants revealed species specific patterns: irrespective of the mathematical model used and irrespective of growing conditions *V. sanguinolenta* featured hyperbolic growth curves (Figure 4.4A), while those of *A. principissa* were sigmoid (with the exception of the linear model for the 1997 data; Figure 4.4B). This type of growth simulation may conceal subtle changes in RGR. However, it is obvious that growth in *V. sanguinolenta* is not retarded considerably in late ontogeny, when approaching maximum size (Figure 4.4, but also Figures 4.2 & 4.3). The most likely explanation is that reproduction is a singular event in the life of a shoot of *V. sanguinolenta* and plants grow until an unknown cue triggers a presumably high investment into reproduction (inflorescence of up to 2 m with up to 98 capsules, each containing about 1000 seeds). While the fruiting ramet finally dies, a dormant meristem develops a new shoot. Notably, there is a c. 30% reduction in expected plant size, i.e. in $LL_{max}$, after fruiting compared to a non-fruiting plant of similar initial size (Figure 4.2). Probably as a consequence of substantial costs of reproduction plants do not flower in subsequent years: not a single *V. sanguinolenta* was encountered flowering twice during the three years of observation. Conversely, reduced growth rates in larger conspecifics of *A. principissa* (Figure 4.3) led to a sigmoid simulation curve of size versus age (Figure 4.4). This suggests a continuously increasing investment into reproduction with increasing size. Indeed, Zimmerman & Aide (1989) found that both flower production and fruit length was positively correlated with shoot length in this orchid. Moreover, the annual probability of fruiting also increased with plant size (Table 4.1) and individuals were observed to be reproductive in subsequent years, although flower production was reduced (Zimmerman & Aide 1989). In contrast to *V. sanguinolenta* changes in plant size subsequent fruiting were less pronounced in *A. principissa* compared (Table 4.4).

**Table 4.4** Comparison of changes in plant size (*V. sanguinolenta*: $LL_{max}$, *A. principissa*: PsbL) for non-reproductive and reproductive plants of similar size. Size differences were calculated as: ln ($size_{year\ n+1}$) – ln ($size_{year\ n}$ ) to compare with data from Zimmerman and Aide (1989).

| **Species** | **Changes in plant size** | | **Source** |
|---|---|---|---|
| | Vegetative | Reproductive | |
| *V. sanguinolenta* | + 0.25 ± 0.16 | - 0.30 ± 0.18 | This study |
| *A. principissa* | - 0.07 ± 0.20 | - 0.32 ± 0.24 | Zimmerman and Aide (1989) |

Combining growth analysis and demographic aspects in the two epiphytic taxa illustrates different strategies to cope with the respective habitat conditions. Retarded growth of larger *A. principissa* and repeated, but moderate investment into reproduction contrasted the comparatively high growth rates in large *V. sanguinolenta*, with a presumably high energetic input into monocarpic shoots. Similarly, growth of non-reproductive plants differed between the two study species: irrespective of a high, size-dependent intraspecific variability maximal growth rates were c. 100% higher in the bromeliad than in the orchid. Differences in growing conditions, however, affected plant growth more substantially. Although the time until maturation matches earlier reports for other epiphytes, high RGRs in small *V. sanguinolenta* under controlled and favorable conditions were comparable to those of annuals, which clearly challenges the notion of an inherently slow growth in vascular epiphytes.

# ANNUAL CARBON BALANCE OF AN EPIPHYTIC BROMELIAD – A MODEL LINKS LEAF PHYSIOLOGY AND PLANT GROWTH

## Chapter abstract

Our understanding of how epiphytes successfully cope with the harsh habitat conditions has advanced substantially in the last decades. However, ecophysiologists mostly explored leaf physiological parameters and, to a lesser extend, whole-plant performance. The model presented here tries to link leaf and whole-plant level mechanistically. In situ gas exchange of differently sized *V. sanguinolenta* was studied as a function of PPFD, precipitation, evaporation, and temperature data, provided by a whether station above the forest canopy. The use of additional morphometric information on the focal species allowed an estimation of net photosynthetic dry mass gain of autotrophic plant parts, respiratory dry mass loss of heterotrophic organs as well as direct dry mass losses. While the first two processes were highly size-dependent direct dry mass loss due to leaf shedding remained rather constant. The resulting annual dry mass balance was also independent of plant size, when expressed per unit leaf area. A subsequent sensitivity analysis showed that from the set of climate parameters that entered the model the number of rain events per year was the best predictor of annual dry mass balance. However, with increasing size plants were less affected by the natural variability of precipitation patterns between years.

Simulations of annual dry mass balances of entire plants did not show any bias for smaller or larger specimens and they adequately reflected changes in climate conditions between years. However, the model underestimated growth under natural conditions consistently. Hence, further analysis using absolute values of annual carbon balance can only be conducted when the model is fully validated. Respective measurements are planned.

## Introduction

One major goal of researchers working with epiphytes is to understand the mechanisms that enable epiphytes to cope with the harsh habitat conditions without accessing the extensive water and nutrient reservoir “ground”. Adaptations to this environment have been repeatedly reported, e.g. in anatomy and morphology (e.g. Lüttge 1989; Benzing 1990; Benzing 2000). Referring to ecophysiology approaching this goal mostly employed a species-specific examination (i.e. without considering size-related changes within a species) of leaf physiological properties under field or controlled conditions (e.g. Sinclair 1983; Winter et al. 1983; Maxwell et al. 1994; Stiles & Martin 1996; Helbsing et al. 2000). Conversely, ecophysiological information on whole-plant performance in situ is scarce. There are only few reports that measured or modeled e.g. epiphyte growth (Zotz 1995; Andrade & Nobel 1996; Chapter 4) and as yet not a single study has tried to link leaf physiological properties with whole-plant performance on a quantitative basis. This lack of information might be due to methodological problems (e.g. difficulties in accessing the canopy for repeated size determination and a high technical and time effort for physiological measurements) as well as the scarcity of basic knowledge on physiology, morphology and demographic aspects of a species. Much of this information is available for the tank bromeliad *Vriesea sanguinolenta*, the focal species of this study. For example, photosynthetic capacity, as determined in the oxygen electrode, scaled with plant size (Schmidt et al. 2001). Also for measurements in situ the size of a plant is an important determinant for leaf physiological properties: maximal photosynthesis rates ($NP_{max}$) correlated positively with plant size (Schmidt & Zotz 2001). On a whole-plant level the efficiency of external water tanks to bridge rainless periods also was a function of plant size (Schmidt & Zotz 2001). Since gas exchange was drastically restricted upon tank water depletion (Schmidt & Zotz 2001) the time available for $CO_2$ gain was prolonged in larger individuals. Consequently, changes in whole-plant carbon gain from small to large should be even more pronounced than size-dependent changes in $NP_{max}$ alone. However, studying in situ growth in *V. sanguinolenta* revealed that relative growth rates (RGR) were highly variable, but did not show a significant trend with increasing plant size. Difference of RGR between years, however, were significant (Chapter 4). Thus, two basic questions arise: (1) which plant processes are responsible for the inconsistent comportment on leaf and whole-plant level, and (2) how are these processes and other processes determining the DM balance of this bromeliad influenced by climate conditions? Thus, the objective of this research was to quantify morphometric characteristics and physiological traits as well as their dependency on climate conditions both on leaf and on whole-plant level for differently sized plants. A model should then link the individual parameters mechanistically. Water relations and key physiological plant processes for carbon turn-over (photosynthesis and respiration) should be the prime targets of climate conditions influencing the DM balance of the epiphyte. Effects on nutrient relations shall not be considered individually, because the uptake of nutrients is via ions and thus coupled to the uptake of water (Chapin 1980). With the development of such a model we should be able to quantify key processes determining the DM balance of differently sized *V. sanguinolenta* under field conditions. Moreover, the model shall represent a simple, but powerful tool to evaluate the impact of various climate parameters on the DM balance of plants of varying size. This shall allow an explanation of the observed year-to-year differences in RGR and it might also be used for modeling growth on the basis of climate measurements.

## Methods

### *Field site and study organism*

Field work was conducted in the Barro Colorado Nature Monument (BCNM, 9°10´N, 79°51´W), Republic of Panama. The reserve is categorized as a tropical moist forest (Holdridge et al. 1971) with a pronounced dry season from late December to late April (Leigh & Wright 1990). Detailed descriptions of vegetation, climate and ecology are provided by Croat (1978) and Leigh et al. (1982). The weather station on Barro Colorado Island (BCI) provided the necessary climate data used in the model.

The study species *Vriesea sanguinolenta* Cogn. & Marchal (Bromeliaceae; syn. *Werauhia sanguinolenta*; Grant 1995) occurs from Costa Rica to Colombia and on various Caribbean islands from lowland up to lower montane wet forests (Croat 1978). This epiphytic bromeliad can be found on a number of different host tree species, but in the BCNM it is particularly abundant on *Annona glabra* L. (Annonaceae) (Zotz et al. 1999a). All parameters related to the phorophyte were recorded in the crown of *A. glabra*, which in the Canal area is restricted to marshes around islands and on the mainland.

Throughout the study I used plant dry mass (DM, in g) as a measure of plant size. For illustrative purposes and for repeated size measurements in the field I used the length of the longest leaf ($LL_{max}$, in cm), a non-destructive measure of plant size that correlated well with DM: $LL_{max} = 10^{0.34 \times \log DM + 0.99}$, $r^2 = 0.99$, $n = 38$, $p < 0.001$. In *V. sanguinolenta* a step change from atmospheric to water-impounding life form occurs during early ontogeny (Schmidt & Zotz 2001). All measurements were conducted with tank individuals covering the entire size range of this stage from 0.1 g – 650 g, i.e. 4 cm – 95 cm $LL_{max}$. More detailed information on morphology, anatomy, and ecophysiology is provided in Schmidt & Zotz (2001, Schmidt et al. 2001).

### *Measurements of maximal tank water content and diel transpiratory water loss*

Prior to the measurements plants were kept wet for five days. Maximum tank water content ($C_{max}$) was gravimetrically determined for 12 differently sized *V. sanguinolenta* after all debris had been removed from the leaf axils. After emptying tanks, leaves were dried with tissue paper and plants were left to dry superficially for a few hours. Subsequently, 24 h mass changes of entire plants exposed in the laboratory clearing of BCI were recorded to determine diel transpiratory water loss. More detailed information on measurements of $C_{max}$ and transpiratory water loss are provided by Zotz & Andrade (1998).

### *Projected leaf catchment area, tank water surface and evaporation rates*

Differently sized plants were digitally photographed from above with a reference disc. The projected leaf catchment area ($A_{proj}$) was then estimated using the pixel-count feature of CorelPaint 7.0 (Corel Ltd., Ontario, Canada).

For the same specimens the tank water surface area was determined by filling all tanks of a plant with warm gelatin of water-like viscosity. After hardening, the upper surface of each of the gelatin parts was traced on paper and their area estimated gravimetrically. The tank water surface was defined as the sum of areas of all individual tanks formed in the leaf axils.

Evaporation above the canopy and at growing sites within the crown of *A. glabra* was measured simultaneously using evapotranspiration gauges (Model A, Etgage company, Loveland, Colorado, USA). To simulate evaporation from the central tank of a *V. sanguinolenta* in situ I shielded the ceramic plate of the gauge that was hung in the tree crown with radially arranged epiphyte leaves. Evaporation at both sites was then recorded and compared for 29 days in the 1999 dry season.

### *Light measurements*

A central slit (width c. 0.5 cm) was cut into a leaf from the apex to the leaf base with a razor blade. The total distance was divided into ten sections. Holding a quantum sensor (LI-190SA, LI-COR Inc., Lincoln, Nebraska, USA) into the slit from underneath photosynthetic photon flux densities (PPFD) were recorded at a specific position in the leaf. Simultaneous readings from a second PPFD sensor, installed above the study plant, allowed the calculation of the relative light interception (rLI). I began applying this procedure to the oldest (= most outward leaves of the rosettes) continuously moving on to the younger leaves. Finally, including all leaves in smaller individuals, or a representative subset of leaves in larger plants, yielded a realistic picture of light distribution within the plant. Measurements were only conducted around noon (between 11:00 and 13:00). Values for the different positions in the leaves were assigned to the corresponding sections (basal, central, apical). For each section, i.e. including all leaves measured, the mean relative light interception ($rLI^{sec}$) was calculated.

For determining light transmission through the phorophyte crown diel courses of PPFD were measured at four different positions in the crowns of two *A. glabra* trees, covering a total of 24 days during the early 1998 rainy season and the 1999 dry season. The measuring sites represented areas where *V. sanguinolenta* was highly abundant. Simultaneously, PPFD was recorded above the canopy. The ratio of PPFD measured above and within the canopy was defined as light transmission through the phorophyte crown (TM).

### *Gas exchange measurements*

For all in situ leaf gas exchange measurements I used a $CO_2/H_2O$-porometer (CQP 130, Walz, Effeltrich, Germany) operating in a continuous open-flow mode, connected to an infrared gas analyzer (IRGA, BINOS 100, Rosemount, Hanau, Germany). For methodological details see Zotz et al. (1995). Gas exchange parameters were calculated following Von Caemmerer & Farquhar (1981). Ambient $CO_2$ concentrations ranged from 340 – 380 µl $l^{-1}$. An artificial light source (Fiber Illuminator F-400 & FL-400, Walz, Effeltrich, Germany) provided homogeneously distributed PPFDs over the leaf area enclosed in the cuvette. Conditions were: saturating PPFD: 700 - 850 µmol $m^{-2}$ $s^{-1}$ (for saturating light conditions compare Schmidt & Zotz 2001), leaf temperature: 28.5 – 30.5°C, relative humidity inside the cuvette: 85 – 95%. All measurements were conducted between 9:30 - 11:30, thus plants had already been induced during preceding exposure to saturating ambient light. All plants were well watered for at least three days prior to experiments.

For measurements on respiration of heterotrophic, i.e. non-green, plant parts a LD2 Leaf Disc Electrode (Hansatech Ltd., King’s Lynn, UK) was used. Freshly punched discs from leaf bases and disc-like pieces from the alive part of the jolted shoot were enclosed in the cuvette of the leaf disc electrode unit (kept at 30°C with a circulating water bath). Samples were subsequently dried at 60°C for seven days and weighed. Triphenyl tetrazolium chloride reduction by disc-like pieces from the shoot was used as a viability assay to estimate the proportion of living and dead tissue of the shoot (Larcher & Eggarter 1959). To explore the relationship of plant size and respiration of heterotrophic plant parts I determined respiration rates of leaf bases and shoots of six specimens ($LL_{max}$: 15 – 80 cm). The temperature dependency of respiration was studied using leaf bases of three plants of similar size ($LL_{max}$: 53 – 61 cm ) by determining respiration at four different temperatures (20, 25, 30 and 35°C).

### *Dry mass loss due to leaf shedding*

To explore the relationship of leaf area (LA) and leaf length (LL) I detached entire leaves (including the chlorophyll free leaf bases) and measured their length and area. I also collected dead leaf parts (from leaves which were not entirely dead) and determined their area and dry mass. Calculated specific leaf weight (SLW) for dead leaves was assumed to be the SLW of leaves that are shed ($SLW^{shed}$; Appendix 5.2), which was significantly lower than SLW for living leaves (ANCOVA, Duncan post hoc test, covariate: plant size, $F_{1,17} = 4.5$, $p = 0.049$).

### *Data analysis*

All results were statistically analyzed with STATISTICA software (STATISTICA 5.1, StatSoft Inc., Tulsa, Oklahoma, USA). The continuous size dependency of various parameters was mainly evaluated by regression analysis including all tank plants. To achieve near-normal distribution most data sets had to be log-transformed before analysis.

The relationship of plant size and potential photosynthetic rates of the sections ($NP_{pot}^{sec}$) was explored by choosing the regression with the highest $r^2$. For the apical section a polynomial regression would have yielded the highest $r^2$, but this function did not represent the measured values for the small individuals adequately: values would have increased for plants smaller $LL_{max}$ = 10 cm. Thus a piecewise linear regression with a software computed breakpoint was used. The $r^2$ of this regression was only marginally lower.

## The model: concept and principle calculations

All abbreviations of the model are summarized and explained in the Appendix 5.1.

### *Temporal plant size adjustment*

Most model-relevant parameters are strongly size-dependent. A positive carbon balance in a specific time period by definition reflects an increase in plant size (in mass units) and will, in turn, affect all size-dependent parameters accordingly. Due to computational simplification and reasons of ecological significance I chose a time interval of two months for plant size adjustment in the model. Carbon gain of autotrophic organs and respiratory carbon losses of heterotrophic plant parts were, depending on (tank) water status, calculated on a daily basis and integrated over two months, while direct DM losses (leaf shed) were determined annually and then adjusted for the two months interval.

### *The leaf section concept*

Along with leaf orientation and leaf position relative to the plant body optical and physiological properties of leaves changed as well. Leaf blades curve outwards resulting in a continuous change in orientation of the leaf surface in relation to the sun at a given time of the day. A leaf blade can thus not be treated as a homogeneous unit in terms of light capture and subsequent acclimation. For practical reasons I divided each leaf into three sections: apical, central and basal. The apical part was more or less horizontal and covered c. 50% of the length of the green part of the leaf (relative leaf length of the apical section, $rLL^{ap}$; Appendix 5.2). The basal section was characterized by steep, but more or less constant angles, averaging 25% of the green part of the leaf (relative leaf length of the basal section, $rLL^{ba}$; Appendix 5.2). The area with the strongest change in orientation was the central section (relative leaf length of the central section, $rLL^{ce}$; Appendix 5.2). This division mode was applied to all (small and medium sized plants) or a representative subset of leaves (large plants). Doing so included intra- and inter-leaf variability due to leaf age, optical properties, light interception, and acclimation thereon without quantifying them individually. For all further analyses on photosynthetic carbon gain of an entire plant the three sections (apical, central and basal) were treated separately.

### *Within plant light distribution*

The light distribution within the epiphyte was defined as the pattern of incident PPFD at the leaf surfaces, i.e. incident PPFD in the three leaf sections ($PPFD^{sec}$). To be able to use routinely monitored PPFD from a weather station above the forest canopy ($PPFD^{above}$) for estimating the amount of PPFD intercepted by the plant required (1) estimates of the averaged relative light interception in reference to the light conditions above the epiphyte for the three different sections ($rLI^{sec}$) and (2) measurements of relative light transmission through the canopy of the phorophyte (TM).

$$PPFD^{sec} = rLI^{sec} \times TM \times PPFD^{above} \tag{1}$$

### *Photosynthetic response to the prevailing light conditions*

Due to ontogenetic stage and previous environmental conditions individual leaves or even parts within a leaf (sections) differed in photosynthetic features. Under well watered conditions with ambient $CO_2$ and rather constant relative humidity and temperature, net photosynthesis of a leaf section ($NP^{sec}$) was a function of incident PPFD and the potential photosynthetic rate under saturating light conditions of the respective section ($NP_{pot}{}^{sec}$). Measured light response curves for different positions in the leaf, different leaves and different plants were standardized: for each curve the maximal photosynthetic rate was set to one (indicated by an asterix in equation (2). Relative values were then fitted after Smith (1937; equation 2; relative light response curve = $rLC^{sec}$, dimensionless: 0 – 1). Although the photosynthetic response to incident PPFD of sections, leaves and plants differed considerably in their performance in absolute terms they were very similar qualitatively (Schmidt & Zotz 2001). These data were used to estimate relative values of photosynthetic yield ($\alpha$) and dark respiration ($R_d$), as the basis of further calculations on absolute net photosynthesis of a section ($NP^{sec}$) of a specific plant according to equation 3. The photosynthetic potential of a section ($NP_{pot}{}^{sec}$) was defined as the mean photosynthesis rate of a section across all (or a representative subset) of leaves of a plant under saturating light conditions, well-watered and ambient $CO_2$ levels.

$$rLC^{sec} = \frac{\alpha \times PPFD^{sec}}{\sqrt{\dfrac{1 + (\alpha \times PPFD^{sec})^2}{(1^* + R_d)^2}}} - R_d \tag{2}$$

$$NP^{sec} = rLC^{sec} \times NP_{pot}{}^{sec} \tag{3}$$

### Diel carbon gain of autotrophic plant parts under well watered conditions

Similar to various epiphytic and non-epiphytic taxa (e.g. Zotz & Winter 1993; Beyschlag 1987) maximal rates of net photosynthesis per day ($NP_{max}$) and the integrated diel carbon gain per unit leaf area ($NP_{24h}$) were closely correlated in *V. sanguinolenta* (Schmidt & Zotz 2001; Appendix 5.2). To use this relationship in the model the highest calculated net photosynthesis rate of a section ($NP^{sec}$) per day was treated as $NP_{max}$. Entering day courses of PPFD above the forest canopy (as hourly means) into the model yielded corresponding day courses of $NP^{sec}$. Under natural conditions maximal values of NP were mostly observed between 9:00 and 13:00. Accordingly, the maximum rate during these late morning hours was chosen to calculate the integrated diel carbon gain per section ($NP_{24h}{}^{sec}$).

$$NP_{24h}{}^{sec} = -5.3 + 17.0 \times \mathrm{Max}\,(NP^{sec})^{9:00-13:00} \tag{4}$$

For the conversion of $NP_{24h}{}^{sec}$ rates (expressed per $m^2$) into the absolute amount of carbon being incorporated by the sections the leaf area of a section (including all leaves) was assumed to scale proportionally to the division of the leaf into a apical (50%), central (25%) and basal (25%) section. Summing up the diel integrals for all sections gave an estimate of whole-plant net photosynthesis ($NP_{24h}{}^{plant}$).

$$NP_{24h}{}^{plant} = (NP_{24h}{}^{ap} \times 0.5 + NP_{24h}{}^{ce} \times 0.25 + NP_{24h}{}^{ba} \times 0.25) \times PLA \tag{5}$$

### Water status

Since water uptake in most tank bromeliads is primarily via leaf trichomes (Benzing 1990), internal water status under natural conditions mainly relies on the permanent supply of externally stored water in the leaf axils of the rosette. In *V. sanguinolenta* maximal tank water content and the efficiency of tanks to bridge rainless periods scaled with plant size (Schmidt & Zotz 2001). Differences in rainfall patterns will thus affect water relations and consequently the carbon balance differently in conspecifics of varying size. Therefore model calculations predicting the actual tank water content for any given day of the year in dependence of climate conditions and plant size are a prerequisite for any further modeling of the annual carbon balance of a tank bromeliad.

The tank water content is, up to capacity, raised by precipitational input and diminished by evaporation from the water surface and uptake by trichomes in the leaf axils forming the tank. Assumptions and computations of tank water content are described in detail in Zotz & Thomas (1999).

### Water status and $CO_2$ exchange of autotrophic plant parts during rainless periods

Initial experiments had to clarify the relationship of the actual tank water content ($C_{act}$) and leaf gas exchange. Greenhouse and field studies revealed that leaf gas exchange is not dependent on tank water content as long as the tank is not empty, i.e. contained less than 10% of the maximal tank water content ($C_{max}$; Table 5.1). Upon tank water depletion gas exchange was substantially reduced in plants of all sizes. In relative terms, however, small individuals reacted less sensitive than larger specimens (Schmidt & Zotz 2001). I used the latter information for the model by introducing a size-dependent correction factor ($d_{\mathrm{empty}\ n}$), which accounts for the relative reduction in net photosynthesis depending on the actual tank water status: for days with $C_{act}$ > 10% of $C_{max}$ photosynthesis values determined for well watered plants were used uncorrected in the model ($d_{empty\ n} = 1$, with n = 0, indicating that there is still water in the tank), while for all days with $C_{act}$ < 10% of $C_{max}$ photosynthetic diel carbon gain was adjusted ($d_{empty\ n}$ = 0 to 1, with n = 1 to 4, referring to the day after tanks were empty; Appendix 5.2). On the fifth day after $C_{act}$ had dropped below 10% of $C_{max}$ diel carbon gain was about zero, irrespective of plant size (Schmidt & Zotz 2001) and thus $d_{empty\ n}$ was set to zero in the simulation.

**Table 5.1** $CO_2$ exchange with decreasing tank water content. Leaf net photosynthesis (NP) of plants with different tank water content ($C_{act}$) under controlled conditions in the greenhouse and under natural conditions. Plants were left to acclimatize for at least three days to the respective tank filling status. Photosynthesis data for plants ($LL_{max}$: 71 – 87 cm) in the controlled environment are means ± SD (n) of instantaneous rates from all leaf sections and all leaf ages. Data under natural conditions are diel integrals estimated from c. hourly measurements on three leaves per plant ($LL_{max}$: 35 – 39 cm).

*Controlled environment*

| $C_{act}$ | NP (µmol $m^{-2}$ $s^{-1}$) |
|---|---|
| 100% | 2.0 ± 1.15 (48) |
| 50% | 1.8 ± 1.30 (12) |
| 25% | 2.2 ± 1.55 (12) |

*Natural conditions*

| 19.4.1998 | | 24.4.1998 | | |
|---|---|---|---|---|
| $C_{act}$ | NP (mmol $m^{-2}$ $d^{-1}$) | $C_{act}$ | NP (mmol $m^{-2}$ $d^{-1}$) | Relative change (%) |
| 100% | 46.4 | 100% | 43.9 | - 5.3 |
| 100% | 36.7 | 80% | 35.8 | - 2.6 |
| 100% | 54.1 | 50% | 49.5 | - 8.6 |
| 100% | 57.3 | 20% | 58.9 | +2.8 |

The $NP_{max}$ – $NP_{24h}$ relationship (Schmidt & Zotz 2001; Appendix 5.2) was derived from gas exchange measurements of plants under well watered as well as under drought conditions, and is thus valid in both cases. Therefore the calculation of $NP_{24h}^{sec}$ under drought conditions simply requires the introduction of the correction factor $d_{empty\ n}$ to equation (4):

$$NP_{24h}^{sec} = \left(-5.3 + 17.0 \times \mathrm{Max}\left[NP^{sec}\right]^{9:00-13:00}\right) \times d_{empty\ \ n} \qquad (4')$$

With the drought adjusted maximal photosynthesis rate per day the diel carbon gain can be calculated for the respective section, and subsequently for the entire plant according to equation (5).

### *Respiratory diel carbon loss of heterotrophic plant parts*

Respiration is generally comprised of two components: a comparatively constant rate of maintenance respiration and a highly variable growth respiration (Lambers et al. 1998). It was necessary to use this distinction in the model as well, because otherwise respiration would have been calculated as entirely independent of plant water status. Respiration, however, shows a gradual decline in times of drought due to a general reduction of carbon assimilation and the overall metabolism associated with slow growth under these conditions (Lambers et al. 1998). But the effects of reduced water availability on growth (and growth respiration) are not immediate. They appear with a lag phase of at least 24 h (Lambers et al. 1998). For model calculations I used the size-dependent correction factor for restricted gas exchange during times of persisting drought with empty tanks ($d_{\text{empty n}}$) with a delay of one day to adjust growth respiration (equation 6). The proportion of growth respiration generally ranges from 40 to 80 % of total respiration (Lambers et al. 1998). In another study Lambers & Poorter (1992) showed that the proportion of growth respiration is associated with RGR. For inherently slow growing herbaceous species (RGR = 113 mg $g^{-1}$ $d^{-1}$ under favorable conditions) the authors gave a mean proportion of 50 %, while fast growing taxa (RGR = 365 mg $g^{-1}$ $d^{-1}$ under favorable conditions) exhibited higher values for growth respiration, i.e. 75%. Relative growth rates under favorable conditions in *V. sanguinolenta* varied between 5 and 190 mg $g^{-1}$ $d^{-1}$ (Chapter 4). Therefore I adopted the proportions of what Lambers & Poorter (1992) called “inherently slow growing species” in the model calculations, i.e. maintenance and growth respiration was each 50% of total respiration.

The respiring, heterotrophic plant parts are represented by the jolted shoot and the chlorophyll-free leaf bases. Therefore respiration had to be determined and calculated for both compartments: maintenance and growth respiration of leaf bases ($mR^{Lb}$ and $gR^{Lb}$) and of the jolted shoot ($mR^{Sh}$ and $gR^{Sh}$). Furthermore, I expected total respiration to be temperature-dependent and adjusted all rates by a factor reflecting nighttime temperatures ($t_n$) and another one for daytime temperatures ($t_d$). For reasons of simplification respiration rates (determined per second) were assumed to be constant over 12 h, i.e. 43200 s, during the day and 12 h during the night. Furthermore, unit DM based rates had to be transformed to the total amount of respiratory carbon loss per plant. Proportions of leaf bases and shoot to whole-plant DM, however, changed with increasing plant size (relative DM of leaf bases, $rDM^{Lb}$, and of the shoot, $rDM^{Sh}$; Appendix 5.2). Respiratory carbon loss of the leaf bases ($R_{24h}{}^{Lb}$) was calculated as:

$$R_{24h}{}^{Lb} = \left[\left(mR^{Lb} + gR^{Lb} \times d_{\text{empty n}}\right) \times \left(t_d + t_n\right)\right] \times rDM^{Lb} \times DM^{plant} \times 43200 \qquad (6)$$

While leaf bases were alive entirely, jolted shoots included dead, non-respiring tissue. Shoot respiration ($R_{24h}{}^{Sh}$) was calculated for the living part only, by quantifying and excluding the dead proportion of shoot DM (relative DM of dead shoot tissue, $rDM^{dSh}$):

$$R_{24h}{}^{Sh} = \left[\left(mR^{sh} + gR^{Sh} \times d_{\text{empty n}}\right) \times \left(t_d + t_n\right)\right] \times \left(rDM^{Sh} - rDM^{dSh}\right) \times DM^{plant} \times 43200 \qquad (7)$$

The total carbon loss of a plant due to respiration of heterotrophic plant parts ($R_{24h}{}^{plant}$) was thus calculated as

$$R_{24h}{}^{plant} = R_{24h}{}^{Lb} + R_{24h}{}^{Sh} \qquad (8)$$

*Direct dry mass loss due to leaf shedding*

Besides dry mass losses due to respiratory $CO_2$ output, direct biomass losses contribute to the overall carbon balance of a plant. Irrespective of size, a *V. sanguinolenta* replaces almost the entire leaf set within one year (Chapter 4). For model calculations it was assumed that all leaves produced during the preceding growing season were shed. The length of the oldest leaf, i.e. the leaf that will be shed next, was c. 83% of $LL_{max}$ (P. Reichling, pers. comm.).

$$LL^{shed} = 0.83 \times LL_{max} \qquad (9)$$

The leaf area of a leaf should be a function of its length (equation 10). Assuming that the leaf area was not changed considerably before leaf shedding I used the correlation of living leaves for the determination of the leaf area for shed leaves as well ($LA^{shed}$; Appendix 5.2).

$$LA^{shed} = f\ LL^{shed} \tag{10}$$

Determination of the specific leaf weight of shed leaves ($SLW^{shed}$; Appendix 5.2) and the number of leaves shed in one year ($nL^{shed}$), allowed the quantification the loss of dry mass for an entire plant per year. No seasonal variation was assumed. Thus, the loss in a two months interval ($DML_{2m}{}^{shed}$) was calculated as:

$$DML_{2m}{}^{shed} = LA^{shed} \times SLW^{shed} \times \frac{nL^{shed}}{6} \tag{11}$$

*Annual dry mass balance of an entire plant*

According to Penning de Vries (1975) a plant has to incorporate 1.88 g $CO_2$ to produce 1 g of dry mass (conversion factor $c = 1 / 1.88$). A two months (2m) carbon budget of a plant resulting from gas exchange processes can thus be converted to a two months DM balance. Together with direct DM loss and initial DM of the plant ($DM_0$), an estimate of the plant size (in g DM) after two months ($DM_{2m}$) was obtained as:

$$DM_{2m} = \sum^{2m} (NP_{24h}{}^{plant} - R_{24h}{}^{plant}) \times c - DML_{2m}{}^{shed} + DM_0{}^{plant} \tag{12}$$

Due to the change in plant size all size-dependent model parameters have to be adequately readjusted for each two months interval. The annual DM balance ($DMB_a$) of an individual of given size was finally calculated as the sum of all two month DM balances in the turn of a year:

$$DMB_a = \sum_{n=1}^{6} (DM_{2m} - DM_0{}^{plant}) \tag{13}$$

*Model assumptions and simplifications*

Measuring PPFD directly on the leaf surface included direct sunlight, diffuse radiation and reflected light from other leaves of the plant. However, PPFD was solely recorded on the adaxial leaf surface. Effects of light incident on the abaxial side were assumed to be integrated in gas exchange measurements using a translucent cuvette. Light interception at different positions on the leaf clearly changes in the course of the day due to a changing angle of the leaf surface to the sun and a varying degree of self shading. However, these changes were assumed to be too subtle to be important. In the tropics the phase of flat angles of the sun to a horizontal line during dawn and dusk is reduced substantially compared to temperate regions, i.e. the sun rises and sets much faster. Specifically between 9:00 and 13:00 (the period of maximal photosynthetic rates; compare equation 4 & 4’) variations in the angle of sun to the leaf surface should not be that different to conditions that were measured around noon (11:00 to 13:00). Nevertheless the position of the sun was considered when determining light transmission through the phorophyte crown by repeated measurements of PPFD during the day (TM; Appendix 5.2).

Although other ways of water uptake, e.g. via roots or via leaf trichomes outside the tanks, could not be ruled out, it was assumed that the main source of water entering the plant body was tank water. Thus, tank water status was treated as a synonym for water availability. Initial tank water content was set to 100% at the beginning of any year to be modeled, irrespective of the preceding climate conditions. Transpiration and $CO_2$ exchange were treated independent of tank water status as long as the tank was not empty, i.e. tank water content had not dropped below 10% of the maximal volume (compare Table 5.1). However, within a plant some tanks, especially the large central tank held water longer than others. For the model all tanks of a plant were treated as one. Upon tank water depletion gas exchange was drastically reduced (Schmidt & Zotz 2001). Transpiration should be affected as well, and in dearth of detailed data transpirational water loss was set to zero on the first day after tanks were empty. The reduction of gas exchange upon tank water depletion was modeled assuming that all sections in the leaves of a plant were affected equally.

Although photosynthesis is clearly dependent on temperature as well, I did not conduct any temperature response measurements. During measurements to establish the relationship of $NP_{max}$ and $NP_{24h}$ (Schmidt & Zotz 2001) leaf temperatures at times of $NP_{max}$ were within a narrow range (27.8°C to 30.7°C), while determining $NP_{24h}$ covered a range from 21.6°C to 37.3°C. Thus, determining $NP_{max}$ for the different sections after the protocol given in the Method section (leaf temperatures ranged from 28.5°C to 30.5°C) and estimating $NP_{24h}$ with the respective correlation (Appendix 5.2) included a wide range of naturally occurring leaf temperatures.

Plant DM can directly be lost as a consequence of herbivore activity, during reproduction (e.g. pollen, nectar, fruit) or as loss of dead vegetative plant parts. For modeling the DM balance of *V. sanguinolenta* the reproductive stage was excluded a priori and herbivory was judged too unpredictable in frequency and extent to be entered as an average value into the model (compare Schmidt & Zotz 2001). Direct DM losses in the model were solely represented by shedding of old leaves. Although there is no information if leaf shedding is seasonal, I assumed that it is not. This expectation arises form the results of growth measurements on a subset of plants during the dry season 1999, which showed that growth is barely retarded compared to the rainy season (data not shown). Other, indirect DM losses such as leaching phenomena were neglected because tropical rain forest plants are not easily leached in general (Mooney 1972). Volatiles like isoprene might be important on ecosystem level but are also negligible in the DM balance of individual plants. Root exudates were not included either, because roots were discarded for the entire analysis. Respiratory $CO_2$ release of shoot and leaf bases, however, was measured for conspecifics covering the entire size range. To be able to model $CO_2$ losses for years with average annual temperatures different from long-term averages (e.g. El Niño) the temperature response of leaf base respiration was quantified. Since respiration rates of leaf bases and shoots were quite similar I assumed identical relative changes for shoot respiration.

Finally, potential size-dependent differences in nutrient uptake were assumed to be integrated into the model without exploring nutrient relations individually: if there was an influence of nutrient availability on investments into the photosynthetic machinery in the long term, effects thereof would be represented by the integrative measure of $NP_{max}$.

## Results

### *Parameter estimates*

An overview of all regression equations and calculated means entering the model is provided in Appendix 5.2.

To be able to use above forest PPFD for modeling photosynthesis I determined light transmission through the phorophyte canopy. At typical growing sites of *V. sanguinolenta*, i.e. in areas of high bromeliad abundance within the canopy of *A. glabra*, hourly transmission through the tree crown (TM) averaged 37 ± 22 % (mean ± SD, n = 584). Particularly low values in the morning and high transmissions in the evening (compare Appendix 5.2) are clearly an effect of the natural setting of the site, i.e. the vicinity of a dense forest in the east and the lake surface in the west. However, during the times of maximal photosynthetic activity (9:00 to 13:00) as used in the model these effects should be negligible.

Light distribution within the epiphyte was also highly variable: irrespective of plant size outer leaf parts (apical section) received most light (on average 81 % of above plant PPFD), while increasing self-shading reduced light interception towards the center of the plants (central: 45%; basal: 17%). Differences were significant at $p < 0.001$ level for the three sections (ANCOVA, Duncan post hoc test, covariate: log plant DM, $F_{2,26} = 332$). Averaged light interception of entire plants was dependent on plant size (relative light interception = 0.056 × log DM + 0.42, $r^2 = 0.80$, n = 10, $p < 0.001$).

An important parameter to calculate the actual tank water content is the evaporation rate of water stored in the tank. Integrated over 29 days evaporation from a simulated tank (evaporation surface was protected by sheathing leaves) at a typical growing site of *V. sanguinolenta* within the phorophyte crown was about 59% of the potential evaporation from an unprotected evaporation surface above the canopy. Hence, evaporation data from above the forest were used in the model and then corrected for a *V. sanguinolenta* tank accordingly. Evaporative water loss from the tank was a function of the surface area of tank water ($S_{max}$), which increased by more than two orders of magnitude ($0.9 \times 10^{-4} – 3.4 \times 10^{-2}$ $m^2$) from the smallest to the largest plant. Similarly, transpirational water loss (T) increased by more than three orders of magnitude ($0.15 – 0.37 \times 10^3$ g $H_2O$ $plant^{-1}$ $d^{-1}$). Parameters related to water input into the tank also scaled with plant size. For example, the catchment area ($A_{proj}$) of the smallest plant was 4 $cm^2$, while the largest plant covered an area of $5.6 \times 10^3$ $cm^2$, again an increase of more than three orders of magnitude. Other morphometric parameters, necessary to simulate gas exchange on whole-plant level, were dependent on plant size as well. The DM of leaves, leaf bases and the jolted shoot clearly increased with increasing plant size (data not shown). When related to whole-plant DM, however, only the proportion of leaf bases correlated positively with plant size, while the proportion of green leaves decreased and shoot proportions remained rather unaffected of changes in plant size (Figure 5.1).

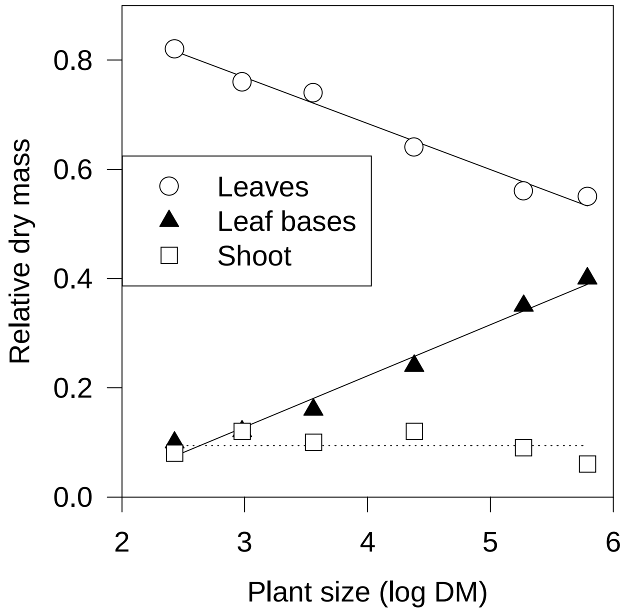

**Figure 5.1** Proportions of leaves, leaf bases and shoot in relation to whole-plant DM (relative DM) as a function of plant size (DM). Solid lines represent significant linear regressions, the dotted line symbolizes a mean. Statistics are given in Appendix 5.2.

Besides the size dependency of various morphometric parameters physiological variables changed with plant size as well. Determined under favorable climate conditions (but with ambient $CO_2$ levels) averaged maximal photosynthetic rates for all values in a section ($NP_{pot}^{sec}$) increased by almost 200% in the case of the apical (2.3 – 6.3 µmol $m^{-2}$ $s^{-1}$) and central section (1.4 – 3.8 µmol $m^{-2}$ $s^{-1}$; Figure 5.2). In the basal section $NP_{pot}$ remained unaffected by plant size and averaged 1.0 µmol $m^{-2}$ $s^{-1}$ (Figure 5.3; Appendix 5.2). The potential net photosynthesis rates differed significantly between the leaf sections (ANCOVA, Duncan posthoc test, covariate: log DM, $F_{2,11} = 60.0$, differences significant at $p < 0.001$) with highest values in the apical and lowest in the basal section (Figure 5.2).

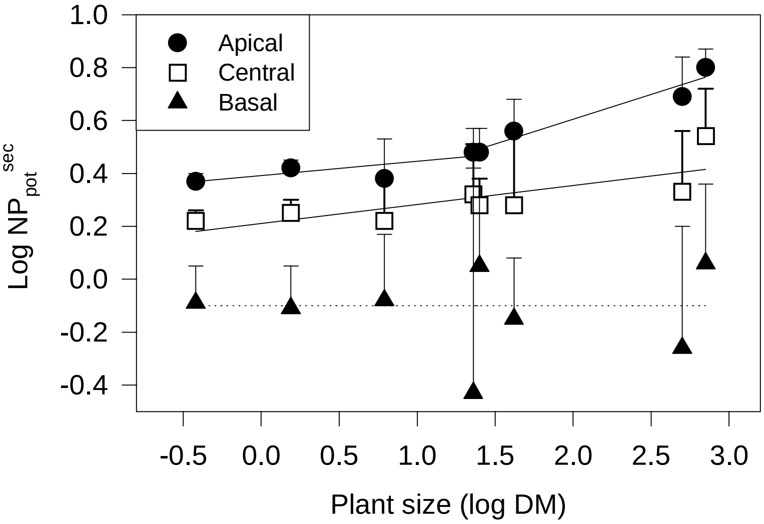

**Figure 5.2** Photosynthetic rates under favorable climate conditions and saturating PPFD for the different leaf sections ($NP_{pot}^{sec}$). Symbols represent averaged values for all or an aliquot of leaves with SD (error bars, n = 4 – 8). Solid lines indicate significant linear (central section) and piecewise linear (apical section) regressions, the dotted line represents a mean. Statistics are given in Appendix 5.2.

Dry mass based respiration rates of both leaf bases and jolted shoot were low and scaled with plant size (Appendix 5.2). While the increase was moderate for the leaf bases (3.3 – 4.8 nmol $g^{-1}$ $s^{-1}$), the change of shoot respiration was more than 100% (3.1 – 7.9 nmol $g^{-1}$ $s^{-1}$). Additionally, leaf base respiration of similarly sized plants was measured at different temperatures. Respiration rates were highly temperature-dependent with an increase of 136% ± 54 (mean ± SD, n = 3) from 20 to 35°C (Figure 5.3). In the model I standardized respiration at 30°C and calculated the temperature-dependent correction factor for respiration of the non-green plant parts and adjusted it for day and night temperatures ($t_d$ and $t_n$; Appendix 5.2).

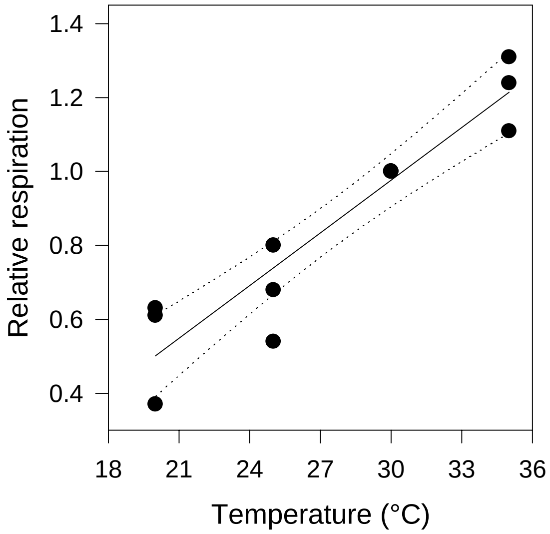

**Figure 5.3** Respiration of leaf bases as a function of temperatures. At four different temperatures respiration rates of three plants of similar size ($LL_{max}$: 53 – 61 cm) were determined and then standardized to rates at 30°C. Given are linear regression (solid line) and 95% confidence intervals (dotted lines). Statistics are provided in Appendix 5.2.

*Model predictions*

The amount of light that a plant received in the course of a year depends on PPFD above the canopy, light penetration through the tree crown and self-shading effects within the epiphyte. According to the model predictions a large plant received about 3.2 ± 0.3 kmol $m^{-2}$ $a^{-1}$ (mean ± SD, n = 8 years), which is about 45 % more light than the smallest plant (2.2 ± 0.17 kmol $m^{-2}$ $a^{-1}$). A second main parameter influencing photosynthetic carbon gain is the tank water content. The necessary rainfall to fill an empty tank is illustrated in Figure 5.4A: large plants required five times the precipitation compared to small plants. However, most rain events fill the tanks of all epiphytes (compare inlet in Figure 5.4A). Water is subsequently lost from a tank by evaporation from the tank water surface and by absorption via trichomes, which was assumed to equal whole plant transpiration. The importance of these two processes in three different evaporative situations for plants of the entire size range is analyzed in Figure 5.4B. In times of high above forest evaporation, evaporation from the tank water surface was similar to whole plant transpiration (Figure 5.4B). With decreasing above forest evaporation the relative importance of transpiration increased, and only very small plants lost more water due to evaporation than to transpiration (Figure 5.4B). The relationship of the different water relations parameters to plant size and to climate conditions allowed an estimation of daily changes in tank water content. Entering climate data of the weather station on BCI for eight consecutive years revealed that the tanks of all plants dried out completely for several days per year. The absolute number of days with empty tank per year strongly depended on plant size (Figure 5.5A): during the El Niño year 1997 (annual precipitation: 1700 kg $m^{-2}$) a large *V. sanguinolenta* of 95 cm $LL_{max}$ was 87 days without water in the tank, while the smallest bromeliad experienced 225 days without tank water, i.e. an increase of 159% (Figure 5.5A). In 1993, a year with annual precipitation close to the log-term average (2700 kg $m^{-2}$) large individuals (33 days with empty tank) were even more efficient in bridging rainless periods compared to small plants (174 days with empty tank), reflected by an increase of 427%. The average relative increase was 235% ± 94 (mean ± SD, n = 8 years). Not surprisingly, within a year most empty tank events occurred in the four months dry season, but even during the rainy season plants had to face empty tank situations. However, the number of such events declined asymptotically towards zero for larger specimens (Figure 5.5B).

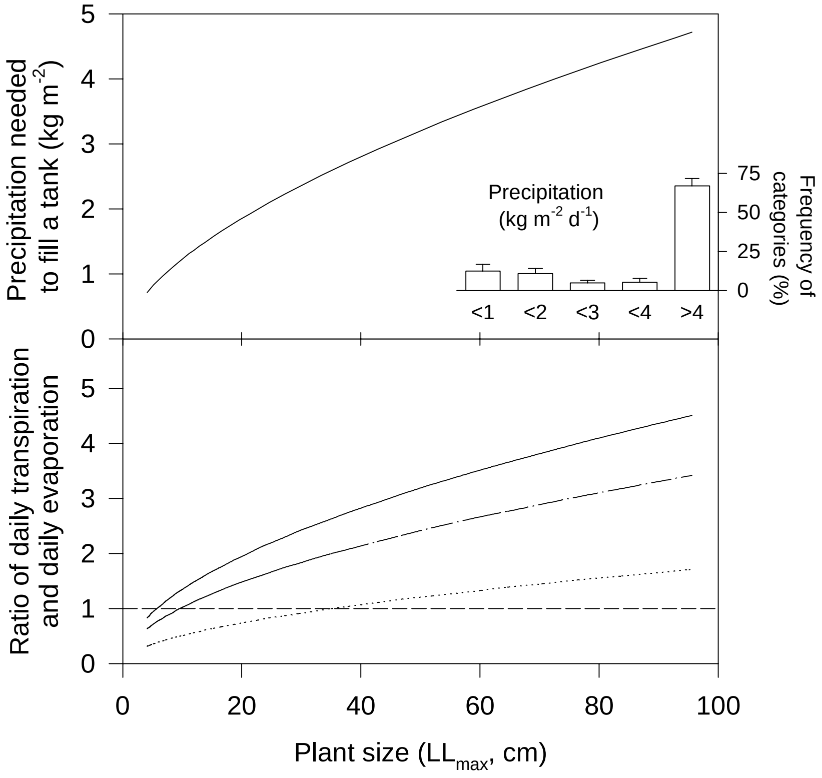

**Figure 5.4** Model predictions of tank water gain and loss as a function of plant size (LL$_{max}$). **Figure 5.4A** Necessary precipitational input to fill an empty tank. The inlet illustrates the frequency of precipitation categories. Values are means (bars) ± SD (error bars; n = 2929 days). **Figure 5.4B** Ratio of transpirational and evaporational tank water loss calculated for three days with different potential above canopy evaporation. The dashed line indicates a one-to-one match of transpiration and evaporation.

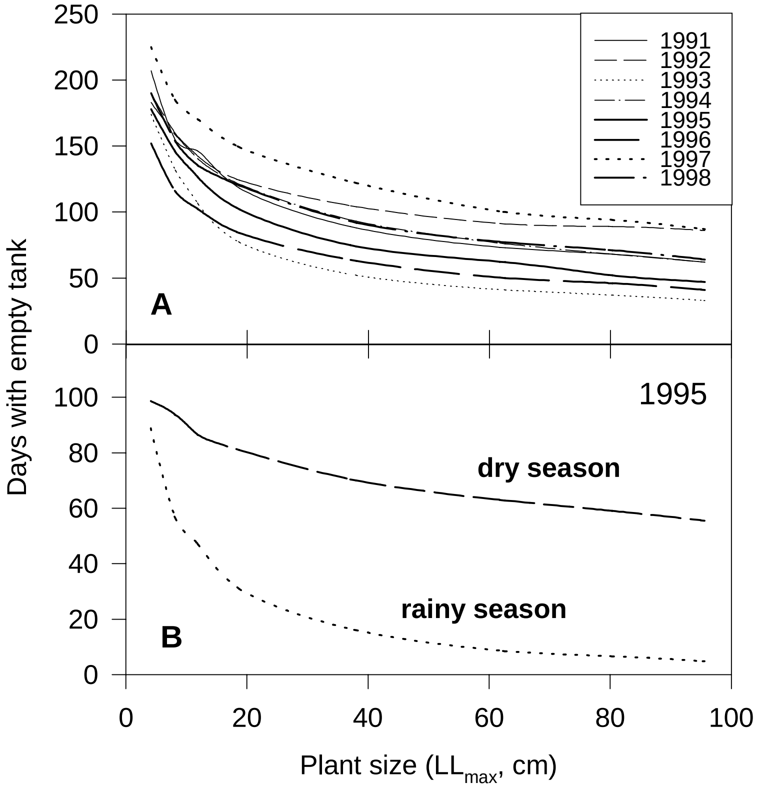

**Figure 5.5** Model predictions for the number of days with empty tank per year as a function of plant size (LL$_{max}$). **Figure 5.5A** Model predictions for 1991 – 1998. Different line symbols represent different years. **Figure 5.5B** Number of days in rainy (dotted line) and dry season (dashed line) with empty tank, estimated for the 'average' year 1995.

Whole-plant carbon and DM gain was highly size-dependent (Figures 5.6A & B). Annual area related rates of net photosynthesis for leaves increased by 334 ± 24% (mean ± SD, n = 8 years) from the smallest to the largest model plant (Figure 5.6A). When expressed on a DM basis increases referring to plant size averaged 116 ± 12% (mean ± SD, n = 8 years; data not shown). Two components contribute to the profound increase in NP: (1) the efficiency to bridge rainless periods increased with plant size, i.e. larger plants prolonged the available time for potential carbon gain and (2) the photosynthetic potential of the most exposed parts of the leaves (Figure 5.2) also increased with plant size. Year-to-year variability of NP was less pronounced: for the smallest plants NP maximally differed by 35% and large specimens by 43% (1993 and 1997; dotted lines in Figure 5.6A).

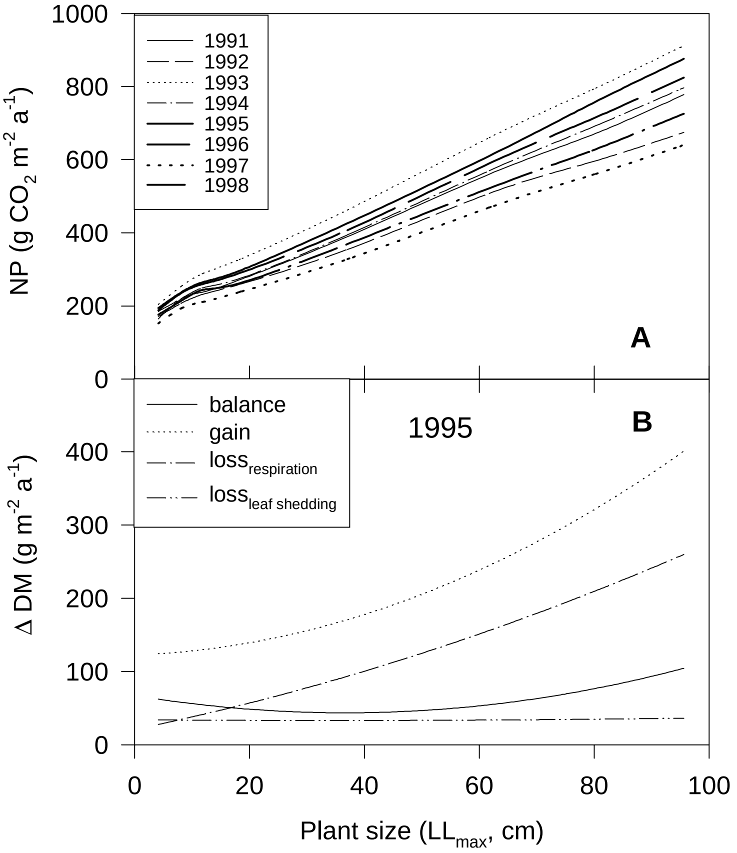

**Figure 5.6** Model predictions as a function of plant size ($LL_{max}$). **Figure 5.6A** Area based annual photosynthetic carbon gain (net photosynthesis, NP). Different line types represent different years. **Figure 5.6B** Area based dry mass changes (ΔDM) during the 'average' year 1995.

Total dry mass losses are comprised of direct biomass loss due to leaf shedding and respiratory $CO_2$ release of the non-green plant parts. The first was by definition independent of plant size, when expressed as DM loss per unit leaf area or per unit DM (Figure 5.6B). Respiratory DM losses, calculated as the sum of growth and maintenance respiration of non-green plant parts, was strongly size-dependent (Figure 5.6B). Both increasing proportions of heterotrophic tissue with plant size (Figure 5.1) and increased size-dependent respiration rates per unit DM determined the relationship of plant size and respiratory DM losses. Estimated for 1995 (Figure 5.6B), a year with annual precipitation of 2530 kg $m^{-2}$, comparable to the long-term average of c. 2600 kg $m^{-2}$, model estimates of DM gain and DM loss resulted in an almost constant DM balance for plants of varying size, when expressed per unit leaf area (Figure 5.6B). Comparing diel carbon balances (not including DM losses due to leaf turn-over) in the course of a year (1995; Figure 5.7) indicates that during the dry season frequent empty tank situations in both small and large specimens prevented positive carbon balances (Figure 5.7; January through April). During the rainy season frequent rainfall led to mostly positive DM balances (Figure 5.7; May through December). But even during the rainy season rainless periods of several days occurred in 1995 (as in other years), which in one case substantially reduced the daily carbon balances of small and large plants (Figure 5.7; beginning of October; indicated by an arrow indexed with “1”). Mostly, however, tanks of large specimens were efficient enough to prevent an empty tank situation, i.e. without water limitations to photosynthetic carbon gain, while small plants showed negative diel balances due to depletion of tank water (Figure 5.7; e.g. second half of August; indicated by an arrow indexed with “2”). Low levels of PPFD may also reduce carbon balances profoundly and sometimes even led to negative diel balances (Figure 5.7; e.g. middle of May and beginning of October; indicated by an arrow indexed with “3”). However, light limitations of photosynthetic carbon gain affected large plants more fundamentally due to high respirational carbon losses (Figure 5.7).

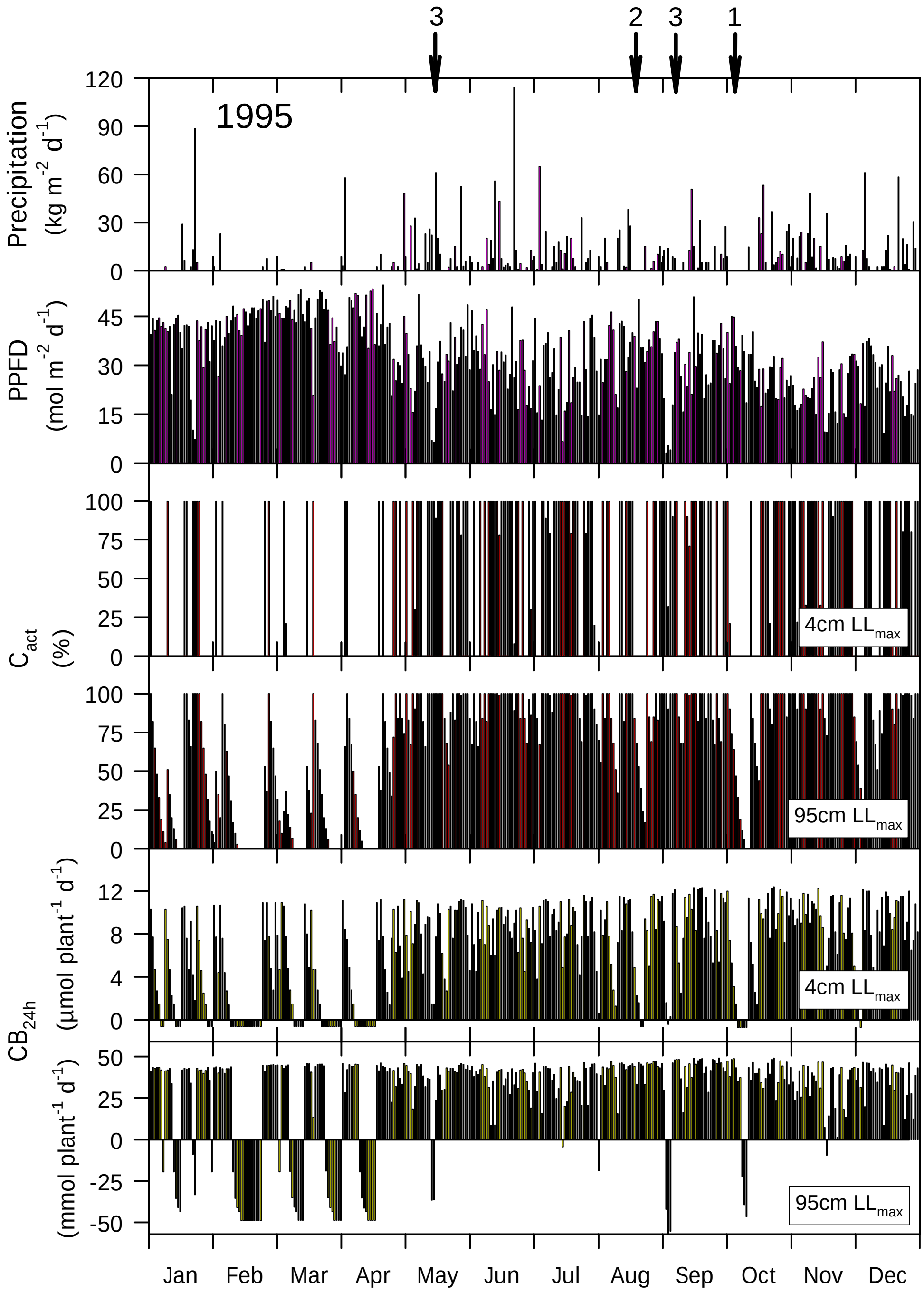

**Figure 5.7** Annual course of climate conditions (precipitation, PPFD) and model predictions (actual tank water content, $C_{act}$; daily carbon balance, $CB_{24h}$) for a small (4 cm $LL_{max}$) and a large plant (95 cm $LL_{max}$) during 1995. Estimates of $CB_{24h}$ do not include DM losses due to leaf shedding, because they are negligible, when expressed on a daily basis. Arrows indicate specific climate situations affecting model predictions, which are referred to in the text.

### *Impact of climate variability on the dry mass balance*

To explore influences of individual climate parameters (within the natural range of the last eight years, 1991 – 1998) on the DM balance of differently sized plants a multiple regression analysis was conducted. Annual DM production of small to medium sized plants was strongly influenced by climate variability (Table 5.2). Partial correlations identified the number of rain days per year and the amount of annual precipitation as the prime determinants of annual DM balance (Table 5.2). Conversely, modeled DM balances for different years of large individuals did not correlate significantly with the change of climate conditions (Table 5.2). This indicates that due to the efficiency to bridge rainless periods large bromeliads were rather independent of rainfall patterns as long as the variability does not exceed the range of the eight years studied.

**Table 5.2** Statistical analysis of the influence of climate variability on the DM balance of *V. sanguinolenta*. Model predictions for four differently sized plants (plant size as $LL_{max}$) during eight consecutive years (1991 – 1998) were analyzed using a multiple regression analysis. Given are significance values (p) and coefficient of determination ($r^2$) of the multiple regressions for different plant sizes. For significant regressions beta-values ± SD and p-values (index) of the partial correlations are provided. Significant partial correlation on a $p < 0.05$ level are printed in italics.

| | **Plant size ($LL_{max}$)** | | | |
|---|---|---|---|---|
| | 4 cm | 12 cm | 38 cm | 95 cm |
| p | = 0.04 | = 0.02 | = 0.04 | = 0.07 |
| $r^2$ | 0.93 | 0.95 | 0.93 | 0.90 |
| Annual precipitation | $-1.04 \pm 0.37^{0.07}$ | *$-1.25 \pm 0.31^{0.03}$* | *$-1.49 \pm 0.38^{0.03}$* | |
| Annual evaporation | $-0.14 \pm 0.35^{0.72}$ | $0.15 \pm 0.29^{0.64}$ | $0.48 \pm 0.35^{0.26}$ | |
| Annual PPFD | $0.72 \pm 0.61^{0.33}$ | $0.08 \pm 0.50^{0.88}$ | $-0.69 \pm 0.62^{0.34}$ | |
| Rain days per year | *$2.33 \pm 0.52^{0.02}$* | *$2.11 \pm 0.43^{0.02}$* | *$1.73 \pm 0.53^{0.05}$* | |

## *Model evaluation*

The sub-model to estimate the actual tank water content ($C_{act}$) was validated separately following the procedure of Zotz & Thomas (1999). I determined the time how long differently sized plants are able to store water in their tanks in situ without precipitational input and compared it with the model predictions under the respective climate conditions. As illustrated in Figure 5.8A the modeled time deviated only marginally from the actual measurements, irrespective of plant size.

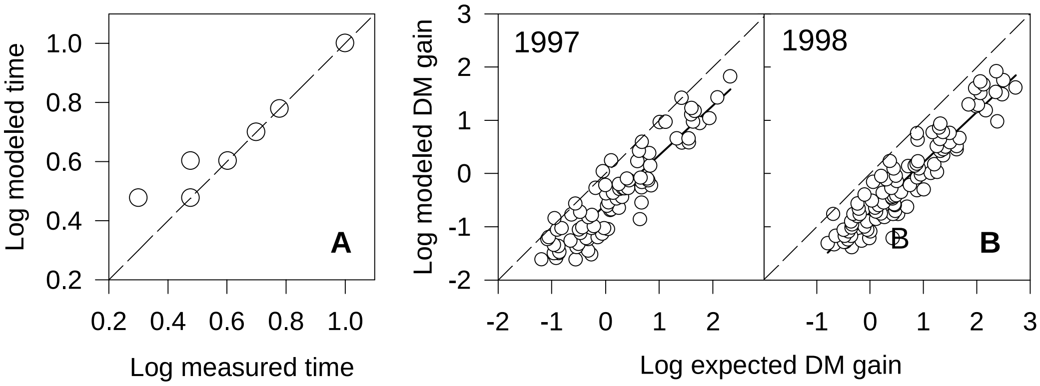

**Figure 5.8** Validation for different parts of the model. Dashed lines indicates a one-to-one match. Data were log-transformed to reach near normal distribution. **Figure 5.8A** Measured versus modeled time (in days, d) for a full tank to dry out. **Figure 5.8B** Dry mass (DM) gain in situ as converted from annual size increments of a non-destructive measure of size versus modeled DM gain (both in g $plant^{-1}a^{-1}$ ) for two consecutive years. Solid lines indicate linear regressions. Regressions are 1997: DM gain (prediction) = $10^{0.93 \times \log \text{DM gain (expectation)} - 0.59}$, $r^2 = 0.85$, n = 101, $p < 0.001$; 1998: DM gain (prediction) = $10^{0.95 \times \log \text{DM gain (expectation)} - 0.74}$, $r^2 = 0.88$, n = 102, $p < 0.001$.

The entire model was evaluated by a comparison of size increments in situ with those calculated by the model. Monitoring a non-destructive measure of plant size ($LL_{max}$) in three subsequent years allowed the calculation of annual size increments for specimens covering the entire size range of the water-impounding phase. Using the regression $LL_{max}$ – plant DM (see Method section) I converted the increase in $LL_{max}$ to an annual DM increment. Comparing these converted field data with the model predictions on annual DM balance (Figures 5.8B & C) allowed three basic conclusions: first, model calculations do not show any obvious systematic bias for smaller or larger conspecifics (the linear regression of observation versus prediction was parallel to the line for a one-to-one match in both years; Figures 5.8B & C). Secondly, year-to-year differences were adequately reflected in the model predictions. Growing conditions during the 1997 “El Niño” year were rather different from those of the consecutive year (compare Chapter 4), so was growth: averaged over the entire size range relative growth rates (RGR) differed by almost 130% between years (RGR in 1997/98: 3.1 ± 3.0, RGR in 1998/99: 7.1 ± 6.0). The same relative position of the regression lines of observation versus prediction suggests that these year-to-year differences were adequately evaluated by the model. Finally, however, the model consistently underestimates growth compared to natural conditions by about 75% in both years.

## Discussion

Although the model reasonably accounts for changes in plant size and climate variability, absolute values were underestimated. A probable source of error may be the respiration measurements for heterotrophic plant parts. (1) Respiration rates of the *jolted shoot* were determined by measuring gas exchange of pieces that were cut out of the shoot. Rates were then multiplied with shoot DM. Although respiration is primarily regulated by demand, via the turn-over of adenylates (Farrar & Williams 1991), it is also possible that other substrates, e.g. oxygen become limiting under certain circumstances. Most parts of the jolted shoot in *V. sanguinolenta* have a permanently high water content or are covered with tank water, which inevitably increases the diffusion resistance for gases. Under such conditions it seems possible that shoot respiration is reduced due to the low partial pressure of oxygen. Cutting pieces out of the shoot tissue will decrease both diffusion distance and resistance for oxygen and thus increase internal $O_2$ concentration compared to the situation in an entire, intact shoot. This might lead to an over estimation of shoot respiration during measurements. (2) Gas exchange of *leaf bases* was measured by cutting discs out of the chlorophyll free part of the leaf bases and placing them in the electrode chamber. In the model measured respiration rates, i.e. released $CO_2$ from the discs, was treated as carbon loss. However, the fate of respiratory $CO_2$ in situ is unclear. Morphological investigations revealed that stomatal density is drastically reduced in the chlorophyll free part of the leaves (leaf bases, data not shown). Considering the leaf anatomy of monocotyledonous leaves of *V. sanguinolenta* (parallel venation) I assumed no major barriers for the diffusion of $CO_2$ along the leaf blade. It is thus very likely that $CO_2$ derived from leaf base (or shoot) respiration is to a high degree fixed in the green parts of the leaves. Nevertheless an unknown proportion of $CO_2$ may be released gradient-dependent through surface tissues of the non-green plant parts even in the absence of stomata. As there is no experimental validation or quantification for this notion. Future measurements on the gas exchange of entire shoots (with and without leaf bases) of differently sized plants shall quantify respiration of the non-green plant parts.

Another potential systematic error is the merely mathematically calculated conversion factor of Penning de Vries (1975), who assumed a chemical composition of an average plant to be $C_{86}H_{160}O_{45}N_7$ and calculated from the molecular weight and the fraction of carbon that 1 g biomass is formed from 1.88 g $CO_2$. Although these calculations were conducted with a large sample size a characterization of the chemical composition of *V. sanguinolenta* biomass is planned and subsequently adjust the Penning de Vries conversion factor.

In contrast to the conceivable lack of accuracy in modeling DM balances, predictions on the tank water content were concordant with field observation (Figure 5.8A). Zotz & Thomas (1999) have already demonstrated that even 'continuously-supplied' tank bromeliads (sensu Benzing 1990) frequently experience empty tanks, i.e. water supply is not really continuous. Irrespective of plant size, *V. sanguinolenta* plants have to cope with numerous empty tank situations per year (Figures 5.5A & 5.7). A more detailed analysis revealed that during the rainy season the decrease of such empty tank situations asymptotically tends towards zero with increasing plant size (Figure 5.5B). This prompts me to hypothesize that in a non-seasonal tropical rain forest with a similar precipitation pattern large tank bromeliads of similar architecture may indeed be continuously supplied, while small conspecifics (or small growing species of similar architecture) have to be referred to as pulse-supplied epiphytes. This means that in such a non-seasonal rain forest water availability could be increasingly limiting for plants of decreasing size. It thus seems likely that the phenomenon of size-related physiological traits (sensu Chapter 7), reflecting long-term acclimation to water availability (Chapter 7), are more widely spread than presently acknowledged.

Besides long-term acclimation, differences in water availability, i.e. in the efficiency of externally stored water to bridge rainless periods, clearly affects short-term photosynthetic performance as well. Although *V. sanguinolenta* does not feature prominent supportive structures (e.g. stems and branches) the model predicts negative diel carbon balances in times of drought (Figure 5.7). Measuring the gas exchange of entire plants Adams III & Martin (1986b) also observed negative diel carbon integrals in *Tillandsia deppeana*. Modeling only leaf carbon budgets annual balances for the 'average year' 1995 ranged from 194 to 876 g $CO_2$ $m^{-2}$ $a^{-1}$, depending on plant size (Figure 5.6A). These numbers seem fairly low compared to data available for other epiphytes (Table 5.3). However, studies on in situ leaf gas exchange of four epiphytic species did, in two cases, not specify plant size. Neither potential differences due to leaf position, leaf age or light interception of different leaf parts were accounted for. In these studies only the most productive part of the leaf was measured (Zotz, pers. comm.). For a comparison the annual leaf carbon balance of *V. sanguinolenta* was recalculated by considering only the most productive part of the leaf as well, i.e. the apical section. Rates were now considerably higher, ranging from 408 to 1414 g $CO_2$ $m^{-2}$ $a^{-1}$ (Table 5.3). Taking into account that (e.g. Zotz & Winter 1994) measured large individuals (Zotz, pers. comm.) annual balances for *V. sanguinolenta* were quite similar to other epiphytes (Table 5.3). Estimations for *V. sanguinolenta* considering all or only the most productive parts of the leaf differed by about 60% in large specimens (Table 5.3), which indicates that leaf carbon balances of the other epiphytes might have been over estimated. However, differences in plant architecture, i.e. differences in self shading and acclimation thereon certainly hamper such a conclusion.

**Table 5.3** Annual leaf carbon balance for different epiphytes. For species of different taxonomic origin and metabolic pathways values on the annual leaf carbon balance ($LCB_a$, in g $CO_2$ $m^{-2}$ $a^{-1}$) are listed. These values include $CO_2$ losses due to dark respiration. While values given by Zotz & Winter (1994a) are integrated from daily integrals determined in situ about monthly, data for *C. bilamellatum* represent model calculations with climate data for BCI, Panama from 1994 under optimal light conditions. All other studies were conducted in central Panama as well.

| **Species** | **Family** | **Pathway** | **$LCB_a$** | **Source** |
|---|---|---|---|---|
| *Clusia uvitana* | Clusiaceae | CAM | 1060 | Zotz & Winter 1994a |
| *Catasetum viridiflavum* | Orchidaceae | C3 | 1090 | Zotz & Winter 1994a |
| *Polypodium crassifolium* | Polypodiaceae | C3 | 840 | Zotz & Winter 1994a |
| *Caularthron bilamellatum* | Orchidaceae | CAM | 1500 | Herz, unpubl. master thesis 1995 |
| *V. sanguinolenta* | Bromeliaceae | C3 | 408 – 1413 | This study, most productive leaf part only |
| *V. sanguinolenta* | Bromeliaceae | C3 | 194 - 876 | This study, model prediction |

Due to the consistency of the deviations (size dependency and year-to-year variability) and despite the absolute underestimation of in situ growth the use of the model as a tool for evaluating environmental influences on the carbon balance of *V. sanguinolenta* seems justified. The results of the sensitivity analysis (Table 5.2) identified the number of rain days per year as the best predictor of plant DM balances but also stated a decreasing importance of this variable on the DM balance for plants of increasing size: the largest specimens were less affected by changes in the precipitation pattern. However, these results have to be considered as preliminary until the model is fully validated.

### *Limitations of the model*

Beyond the problem of validation there are other, conceptual limitations to the model. Although the model includes physiological and morphological acclimation to long-term resource availability, reflected by differences in leaf physiology during ontogeny (compare Chapter 7), short-term acclimation to differences in climate conditions (e.g. dry versus rainy season, year-to-year) were not considered. Neither was compensatory potential of a plant subsequent to stress (e.g. Chiariello & Gulmon 1991). Although I did not focus on such processes, they were assessed as rather insignificant for modeling photosynthesis. Studying diel carbon gain during a drought cycle demonstrated that after 11 days with empty tank, i.e. stomata were closed for about one week, photosynthetic response upon watering was almost immediate without an obvious hysteresis (Schmidt & Zotz 2001).

In the same study Schmidt & Zotz (2001) reported that *V. sanguinolenta* conducts a phase shift from an early atmospheric stage towards the tank-forming phase. Due to technical and methodological problems of measuring water status and gas exchange of atmospherics the model was only parameterized for vegetative, water-impounding specimens. Thus, any attempt of estimating longevity of individual plants has to include further studies focussing on the atmospheric stage.

For specimens in the tank phase DM losses might be systematically underestimated, because roots were not considered in the model. There were three major reasons for the exclusion: (1) The prime function of roots of tank bromeliads is to securely attach the plants on the substrate, while water and nutrient uptake via roots is ecologically insignificant (Benzing 2000). This indicates that gas exchange through the root surface should be low. (2) Roots represent only a minute proportion of whole-plant DM (pers. observ.). (3) Detaching roots from the bark of the phorophyte is extremely time consuming and subject to a high sampling and measuring error.

In their natural habitat tanks of *V. sanguinolenta* normally accumulate organic material that might displace water or influence evaporation. In the model such potential effects were neglected.

### *Further analyses and potential of the model*

One major application of the model in the future is to quantify growth under constantly changing climate conditions at a given site. With the model developed so far it is possible to estimate annual DM increments for plants in the vegetative, water-impounding phase, ranging from 4 cm $LL_{max}$ to 95 cm $LL_{max}$, under the climate conditions in the study area (BCNM) in a given year. Repeated modeling with climate data of subsequent years will thus simulate growth in the long term and estimate the time a small tank plant (4 cm $LL_{max}$) requires to reach a potentially reproductive size, i.e. 60 – 70 cm $LL_{max}$. Since data on size-dependent mortality are available for *V. sanguinolenta* (Chapter 4) even the probability that a small tank plant reaches the reproductive stage can be calculated. However, to outline a realistic scenario for the entire ontogeny additional information on germination, seedling growth and growth during transition from the atmospheric to the tank stage is needed. Initial germination experiments in the greenhouse indicate that germination is almost immediate (two weeks after exposure to moist conditions; A. Baier, pers. comm.) and that germination rates were high (almost 100% of the seed germinated). Monitoring growth of atmospherics in situ from 1997 to 1999 revealed that all seedlings ($LL_{max}$ < 1 cm) remained in the atmospheric stage for the entire observation period, while most initially larger atmospherics ($LL_{max}$ > 1 cm in 1997) shifted life from to tank plants in 1998 or 1999. I thus hypothesize that it takes about 3 - 5 years until a seedling reaches the water-impounding phase. Considering that a small tank plant needs 10 - 35 years to reach maximum size (depending on climate conditions; Chapter 4) the time from germination to transition seems comparatively short. However, we do not have any information about the plastic growth response of atmospherics to environmental conditions and how these conditions influence (or trigger) the transition to the tank-forming stage. Moreover, if one wants to estimate the duration of entire life cycles of this species a accurate quantification of mortality during the atmospheric phase is required as well, which may substantially reduce the probability of a plant to complete its life cycle.

Another principle option of growth simulation is to estimate DM changes under conditions different from those in the study area, i.e. to model growth by processing climate data from other areas where *V. sanguinolenta* naturally occurs. A prerequisite for a successful simulation clearly are long-term records for the respective climate data. In addition, the relationship of $LL_{max}$ and plant DM has to be re-determined. As studies in the greenhouse already showed DM allocation patterns responded highly plastic to a better availability of resources (Chapter 4).

The potential of the model to simulate entire life cycles of *V. sanguinolenta* in dependence of climate parameters (in the study area and elsewhere) encourages to think about growth of the focal species under a generally altered macro-climate in the context of global change. A rise in temperature within the expected range associated with global warming increases growth because metabolism, and in particular cell division, is highly temperature sensitive (Farrar &

Gunn 1996) whereas photosynthesis is not (Farrar 1988). Thus, the plastic response of growth would have to be accounted for by re-assessing the relationship of plants DM and $LL_{max}$ at different temperatures. Furthermore, the photosynthetic response to changes in $CO_2$ concentrations will have to be studied. As yet, the model was developed with photosynthesis data measured under more or less constant, ambient $CO_2$ levels, i.e. in the naturally occurring range at the study site (340 – 380 µl $l^{-1}$).

As a second major field of application the model may aid to understand the distributional limits of the species both within the canopy and in geographical terms. Dispersal limitation and physiological incompetence are the main explanations for the absence of a species at certain sites. in *V. sanguinolenta* dispersal limitations seems unlikely, because the numerous and light seeds of tillandsioid bromeliads are plumed and can be carried great distances by air turbulences (Benzing 1990). Other processes such as interspecific competition neither seem to play a major role among epiphytic populations (Benzing 1990) and herbivory may be substantial at the level of individual plants (Schmidt & Zotz 2001) but cannot explain an exclusion from a given site. Clearly, other biotic interactions which e.g. may inhibit germination cannot be ruled out. It is thus assumed that in the case of *V. sanguinolenta* the physiological competence of the species to be of major significance for its distribution pattern. For example, the drought induced death of a plant mostly illustrates its ecophysiological incompetence to cope with the habitat conditions. In a natural population of *V. sanguinolenta* mortality in the water-impounding stage can be more than 35% in small and medium size categories (Chapter 4). Analyzing the entire population the main reason for death was drought. Large individuals, however, were never found dry (Chapter 4). In other words, naturally occurring short-term drought mainly affected smaller individuals. Similarly, the model predicts that the tanks of small plants are empty during most of the year while larger plants experience empty tanks less frequently (Figure 5.4). However, linking tank water status and drought induced mortality is difficult due to continuos size-dependent changes in leaf anatomy (e.g. decreasing proportion of hydrenchyma relative to leaf thickness) and physiology (e.g. increasing residual transpiration when stomata are closed) during the tank phase (Schmidt & Zotz 2001). Similarly, atmospheric plants, which have to rely on tissue water only, feature more xeromorphic characteristics than small tank plants (Schmidt & Zotz 2001). Considering the inefficiency of the tanks of small individuals leads to the hypothesis that atmospherics are more resistant to drought than small tank plants. Indeed, mortality rates are lower during this phase than in the water-impounding stage (Schmidt & Zotz 2001). Clearly, information on mortality as a function of the (tank) water status of a plant are essential in order to use the model for a deeper understanding of distributional limits of the species in dependence of climate parameters.

Constantly negative DM balances of plants due to unfavorable conditions other than a lethal water status may be another example for physiological incompetence, probably leading to exclusion from a given site. As yet the relationship of model predictions and climate parameters was explored with a multiple regression analysis. However, to exceed the correlational level a direct sensitivity analysis would be desirable as well, i.e. all climate parameters that enter the model have to be kept constant except one. Inserting the extremes of e.g. annual precipitation on BCI during the last 50 years while PPFD, evaporation and temperature are entered as constant long-term averages will yield the range of DM balances as a function of annual precipitation. Varying other parameters while the rest remains constant will show the impact of the respective parameter on the plants DM balance. Comparing the variability in DM balances as responses of climate variability accomplishes the results of the statistical sensitivity analysis by circumventing the natural relatedness of certain climate parameters (e.g. annual PPFD and annual precipitation). Being able to estimate DM balances in response to individual climate parameters allows the determination of threshold values for the respective parameter beyond which bromeliad growth is impossible, i.e. DM balances ▶ 0. Similarly, climatic threshold values can be calculated at which (small) plants desiccate. This type of analysis offers two major possibilities. (1) Comparing these threshold values with climate changes within the forest canopy might provide valuable information on growth limitations and mortality risks within the epiphytic habitat. Scaling down to individual trees Zotz (1997b) already gave a clear description of within crown distribution of *V. sanguinolenta.* Additional measurements on microclimate conditions as a function of position within the crown of *A. glabra* are planned. Results thereof and the comparison with the threshold values from the direct sensitivity analysis may reveal if within crown distribution of *V. sanguinolenta* is determined by drought induced mortality, by microclimatic growth limitations or if other processes like seed distribution or germination preferences are more important. (2) Comparing these threshold values with climate characteristics of different regions of the tropics will determine the geographic area in which water relations potentially ensure survival and vegetative growth of *V. sanguinolenta* is potentially possible.

In conclusion, the model presented here is a potentially powerful tool to identify climatic key parameters and quantitatively evaluate their impact on the DM balance of differently sized *V. sanguinolenta*. Given a full validation in the near future applications can be manifold and may aid to understand the relationship of physiological traits and climate parameters affecting growth and distribution of *V. sanguinolenta.*

## Appendices

### *Appendix 5.1: Abbreviation list for the model*

| Abbreviation | Unit | Explanation |
|---|---|---|
| **Index** $^{sec}$ | | Indexed variable refers to all of the three sections, i.e. apical, central, basal |
| **Indices** $^{ap\ ,\ ce,\ ba}$ | | Indexed variable refers to either the apical, the central or the basal section |
| **Index** $^{Sh}$ | | Indexed variable refers to the shoot |
| **Index** $^{Lb}$ | | Indexed variable refers to leaf bases |
| **Index** $^{plant}$ | | Indexed variable refers to the entire plant |
| **Index** $^{shed}$ | | Indexed variable refers to shed leaves |
| **Subscript** $_{2m}$ | | Value of variable after two months |
| **Subscript** $_{0}$ | | Value of variable at the beginning (of calculations) |
| $\alpha$ | (0 – 1) | Photosynthetic yield |
| $\mathbf{A_{proj}}$ | cm$^2$ | Projected plant area |
| ***c*** | | Conversion factor for incorporated $CO_2$ to DM gain after Penning de Vries (1975) |
| $\mathbf{C_{act}}$ | % | Actual tank water content |
| $\mathbf{CB_{24h}}$ | mmol $CO_2$ plant$^{-1}$ d$^{-1}$ | Daily carbon balance (excluding DM losses due to leaf shedding) |
| $\mathbf{C_{max}}$ | ml | Maximal tank water content of a plant |
| $\boldsymbol{d}_{\mathbf{empty\ n}}$ | (0 – 1) | Correction factor accounting for the relative reduction of gas exchange for day n after the tank is empty, i.e. $C_{act}$ has dropped below 10% of $C_{max}$ |
| $\mathbf{\Delta DM}$ | g m$^{-2}$ a$^{-1}$ | Annual change in dry mass |
| **DM** | g | Dry mass |
| $\mathbf{DMB_a}$ | mmol $CO_2$ plant$^{1}$ a$^{-1}$ | Annual DM balance |
| **DML** | g | Dry mass loss |
| **F** | (0 – 1) | Rain throughfall through the canopy after Frank & Curtis (1981) |
| **gR** | nmol $O_2$ m$^{-2}$ s$^{-1}$ | Growth respiration |
| ***k*** | (0 – 1) | Correction factor accounting for the reduction of evaporation in the crown of *A. glabra* compared to evaporation above the canopy |
| **LA** | cm$^2$ | Leaf area |
| **LL** | cm | Leaf length |
| $\mathbf{LL_{max}}$ | cm | Length of the longest leaf of a plant |
| **mR** | nmol $O_2$ m$^{-2}$ s$^{-1}$ | Maintenance respiration |
| $\mathbf{nL^{shed}}$ | a$^{-1}$ | Number of leaves shed within one year |
| **NP** | µmol $CO_2$ m$^{-2}$ s$^{-1}$ | Net photosynthesis |
| $\mathbf{NP_{24h}}$ | mmol $CO_2$ m$^{-2}$ d$^{-1}$ | Net photosynthesis per 24h |
| $\mathbf{NP_{max}}$ | µmol $CO_2$ m$^{-2}$ s$^{-1}$ | Maximal net photosynthesis under favorable conditions |

| | | |
|---|---|---|
| **$NP_{pot}^{sec}$** | µmol $CO_2$ $m^{-2}$ $s^{-1}$ | Potential $NP_{max}$ of a section under saturating PPFD |
| **PLA** | cm² | Plant leaf area |
| **PPFD** | µmol photons $m^{-2}$ $s^{-1}$ | Photosynthetic photon flux density |
| **R** | | Respiration |
| **$R_{24h}$** | mmol $CO_2$ $plant^{-1}$ $d^{-1}$ | Respiration per 24h |
| **$R_d$** | nmol $CO_2$ $m^{-2}$ $s^{-1}$ | Dark respiration |
| **$R_{lb}$** | nmol $O_2$ $g^{-1}$ $s^{-1}$ | Respiration rate of leaf bases |
| **$R_{sh}$** | nmol $O_2$ $g^{-1}$ $s^{-1}$ | Respiration rate of the shoot |
| **rDM** | (0 – 1) | Relative DM |
| **$rDM^{dSh}$** | (0 – 1) | Relative DM of dead shoot tissue (related to shoot DM) |
| **RGR** | | Relative growth rate after Kluge & Ting (1978) |
| **rLC** | (0 – 1) | Relative light response curve |
| **rLI** | (0 - 1) | Relative light interception of a plant part (related to PPFD above the plant) |
| **rLL** | (0 – 1) | Relative leaf length (in relation to total length of the leaf) |
| **SLW** | g $cm^{-2}$ | Specific leaf weight |
| **$S_{max}$** | $cm^2$ | Maximal tank water surface of a plant |
| **T** | g $H_2O$ $plant^{-1}$ $d^{-1}$ | Daily transpirational water loss of a plant |
| ***t*** | | Correction factor that accounts for temperature dependent changes in respiration |
| **$t_d$** | (0 – 1) | By adjusting respiration to average day time temperature |
| **$t_n$** | (0 – 1) | By adjusting respiration to average night time temperature |
| **TM** | (0 – 1) | Mean hourly transmission through the tree crown |
| **Tp** | | Temperature |

## *Appendix 5.2 Model parameter estimates*

Variables and parameter estimates as used in the model. Estimates are for plants, covering the entire size range of the tank phase, i.e. DM = 0.1 – 650 g or $LL_{max}$ = 4 – 95 cm. If there is no regression type provided parameter estimates are means ± SD. Also listed are coefficients of determination ($r^2$), sample size (n), significance levels (p), and data origin. In case of piecewise linear regressions two equations are given, one for parameters before and one for parameters after the breakpoint (BP), which is listed in the SD column.

| **Variable** | **Type of equation /** | **Parameter** | | | | **$r^2$** | **n** | **p** | **SD** | **Source** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Specification** | **a / Mean** | **b / SD** | **c** | **d** | | | | | |
| $\alpha$ | after Smith (1937) | 0.66 | | | | 0.98 | 51 | | | This study |
| $\mathbf{A_{proj}}$ | $10^{a \times \log DM + b}$ | 0.85 | 1.49 | | | 0.99 | 10 | < 0.001 | | This study |
| ***c*** | | 0.53 | | | | | | | | Penning de Vries 1975) |
| $\mathbf{C_{act\ 0}}$ | by definition | 100 | | | | | | | | This study |
| $\mathbf{C_{max}}$ | $10^{a \times \log DM + b}$ | 1.06 | 0.50 | | | 0.97 | 11 | < 0.001 | | This study |
| $\boldsymbol{d}_{\mathbf{empty\ n}}$ | | | | | | | | | | |
| n = 1 | $a \times \log DM + b$ | -0.11 | 0.62 | | | 0.99 | 3 | | | Schmidt & Zotz (2001) |
| n = 2 | $a \times \log DM + b$ | -0.08 | 0.38 | | | 0.98 | 3 | | | Schmidt & Zotz (2001) |
| n = 3 | $a \times \log DM + b$ | -0.05 | 0.23 | | | 0.9 | 3 | | | Schmidt & Zotz (2001) |
| n = 4 | $a \times \log DM + b$ | -0.03 | 0.15 | | | 0.76 | 3 | | | Schmidt & Zotz (2001) |
| **DM** | $10^{a \times \log LLmax + b}$ | 2.74 | -2.65 | | | 0.99 | 46 | < 0.001 | | Schmidt & Zotz (2001) |
| **F** | | 0.88 | | | | | | | | Frank & Curtis (1981) |
| ***k*** | | 0.59 | | | | | | | | This study |
| **LA** | $10^{a \times \log LL + b}$ | 1.68 | -0.32 | | | 0.98 | 12 | < 0.001 | | This study |
| $\mathbf{LL_{max}}$ | $10^{a \times \log DM + b}$ | 0.36 | 0.97 | | | 0.99 | 46 | < 0.001 | | Schmidt & Zotz (2001) |
| $\mathbf{nL^{shed}}$ | $10^{a \times \log DM + b}$ | 0.10 | 0.77 | | | 0.39 | 208 | < 0.001 | | This study |
| $\mathbf{NP_{24h}}$ | $a \times NP_{max} + b$ | 17.0 | -5.3 | | | 0.86 | 116 | < 0.001 | | Schmidt & Zotz (2001) |
| $\mathbf{NP_{max}^{sec}}$ | | | | | | | | | | |
| sec = ap | $10^{a \times \log DM + b}$ / $10^{c \times \log DM + d}$ | 0.19 | 0.23 | 0.05 | 0.39 | 0.96 | 8 | | at DM = 24 g | This study |
| sec = ce | $10^{a \times \log DM + b}$ | 0.11 | 0.16 | | | 0.85 | 8 | = 0.001 | | This study |
| sec = ba | | 1.01 | 0.24 | | | | 8 | | | This study |
| $\mathbf{rLL^{sec}}$ | | | | | | | | | | |
| sec = ap | | 50 | | | | | | | | This study |
| sec = ce | | 25 | | | | | | | | This study |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| sec = ba | | 25 | | | | | | This study |
| **PLA** | $10^{a \times \log DM + b}$ | 0.92 | 1.81 | | 0.98 | 37 | < 0.001 | This study |
| **rDM** | | | | | | | | |
| L | $a \times \log DM + b$ | - 0.08 | 0.77 | | 0.98 | 6 | < 0.001 | |
| Lb | $a \times \log DM + b$ | 0.09 | 0.13 | | 0.94 | 6 | < 0.001 | This study |
| Sh | | 25.5 | 5.57 | | | 4 | | This study |
| dSh | | 9.4 | 0.025 | | | 6 | | This study |
| **$R_d$** | after Smith (1937) | 9.3 | | | 0.98 | 51 | | This study |
| **R** | | | | | | | | |
| Lb | $10^{a \times \log DM + b}$ | 0.08 | 0.46 | | 0.7 | 6 | = 0.038 | This study |
| Sh | $10^{a \times \log DM + b}$ | 0.19 | 0.34 | | 0.76 | 6 | = 0.023 | This study |
| **$rLI^{sec}$** | | | | | | | | |
| sec = ap | $a \times \log DM + b$ | 0.05 | 0.77 | | 0.66 | 10 | = 0.004 | This study |
| sec = ce | $a \times \log DM + b$ | 0.08 | 0.37 | | 0.72 | 10 | = 0.002 | This study |
| sec = ba | $a \times \log DM + b$ | 0.05 | 0.12 | | 0.56 | 10 | = 0.013 | This study |
| **$rLL^{shed}$** | | 0.83 | 0.12 | | | 8 | | This study |
| **$rNP_{max}$** | after Smith (1937) | 98.1 | | | 0.98 | 51 | | Schmidt & Zotz (2001) |
| **$SLW^{shed}$** | $10^{a \times \log DM + b}$ | 0.61 | 0.92 | | 0.85 | 8 | < 0.001 | This study |
| **$S_{max}$** | $10^{a \times \log DM + b}$ | 0.70 | 0.73 | | 0.99 | 9 | < 0.001 | This study |
| **T** | $10^{a \times \log DM + b}$ | 0.89 | 0.09 | | 0.99 | 45 | < 0.001 | This study |
| ***t*** | | | | | | | | |
| day | $10^{a \times \log Tp\ day + b}$ | 1.57 | -2.34 | | 0.88 | 12 | < 0.001 | This study |
| night | $10^{a \times \log Tp\ night + b}$ | 1.57 | -2.34 | | 0.88 | 12 | < 0.001 | This study |
| **TM** | mean at 06:00 | 0.39 | 0.251 | | | 48 | | This study |
| | 07:00 | 0.23 | 0.119 | | | 48 | | This study |
| | 08:00 | 0.26 | 0.126 | | | 48 | | This study |
| | 09:00 | 0.28 | 0.149 | | | 48 | | This study |
| | 10:00 | 0.32 | 0.151 | | | 48 | | This study |
| | 11:00 | 0.34 | 0.137 | | | 48 | | This study |
| | 12:00 | 0.41 | 0.149 | | | 48 | | This study |
| | 13:00 | 0.36 | 0.196 | | | 48 | | This study |
| | 14:00 | 0.37 | 0.178 | | | 48 | | This study |
| | 15:00 | 0.37 | 0.163 | | | 46 | | This study |
| | 16:00 | 0.44 | 0.200 | | | 46 | | This study |
| | 17:00 | 0.66 | 0.374 | | | 44 | | This study |
| | 18:00 | 0.40 | 0.045 | | | 16 | | This study |

# PLANT SIZE AND BIOTIC INTERACTIONS – A CASE STUDY OF HERBIVORY IN AN EPIPHYTIC BROMELIAD

## Chapter abstract

Although herbivory in ground-rooted flora is well documented, current knowledge of the herbivore pressure on vascular epiphytes remains mostly anecdotal. Here, the results of a three year study on the herbivory in a population of the epiphytic bromeliad *Vriesea sanguinolenta* are presented. In different years, 26 – 61% of all epiphytes showed traces of herbivore attack, while up to 4.4 % of the entire leaf area of the epiphyte population were consumed annually. Almost all large specimens showed signs of predation but damage was minimal. Conversely, smaller plants were less frequently but more severely affected. The recorded levels of damage to photosynthetic tissue, mostly caused by the larvae of *Napaea eucharilla* (Riodinidae, Lepidoptera), indicate that vascular epiphytes may be regularly and sometimes even lethally attacked by insect herbivores. The level of damage was comparable to ground-rooted tropical flora, which certainly does not support the prevalent notion of low and negligible levels of herbivory in vascular epiphytes.

## Introduction

While soil-rooted plants have been extensively studied with respect to plant-herbivore interactions (e.g. Barbosa & Schultz 1987; Howe & Westley 1988; Schoonhoven et al. 1998), little information is available on herbivory among epiphytes in situ, despite a recent increase in interest in this group (e.g. Lüttge 1989; Benzing 1990). Researchers working on the demography of epiphytes rarely report leaf damage or mortality due to herbivore activity (e.g. Benzing 1981; Larson 1992; Hietz 1997; Hietz & Ausserer 1998). Other authors discuss the possibility of protection against herbivores by associated ants without actual evidence of herbivore attack on their study organisms (Fisher & Zimmerman 1988; Fisher et al. 1990). It thus remains an open question, whether signs of herbivory were indeed lacking or if these authors considered it too irrelevant to be mentioned.

Some authors, however, do report herbivory among vascular epiphytes. Benzing (1990), for example, observed that signs of predation are common among some epiphytic taxa, but considered extensive defoliation as rare in neotropical epiphytes. In conjunction with ant-epiphyte associations Janzen (1974) and Huxley (1978) mentioned leaf-consuming insects on *Hydnophytum*, Koptur et al. (1998) demonstrated that ants visiting nectaries on *Polypodium plebeium* Schldl. & Cham. protected the fern against foliage-feeding sawfly and lepidopteran caterpillars and from work of Dejean et al. (1992) we know that epiphytes are a potential foraging target for leaf cutter ants. Damage caused by leaf miners (*Scelonopla* sp. and *Octotoma* sp.) was observed on *Guzmania sp.* (D. Windsor in Zotz & Andrade 2000) or occasionally on *Tillandsia fasciculata* Sw. (M. Matzat, pers. comm.). On other bromeliads, i.e. *Tillandsia* spp. (Rauh 1990) and *Tillandsia ionantha* Planchon (García-Franco & Rico-Gray 1992) root galls were found. The latter authors reported the highest infestation frequencies for intermediate plant sizes. In a census of more than 300 individuals of *Dimerandra emarginata* (G. Meyer) Hoehne (Orchidaceae) Zotz (1998) found only three plants showing signs of herbivory. The only herbivore found was *Cremna thasus* Stichel (Riodinidae). Data are also available from Lowman et al. (1996 & 1999): they sampled five plants in 1995 and 1997 and found that 10.4 % and 9 %, respectively, of the leaf area of *Aechmea nallyi* L.B. Smith were mined or missing.

In summary, most of the above reports describe herbivory as an anecdotal event, and rarely exceed an observational level. In spite of the dearth of actual data, the described accounts have led to the assumption that herbivory - in general - has a negligible impact on individual epiphytes as well as on epiphyte populations. The aim of the present work is to challenge this notion with a quantitative evaluation of herbivory levels in an epiphyte population over three years. It is proposed that herbivory among vascular epiphytes is more common and probably more important than currently acknowledged.

## Methods

### *Study site*

The study was carried out in the Barro Colorado Nature Monument (9°10´N, 79°51´W), Republic of Panama. The forest of this biological reserve is classified as a tropical moist forest (Holdridge et al. 1971). Mean annual rainfall is c. 2600 kg $m^{-2}$ with a pronounced dry season from late December to late April (Windsor 1990). Detailed descriptions of vegetation, climate and ecology are reported by Croat (1978) and Leigh et al. (1982).

### *Study species*

**Phorophyte**. *Annona glabra* L. (Annonaceae) is a flood-tolerant, evergreen tree with a sparse canopy and a maximum height of c. 8 m. It occurs in the New World from the southern United States to the Amazon, in the Old World in parts of Africa and has been introduced recently to Australia (Croat 1978; Swarbrick & Skarratt 1994). In the Canal Area, *A. glabra* is restricted to marshes around islands and the mainland. It frequently supports a large epiphytic load (Croat 1978; Zotz et al. 1999a).

**Epiphyte**. *Vriesea sanguinolenta* Cogn. & Marchal (Bromeliaceae, syn. *Werauhia sanguinolenta* Cogn. & Marchal; Grant 1995) occurs from Costa Rica to Colombia and on various Caribbean islands from lowland up to lower montane wet forests (Croat 1978). Its growth habit is best characterized as a water impounding multi-tank rosette (Benzing 1980). *Vriesea sanguinolenta* colonizes many different host trees, but is particularly abundant on *A. glabra* (Zotz et al. 1999a). Maximal leaf length ($LL_{max}$) as a measure of plant size was used throughout the study. This parameter correlated closely with the plant leaf area (PLA) of the corresponding individual: PLA ($cm^2$) = $10^{-0.55 + 2.45 \times \log LLmax\,(cm)}$, $r^2 = 0.99$, $p < 0.001$, $n = 55$). I distinguished size categories (SC) that correspond to the ontogenetic stages: a) atmospheric, non-impounding, b) tank-forming, vegetative, c) tank-forming, reproductive. To subdivide the large category of non-reproducing tank bromeliads I applied the procedure suggested by Vandermeer (1978), which resulted in a total of six size categories (Table 6.1). For SC 1, reliable herbivory data were difficult to obtain in the field: the atmospheric stage was thus excluded from further analysis.

**Table 6.1.** Classification of size categories after ontogenetic criteria and maximal leaf length ($LL_{max}$, in cm) as measure of plant size.

| **size category** | **Size range ($LL_{max}$, cm)** | **ontogenetic characteristics** |
|---|---|---|
| 1 | 0 – 2.0 | atmospheric |
| 2 | 2.1 – 7.0 | tank-forming, vegetative |
| 3 | 7.1 – 13.0 | tank-forming, vegetative |
| 4 | 13.1 – 25.0 | tank-forming, vegetative |
| 5 | 25.1 – 60 | tank-forming, vegetative |
| 6 | > 60 | tank-forming, reproductive |

**Herbivore.** *Napaea eucharilla* Bates (Riodinidae, Lepidoptera) ranges from Mexico to Brazil (DeVries 1997). Known host plants of this oligophagous lepidopteran encompass various bromeliads, such as *Ananas comosus*, *Aechmea* spp., *Vriesea* spp., and *Guzmania* spp. (DeVries 1997) stated that larvae graze on the underside of the leaf, leaving the upper epidermis intact to form windows. In contrast, own observations indicate that *N. eucharilla* often feeds on the upper leaf side as well. The characteristic feeding habit facilitated the quantification of herbivore damage. More than 95% of the total amount of recorded leaf area consumption could unequivocally be attributed to feeding of *N. eucharilla*, while the remaining leaf damage is of unknown origin. For all data analysis total herbivory was used.

### *Demographic studies*

In 1997, 31 plots with a total of 295 specimens of *V. sanguinolenta* were set up in an area of c.1 ha. Each plot consisted of branch sections (1 – 2.5 m in length) within the crown of *A. glabra*, sometimes with more than one plot per tree. Herbivory was recorded at the end of every dry season from 1997 to 1999. Briefly, length and width of the window-like damage were estimated. Adding the calculated areas of each patch yielded an estimate of damaged leaf area per plant. In almost all cases, the entire set of leaves is replaced within a year (Chapter 4). Leaf longevity is thus about one year, and the estimated damage reflects the herbivore activity on *V. sanguinolenta* during the preceding year. Plants that died between two observation intervals were replaced by plants of comparable size. The death of plants between two observations was assumed to be related to herbivore activity when damage exceeded 70% PLA.

In order to quantify the leaf area that is potentially consumed during the larval stage of *N. eucharilla*, the leaf part on which a female adult just oviposited was transferred into a petri dish and kept in the shade. The emerging larvae were fed with leaf pieces of *V. sanguinolenta* (SC 5), which were replaced and analyzed for consumed leaf area daily.

## Results

### *Herbivory on the population level*

During the observation period from 1997 to 1999, the size category distribution remained constant (Figure 6.1, open bars). Likewise, the frequency pattern of plants being attacked (irrespective of the amount of damage) was qualitatively similar among years: plants in the smaller size categories (SC 2 & 3) were rarely affected, while every individual in SC 6 was damaged (Figure 6.1, gray bars). The proportion of plants with signs of herbivory ranged from 26 to 61% for the respective years (Table 6.2).

**Table 6.2** Herbivory characteristics from 1997 to 1999: given are proportions of plants which show signs of herbivory (in % of all individuals in the specified group) and the consumed leaf area related to the total leaf area of a given set of *V. sanguinolenta* (in % of total leaf area).

| | **1997** | **1998** | **1999** |
|---|---|---|---|
| Attacked plants in the entire population (%) | 26.3 | 29.9 | 60.8 |
| Attacked plants in the subpopulation (%) | 16.1 | 63.3 | 27.8 |
| Consumed leaf area in the entire population (%) | 2.4 | 1.7 | 4.4 |
| Consumed leaf area in the subpopulation (%) | 2.2 | 18.4 | 5.5 |

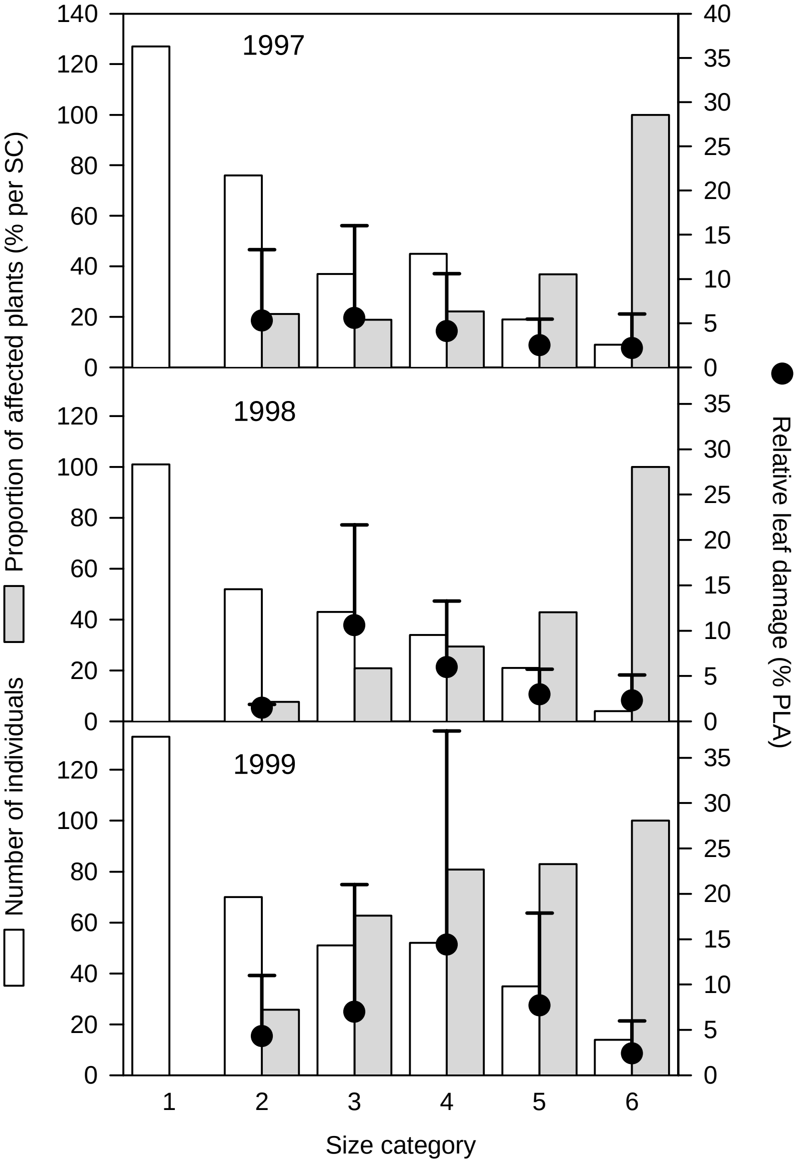

**Figure 6.1** Size category frequencies and herbivory characteristics in the entire population of *V. sanguinolenta* within the plot area over three consecutive years. Given are the absolute numbers of individuals within the respective SC (open bars), the percentage of plants showing signs of herbivory, irrespective of the amount of damage (in %; gray bars) and the relative leaf damage of individual plants as the mean ± SD of all specimen within a SC (in % PLA; circles with error bars). Note that damage in SC 1 was not determined.

Affected individuals experienced highly variable relative leaf damage (in % PLA), and thus, the mean relative leaf damages for plants within the respective size categories were also subject to a high variability (Figure 6.1, circles with error bars). During the three years of observations no obvious frequency pattern of relative leaf damage among the different size categories emerged, but variability seemed to be lower in larger plants (SC 5 & 6, compare Figure 6.1). The mean annual leaf area consumed per plant ranged from 4.1 to 8.4% for the respective years (Figure 6.2).

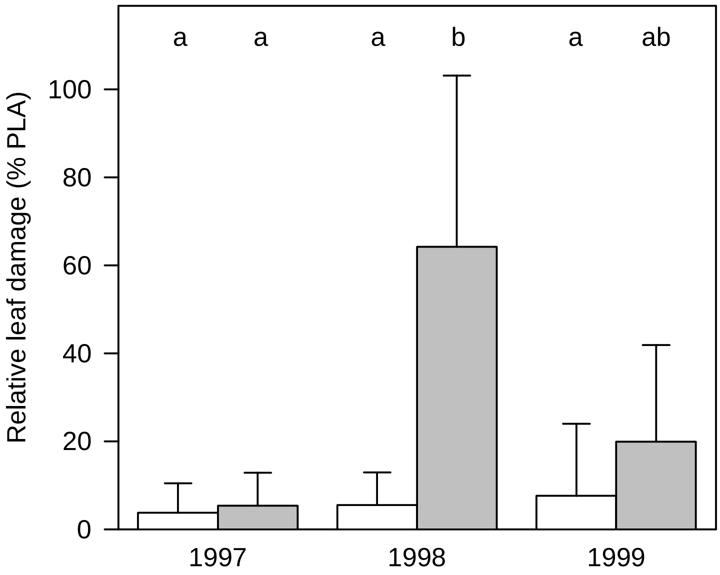

**Figure 6.2** Relative leaf damage (in % of PLA) as annual means ± SD for the entire population (open bars) and the subpopulation (gray bars). Significant differences between groups are indicated with different letters ($p < 0.05$, one-way ANOVA, Scheffé posthoc test). Data were log-transformed prior to analysis.

The loss of photosynthetic tissue due to herbivores can also be related to the total leaf area offered by a set of *V. sanguinolenta* (= leaf area related damage; in % of the total leaf area). As shown in Figure 6.3, the amount of consumed leaf area per SC was a function of the leaf area offered by *V. sanguinolenta* within the respective category. On the population level the annual leaf area related damage varied between 1.7 and 4.4% (Table 6.2). Annual mortality due to herbivore activity was 2.6% from 1997 to 1998 and 1.8% from 1998 to 1999.

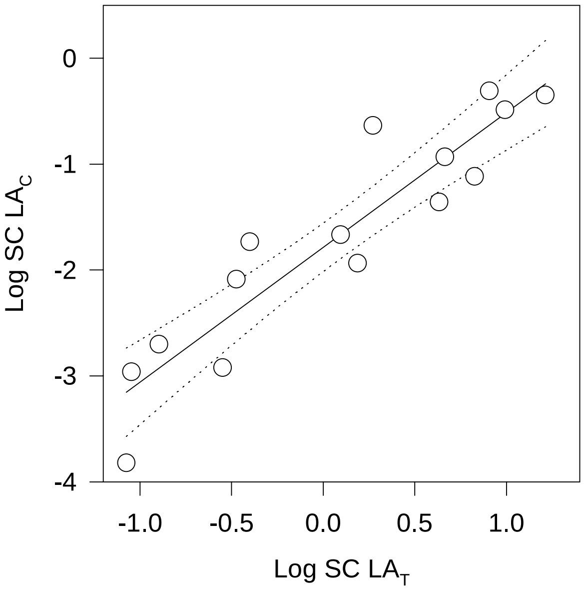

**Figure 6.3** Relationship of total leaf area (SC $LA_T$) offered by the epiphyte *V. sanguinolenta* within the same size category (SC) and consumed leaf area within a size category (SC $LA_C$). Each circle symbolizes the mean value of all individuals within a SC. Given are regression line (solid) and 95% confidence intervals (dotted). The linear regression is: log SC $LA_C$ = 1.28 log SC PLA – 1.79, $r^2 = 0.86$, $p < 0.001$, $n = 15$.

*Outbreak-like situation in a subpopulation*

During the 1998 census, the subpopulation of *V. sanguinolenta* on one phorophyte (represented by two non-adjacent plots, n = 32 plants) showed outstandingly high herbivore presence. Leaf damage was observed on 63% of all *V. sanguinolenta*. Even in SC 2 the proportion of plants affected was 50% (Figure 6.4).

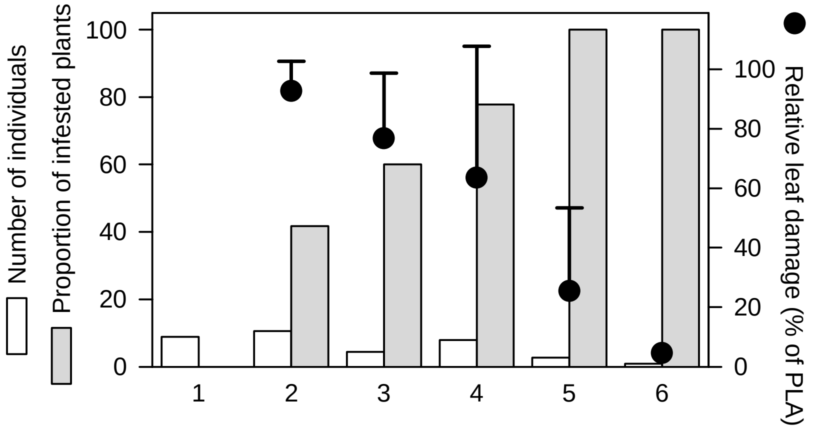

**Figure 6.4** Size category frequencies and herbivory characteristics in a sub-population of *V. sanguinolenta* in 1998. Given are the absolute numbers of individuals within the respective SC (open bars), the percentage of plants showing signs of herbivory, irrespective of the amount of damage (in %, gray bars) and relative leaf damage of individual plants as the mean ± SD of all specimen within a SC (in % PLA; circles with error bars).

Once attacked, small individuals of size categories 2 to 4 were almost always completely defoliated, while large conspecifics (SC 5 & 6) lost less than 20% PLA (Figure 6.4). Mean annually consumed leaf area per plant across the subpopulation was significantly increased in 1998 (64.2%), whereas the values for 1997 and 1999 remained at the level of the entire population (Figure 6.3).

In contrast to the results for the entire population, the total leaf area offered by plants constituting the six size categories could not predict the amount of consumed leaf area (Pearson product-moment correlation, $r^2 = 0.64$, $p = 0.10$). Presumably as a consequence of this considerable damage, 65% of the infested plants, or 50% of all individuals in the subpopulation, died before the next census in 1999.

### *The main herbivore*

More than 95% of all leaf damage could be attributed to feeding of the caterpillar of *Napaea eucharilla* (Riodinidae, Lepidoptera). Adults were observed ovipositing clusters of up to seven separate eggs, from which small, c. 2 mm long, larvae hatched after two weeks. Larval development, involving four to five instars required 45 to 55 days, followed by a two-week pupation. Due to high larval mortality, these and the following data are based on just three individuals and should be taken as preliminary. From hatching to pupation, larvae consumed 61 ± 20 $cm^2$ (mean ± SD) of leaf area. Comparing this nutritional need with PLA suggests that the minimum plant size for a larva of *N. eucharilla* to complete its development would be 7.5 cm $LL_{max}$. This suggests that larvae feeding on smaller plants, for example on plants of SC 2, must either switch host plants, will fail to develop fully, i.e. they die, or they are more flexible in their feeding behavior than the initial observations indicate. Larvae feeding on large bromeliads such as SC 6 will not have to change host plants. The estimated number of caterpillars per plant of SC 6 was 5.4 ± 0.4 $a^{-1}$ (total number of plants = 28).

## Discussion

Between one and two thirds of all individuals of *V. sanguinolenta* were affected by herbivory. Annual loss of photosynthetic tissue due to herbivore activity on the population level ranged from 1.7 to 4.4% of the total leaf area with a mean herbivore induced mortality rate of 2.2% $a^{-1}$. Leaf damage in soil-rooted plants in the tropics ranges from 0.0003 to 0.8% $d^{-1}$ (Coley et al. 1985). Transformed to daily rates, *V. sanguinolenta* suffered 0.007 to 0.012% damage, which does certainly not support the notion of low and negligible levels of herbivory in vascular epiphytes. This is even more so, when focusing on the herbivore damage in the subpopulation on one phorophyte in 1998. About two thirds of herbivore-infested individuals (Table 6.2) and an annual consumption rate of more than 18% of the total leaf area (Table 6.2) killed 50% of all *V. sanguinolenta* in the population of this tree.

Using mean leaf area consumption during the larval development and observed leaf damage allows a rough estimate of herbivore abundance. For the entire epiphyte population 0.6 ± 0.27 (mean ± SD, n = 3 years) caterpillars of *N. eucharilla* per plant are obtained. During the peak abundance in the 1998 outbreak in a subpopulation 1.8 herbivores per plant were reached. According to Berryman (1987) an outbreak can be defined as an explosive increase in the abundance of a particular species that occurs over a relatively brief interval. Moreover, outbreak species have to defoliate plants in order to be categorized as such. Although the overall ecology of *N. eucharilla* does not justify classification as an outbreak species, the high plant mortality rates and the strongly increased herbivore abundance suggests that outbreak-like situations can develop locally.

In summary, *V. sanguinolenta* is regularly and sometimes even lethally attacked by an insect herbivore at the study site. Whether or not this report is exceptional for vascular epiphytes is unclear in view of the lack of pertinent data from other studies. Preliminary results from another epiphyte (unpubl. data) indicate similar herbivory levels: almost 75% of 221 individuals of the understory epiphyte *Aspasia principissa* Reichb.f. (Orchidaceae) showed signs of herbivory. Among affected plants the relative leaf damage averaged 4.1% ± 9.0 (mean ± SD, n = 162 plants). A total of 2.8% of the entire leaf area of the epiphyte population was consumed. Although damage could not be attributed to a specific herbivore, these data corroborate the hypothesis that herbivory among epiphytes is more widespread and probably more important than previously thought.

The expectation of an overall low herbivore pressure seems mostly due to ecological theory, which predicts that resource-poor habitats (such as the epiphytic habitat on *A. glabra*) will coincide with low nutrient content in the tissues of inhabiting plants, leading to weak photosynthetic performance and slow inherent growth. Long-lived leaves are well protected by a high investment in defense metabolites (Coley et al. 1985). In soil-rooted trees, which have access to better resources, the opposite should apply. Herbivores should thus prefer the softer and more nutritious leaves of the phorophyte over the low-quality food of epiphyte leaves. This general scenario does not seem to be valid in the *Annona* – *Vriesea* system. Although the leaf nitrogen content of *A. glabra* (2.6% ± 0.1, mean ± SD, n = 3; unpubl. data) is indeed five times higher than in *V. sanguinolenta* (0.53% ± 0.2, mean ± SD, n = 14, (Schmidt et al. 2001), herbivory levels of the host tree appear to be low (pers. observ.).

However, this statement must be considered preliminary without actual quantification of the herbivory levels on the host tree.

In conclusion, epiphyte-herbivore relationships are clearly an under-studied area of epiphyte ecology. In this report it was shown, that herbivore pressure can be substantial, at least in certain years and in some species such as *V. sanguinolenta*. To date, however, the lack of comparable data for other species of vascular epiphytes precludes generalizations. Presently, the results of this study are used as the basis for further investigations: (1) experimental studies which address the impact of *Napaea* on the population dynamics of *V. sanguinolenta* and (2) a detailed assessment of the effect of herbivory on the physiology of this bromeliad..

# WHAT IS THE DRIVING FORCE BEHIND THE OBSERVED PHENOMENON OF SIZE-DEPENDENT ECOPHYSIOLOGICAL CHANGES IN VASCULAR EPIPHYTES?

## Chapter abstract

Recent reports have demonstrated that physiological properties in vascular epiphytes scale with plant size. The objective of this paper was to test if (1) water availability (2) nutrient availability or (3) age or developmental stage are the driving force behind the observed phenomenon. Since water and nutrient uptake are coupled in nature I chose a multiple experimental approach, and found that a) different from larger conspecifics, small individuals of two epiphytic orchid species responded in situ to improved water availability (natural and experimental shading) with a significant increase in both photosynthetic capacity (PC) and leaf nitrogen content, b) stored nitrogen pools in the stems of the studied orchid were large enough to provide a possible explanation for these size-related changes in leaf N, and c) under controlled greenhouse conditions, PC, leaf N, and relative growth rates were significantly higher in well-watered plants compared to poorly watered conspecifics, irrespective of nutrient supply.

The profound physiological response of the studied epiphytes to resource availability precludes the possibility that the “size effect” is an “age effect”. Similarly, the results make nutrient limitation in smaller plants an unlikely explanation. This suggests that differences in water availability in smaller and larger conspecifics govern the magnitude of the investments in photosynthesis and other functions, thus explaining the observed size-dependent variation in physiological parameters.

## Introduction

Recent studies with vascular epiphytes have shown that plant size may be an important source of intraspecific variation of the physiological characteristics of plant organs. For example, Zotz (1997a) reported a continuous size-related increase in both leaf nitrogen content (leaf N) and photosynthetic capacity (PC) measured with the oxygen electrode under non-limiting conditions in the epiphytic orchid *Dimerandra emarginata*. For the same species, a continuous increase in carbon isotope discrimination from smaller to larger plants was found (Zotz & Ziegler 1999), indicating consistently decreasing stomatal limitations with increasing plant size. That this size-dependency of certain physiological parameters is not restricted to an exceptional species was shown by Schmidt et al. (2001), who observed similar increases in leaf N and PC in eight of nine other species, including all major epiphyte taxa, i.e. orchids, bromeliads, aroids and ferns. Evaluating the relevance of these findings for the comportment under natural conditions, Schmidt and Zotz (2000a) measured the in situ $CO_2$ exchange of leaves of differently sized *Vriesea sanguinolenta* (Bromeliaceae): under well-watered conditions both maximal rates of net photosynthesis and diurnal carbon gain increased with plant size.

At the level of entire plants, Zotz & Thomas (1999) and Schmidt & Zotz (2000a) showed for three epiphytic tank bromeliads that the efficiency of externally stored water in bridging rainless periods also scaled with plant size, i.e. water availability correlates positively with plant size even in so-called ‘continuously supplied’ species (sensu Benzing 1990). As a corollary, internal water storage capacities, as indicated by the thickness of leaf hydrenchymas, decreased with plant size in *V. sanguinolenta* (Schmidt & Zotz 2000a), indicating a shift in water storing strategies. The latter authors also showed that stomatal regulation differs with plant size: upon tank water depletion, small plants (compared to larger conspecifics) reacted to further water shortage with a less severe restriction of stomatal conductance, allowing larger fluctuations in tissue water content but prolonging the period of active carbon fixation. Finally, a field study on the abscisic acid metabolism of *D. emarginata* during drought cycles (Zotz et al., submitted) revealed pronounced and consistent differences in root hormone levels and xylem hormone transport in small, medium-sized and larger plants.

In summary, size-related differences in ecophysiological properties of both plant organs and entire plants are consistently observed in vascular epiphytes, and are also relevant in situ. Thus, the question arises: what is the mechanism behind the observed size-dependent differences? One plausible hypothesis was first proposed by Zotz (1997a): bark epiphytes cannot use soil as a vast external water reservoir, causing water stress even after very short rainless periods (Benzing 1990). Changes in surface/volume ratios, i.e. the ratio of leaf surfaces to plant water content (Zotz 1997a; Zotz & Andrade 1998; Schmidt et al. 2001) should therefore substantially affect plant water relations: under identical microclimate conditions small plants will deplete their internal water reservoirs much earlier than larger conspecifics. The optimal partitioning theory (Bloom et al. 1985) predicts that plants respond to limiting environmental factors in such a way as to make all resources equally limiting. Analogously, the functional convergence hypothesis (Mooney & Gulmon 1979) concludes that the biochemical capacity for $CO_2$ fixation should be curtailed whenever a limitation in the availability of any resource prevents the efficient exploitation of additional capacity. Assuming an earlier onset of stomatal closure in smaller plants (compared to larger conspecifics) due to a less favorable water status, long-term carbon gain will be more restricted. Additional investment of important resources such as nitrogen into the photosynthetic apparatus, i.e. in comparatively short lived organs, will not adequately increase productivity. Because nitrogen is in short supply in most plant communities (Chapin 1980) plants should invest only as much nitrogen into their photosynthetic apparatus as needed given other limitations. Data on leaf N in differently sized conspecifics support this notion: smaller plants exhibit lower concentrations of leaf N compared to their larger conspecifics (Zotz 1997a; Zotz & Andrade 1998; Schmidt et al. 2001). Given the well documented correlation between leaf N and PC (e.g. Field & Mooney 1986; Evans 1989) a size-related change in PC is expected. Although the quantification of key parameters (surface/volume ratio, leaf N, PC) already supports this hypothesis, alternative explanations cannot be ruled out. For example, the observed size-related changes could also be due to nutrient accumulation in the plant body during ontogeny. Generally, the epiphytic habitat is categorized as both drought-prone and nutrient poor (Benzing 1990). If productivity was mainly nutrient limited small plants might not have had the time to take up and accumulate sufficient amounts of nutrients. As a third possibility the “size effect” could also be an effect of age or developmental stage.

This question was addressed by using three different approaches: (1) a comparison of ecophysiological parameters of individuals from shaded versus exposed sites, (2) the quantification of nitrogen pools in different plant organs and (3) by altering water and nutrient supply for plants under controlled greenhouse conditions.

Epiphytic habitats in the upper canopy are characterized by intense radiation, associated with elevated leaf and substrate temperatures and low relative humidity. In addition, these growing sites are subject to strong winds, which also contributes to an overall high evaporational demand for the plants living in such an environment (Benzing 1990). Conversely, conspecifics growing in more shaded sites will experience water deficiencies less frequently and less severely (Pittendrigh 1948; Benzing 1990). This can, in turn, influence photosynthetic performance both in the short term (by stomatal regulation) and in the long term (by acclimatization; Jones 1973). Clearly, other factors such as long-term light conditions will also influence photosynthetic performance: individuals growing under continuous low light conditions frequently exhibit lower rates of photosynthesis than conspecifics growing more exposed (e.g. Björkman & Holmgren 1966; Björkman 1981; Ögren & Evans 1993). For the comparison of shaded versus exposed sites it was hypothesized that water availability would be of superior importance in smaller individuals, where higher PCs were expected in the shaded site due to lower evaporational demand. For larger specimens, in contrast, I anticipated an acclimation to prevailing light conditions.

Secondly, size-dependent variation in nitrogen pools in leaves, stems and roots of an orchid species were quantified: if nitrogen pools in stems were large enough to account for the size-dependent increase in leaf N (compare Schmidt et al. 2001) it would be unlikely that smaller plants are nitrogen limited.

Finally, a greenhouse experiment was conducted with epiphytes grown under different nutrient and water supply. According to the above line of reasoning only water supply should have a strong effect on PC and leaf N.

## Materials and Methods

### *Site comparison*

Field work was carried out in the Barro Colorado Nature Monument (BCNM; 9°10´N, 79°51´W), Republic of Panama. The tropical moist forest (Holdridge et al. 1971) of this biological reserve in and around Lake Gatun receives about 2600 kg $m^{-2}$ annual rainfall with a pronounced dry season from late December to late April (Windsor 1990). Detailed descriptions of vegetation, climate and ecology are reported by Croat (1978) and Leigh et al. (1982).

The first study species, *Polystachya foliosa* (Lindl.) Reichb.f. (Orchidaceae), occurs from Mexico through South America inhabiting mid-level growing sites as well as exposed branches in the upper canopy and along the shore line (Croat 1978; Zotz & Ziegler 1997). Two populations of, respectively, 11 and 13 individuals were chosen, growing close to the lake on *Annona glabra* L. (Annonaceae) and on a branch of *Anacardium excelsum* (Bertero & Balb.) Skeels (Anacardiaceae). The former is a small flood-tolerant, evergreen tree with a sparse canopy, while the latter is a large species with a dense crown. Microclimate conditions in *A. glabra* were: PPFD (as daily integrals; n = 32 days): 9 to 27 mol $m^{-2}$ $d^{-1}$, average transmission through the canopy (mean ± SD, n = 32 days): 45 % ± 5.9, air temperature (as daily means; n = 14 days): 25.6 to 30.7 °C, relative humidity (as daily minimum; n = 14 days): 65 to 78 %. Since the *Anacardium* branch was only about 1.5 m above the lake surface I assume quite similar microclimate conditions in terms of relative humidity, air temperatures, while measured PPFD was much lower (as daily integrals; n = 5 days): 1.5 to 2.6 mol $m^{-2}$ $d^{-1}$, representing a transmission through the crown of 4.5 %, ± 0.5 (mean ± SD, n = 5 days). For all samples PC and leaf N were determined in the 1996 rainy season.

### *Artificial shading in a natural habitat*

A manipulative field study was conducted with *Dimerandra emarginata* (G. Meyer) Hoehne, which is mainly found at rather exposed sites in BCNM. The treatment comparison was conducted on individuals growing in *A. glabra*. For repeated measurements I chose the length of the most recent stem (stem length, SL, cm) as a non-destructive measure of plant size, which closely correlates with plant dry mass (DM, g), a more rigorous measure for size: $DM = 0.0071 \times SL^2 + 0.1254 \times SL$, $r^2 = 0.84$, n = 36.

For a comparison 28 pairs of *D. emarginata* were chosen, growing next to each other within the crown of two adjacent phorophytes. Pairs (of similar size) covered the entire size range of the species. Morphometric data were taken for all specimens at the end of the 1998 dry season. Of each pair, one was covered with shade cloth, including its roots. Microclimate conditions determined for the exposed and the shaded group are listed in Table 7.1. Potential evaporation was measured using an evapotranspiration gauge (Model A, ETgage Company, Loveland, Colorado, USA). After exposure to treatment conditions for one year, the most recent stem (1998) was collected in the 1999 dry season and transferred to the lab in a moist zip-lock bag, where PC, leaf N, and SL were determined.

**Table 7.1** Treatments in the ‘artificial shading’ – experiment. Microclimate parameters for sites with (shaded) or without (exposed) artificial shading were recorded on, respectively, 16 days (PPFD) and on 30 days (evaporation) in the 1999 dry season. Values for transmission are means ± SD.

| **Microclimate parameter** | **Shaded** | **Exposed** |
|---|---|---|
| Range of daily integrated PPFD (mol $m^{-2}$ $d^{-1}$) | 5 - 9 | 14 - 19 |
| Transmission (% of PPFD above the canopy) | 16 ± 8 | 39 ± 15 |
| Evaporation over 30 days (kg $m^{-2}$) | 77 | 113 |
| Relative evaporation (% of evaporation above the canopy) | 48 | 70 |

### *Controlled environment*

*Dimerandra emarginata* plants of similar size (SL = 12 - 19 cm) were collected in the BCNM, Panama, transferred to Würzburg and potted in “Blähton”. After an acclimation phase of several months in the greenhouse under identical conditions (Table 7.3), plants were randomly assigned to different water and fertilizer regimes. Arrangement of plants on the greenhouse bench was altered several times during the course of the experiment to avoid influences of possible microclimatic small-scale differences in the greenhouse (mean daily PPFD: c. 15 mol $m^{-2}$ $s^{-1}$, air temperature 24 to 26°C, relative humidity: 60 to 80 %). At the start of the experiment (December 1997) and after one year samples were taken for determination of leaf N, PC and relative growth rate.

### *Oxygen evolution, leaf nitrogen content and relative growth rate*

Oxygen exchange was measured in the laboratory using a LD2 Leaf Disc Electrode (Hansatech Ltd., King's Lynn, UK). Freshly punched leaf discs from the youngest fully expanded leaves were enclosed in the cuvette of the leaf disc electrode unit (kept at 30°C using a circulating water bath). Subsequently, the cuvette was charged with c. 5% $CO_2$. After calibration, the leaf discs were induced in six light-dark cycles of 5 min (c. 550 µmol $m^{-2}$ $s^{-1}$) and 5 min (0 µmol $m^{-2}$ $s^{-1}$). Previously, saturating but not inhibiting light conditions had been determined for each species by studying the response of $O_2$ evolution to PPFDs of up to 1800 µmol $m^{-2}$ $s^{-1}$ with specimens of intermediate size. The actual measurement of maximal $O_2$ evolution (defined as photosynthetic capacity, PC) was then determined at 1000 - 1200 µmol $m^{-2}$ $s^{-1}$ PPFD. Samples were subsequently dried at 60°C for 5 to 7 days, weighed and nitrogen content was determined at the University of Würzburg using a CHN-O Element Analyzer (Heraeus, Hanau, Germany). To quantify dry mass and nitrogen content of different plant parts, 12 *D. emarginata* plants were carefully removed from the bark surface with forceps. Subsequently, plants were divided into (1) all leaves of the most recent stem, (2) leaves of backshoots, (3) stems, (4) roots, and (5) fruits. Plant parts were dried at 60°C for at least one week, weighed and analyzed for N content. Older leaves and fruits were not used in the further analysis. Relative growth rate ($RGR_{SL}$, in cm $cm^{-1}$ $a^{-1}$) was defined as $\Delta SL / SL_{year\ n-1}$, with $\Delta SL$ = increase of stem length (cm) in two subsequent years and $SL_{year\ n-1}$ = stem length (cm) of the preceding year.

### *Data analysis*

All results were statistically analyzed with STATISTICA software (STATISTICA 5.1, StatSoft Inc. Tulsa, Oklahoma, USA). Whenever data were not normally distributed a log-transformation was performed prior to analysis. Nevertheless data are depicted unlogged for reasons of clarity .

## **Results**

### *Site comparison*

Both the orchid population in *Anacardium excelsum*, representing a shaded site, and the one growing in the light crown of *Annona glabra* showed significant increases of area related PC ($PC_{LA}$) with plant size (Figure 7.1). The slopes of the regressions, however, were significantly different ($p < 0.05$). The increase in $PC_{LA}$ from smallest to largest individual was only 75% in the shaded site, whereas changes in $PC_{LA}$ for plants at the exposed site were much more pronounced, representing a 320% increase for the same size range. Regression lines intercepted at stem length (SL) = 3 cm (Figure 7.1). When expressed on a dry mass basis ($PC_{DM}$), photosynthetic capacity scaled with plant size in specimens at the exposed site ($PC_{DM} = 10^{0.24 \times \log SL + 2.23}$, $r^2 = 0.33$, $p = 0.031$, $n = 13$) but not at the shaded site ($p = 0.49$, $n = 11$). Analyzing only specimens smaller than 2 cm SL, i.e. individuals evidently smaller than those at the intercept of the regression lines, area and dry mass related PC were > 50% higher at the shaded than at the exposed site (Table 7.2). Leaf N of the shade plants was elevated roughly proportionally (Table 7.2).

**Table 7.2** Comparison of small individuals from shaded versus exposed sites. Listed are results for *P. foliosa* (SL < 2 cm) growing in *A. excelsum* (naturally shaded) or in *A. glabra* (naturally exposed). Also given are results for *D. emarginata* (SL < 5 cm) in *A. glabra*, with (shaded) or without (exposed) artificial shading. Values for photosynthetic capacity (PC), leaf N and relative growth rate ($RGR_{SL}$) are means ± SD. For statistical analysis I employed a t-test for independent samples, listing degrees of freedom (df) and significance values (p).

| **Species** | **Parameter** | **Shaded** | **Exposed** | **df** | **p** |
|---|---|---|---|---|---|
| *P. foliosa* | $PC_{LA}$ (µmol $m^{-2}$ $s^{-1}$) | 8.2 ± 1.3 | 5.4 ± 1.7 | 8 | = 0.017 |
| | $PC_{DM}$ (nmol $g^{-1}$ $s^{-1}$) | 319 ± 25 | 185 ± 69 | 8 | = 0.003 |
| | Leaf N (%) | 1.93 ± 0.16 | 1.43 ± 0.34 | 8 | = 0.018 |
| *D. emarginata* | $PC_{LA}$ (µmol $m^{-2}$ $s^{-1}$) | 3.9 ± 0.5 | 3.2 ± 0.5 | 13 | = 0.019 |
| | $PC_{DM}$ (nmol $g^{-1}$ $s^{-1}$) | 42.6 ± 5.4 | 28.8 ± 6.7 | 13 | = 0.002 |
| | Leaf N (%) | 1.12 ± 0.12 | 0.98 ± 0.10 | 13 | = 0.023 |
| | $RGR_{SL}$ (cm $cm^{-1}$ $a^{-1}$) | 0.77 ± 0.32 | 0.38 ± 0.31 | 18 | = 0.015 |

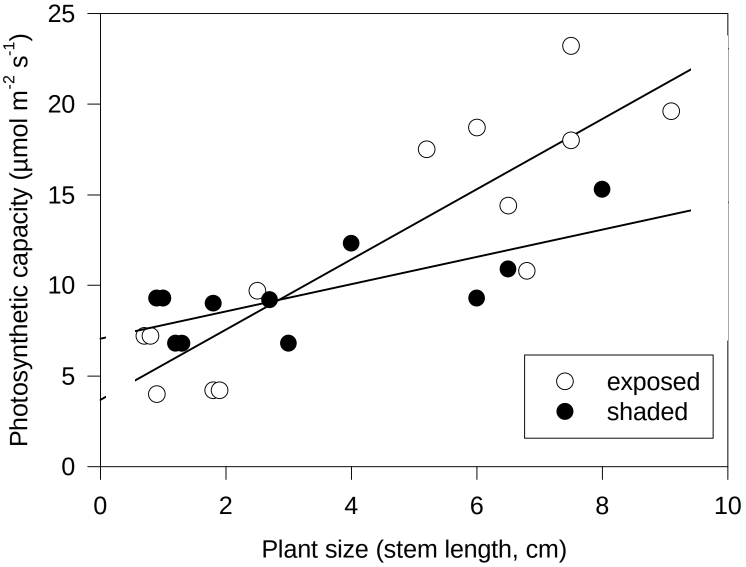

**Figure 7.1** Area based Photosynthetic capacity as a function of plant size for *P. foliosa*, growing in *Anacardium excelsum* (shaded; closed circles) and in *Annona glabra* (exposed; open circles). Each symbol represents a different plant. Unlogged data are depicted for illustrative purposes. Regressions were, for the shade group: $PC_{LA} = 10^{0.20 \times \log SL + 0.89}$, $r^2 = 0.39$, $p = 0.041$, $n = 11$ and for the exposed group: $PC_{LA} = 10^{0.56 \times \log SL + 0.74}$, $r^2 = 0.68$, $p < 0.001$, $n = 13$.

### *Artificial shading in a natural habitat*

Irrespective of treatment (shaded or exposed) $PC_{LA}$ scaled with plant size in *D. emarginata* (Figure 7.2A). Again, the slope of the regression was significantly steeper ($p < 0.05$) in the exposed individuals, while regression lines intercepted at SL = 8.5 cm (Figure 7.2A). The increase of PC from the smallest to the largest plant was about 100% in the shade treatment, but almost 200% in the exposed treatment. Relating PC to DM yielded similar trends: $PC_{DM} = 10^{0.26 \times \log SL + 1.5}$, $r^2 = 0.65$, $p < 0.001$, $n = 27$ for exposed specimens and $PC_{DM} = 10^{0.16 \times \log SL + 1.6}$, $r^2 = 0.41$, $p = 0.005$, $n = 17$ for shaded individuals. Likewise, leaf N scaled with plant size with an increase of 21% and 68%, respectively (Figure 7.2B). Again, analyzing only specimens evidently smaller than those at the intercept (Figure 7.2A; SL < 5 cm), PC and leaf N were significantly reduced in the exposed group; changes were small but significant (Table 7.2). The same trend was detectable in regard to relative growth rates: the annual size increase among shade plants was twice that of exposed plants (Table 7.2).

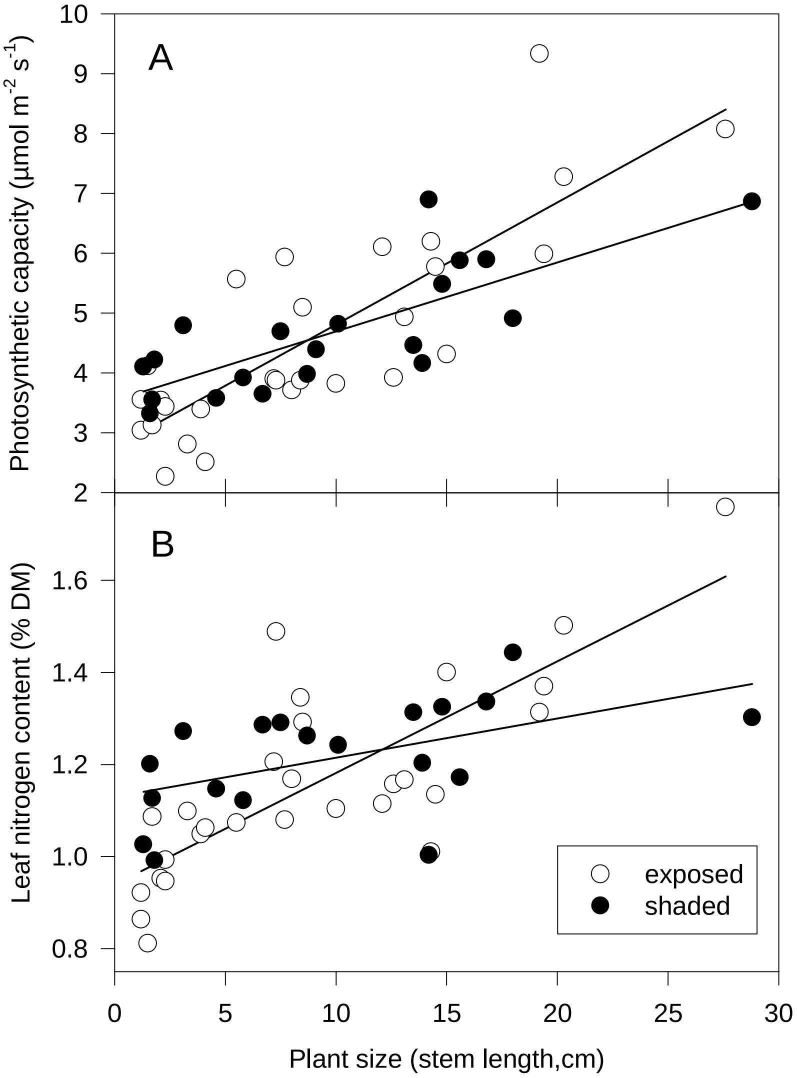

**Figure 7.2A** Area based Photosynthetic capacity and **Figure 7.2B** leaf nitrogen content (leaf N) as a function of plant size for *D. emarginata* with (shaded; closed circles) or without (exposed; open circles) artificial shading. Each symbol represents a different plant. Unlogged data are depicted for illustrative purposes. Regressions were, for the shade group: $PC_{LA} = 10^{0.16 \times \log SL + 0.52}$, $r^2 = 0.49$, $p < 0.001$, $n = 20$ and leaf $N = 10^{0.061 \times \log SL + 0.031}$, $r^2 = 0.32$, $p = 0.012$, $n = 19$, and for the exposed group: $PC_{LA} = 10^{0.28 \times \log SL + 0.41}$, $r^2 = 0.56$, $p < 0.001$, $n = 28$ and $N = 10^{0.15 \times \log SL - 0.062}$, $r^2 = 0.63$, $p < 0.001$, $n = 28$.

For individuals of *D. emarginata* growing in another *A. glabra* (without experimental shading), nitrogen content and dry mass were determined for the different plant organs. The root-shoot ratio negatively scaled with plant size (root-shoot ratio = $10^{-0.40 \times \log SL + 0.28}$, $r^2 = 0.52$, $n = 36$, $p < 0.001$). Average nitrogen content of all leaves of the most recent stem of a plant scaled linearly with plant size, whereas the nitrogen content of stems and roots was consistently lower and did not show any size dependency (Figure 7.3A). An increase in total dry mass from 0.168 g to 10.775 g, i.e. by almost two orders of magnitude, was associated by a shift in proportions: investment in leaves and roots decreased, while the that in stems scaled positively with plant size (Figure 7.3B).

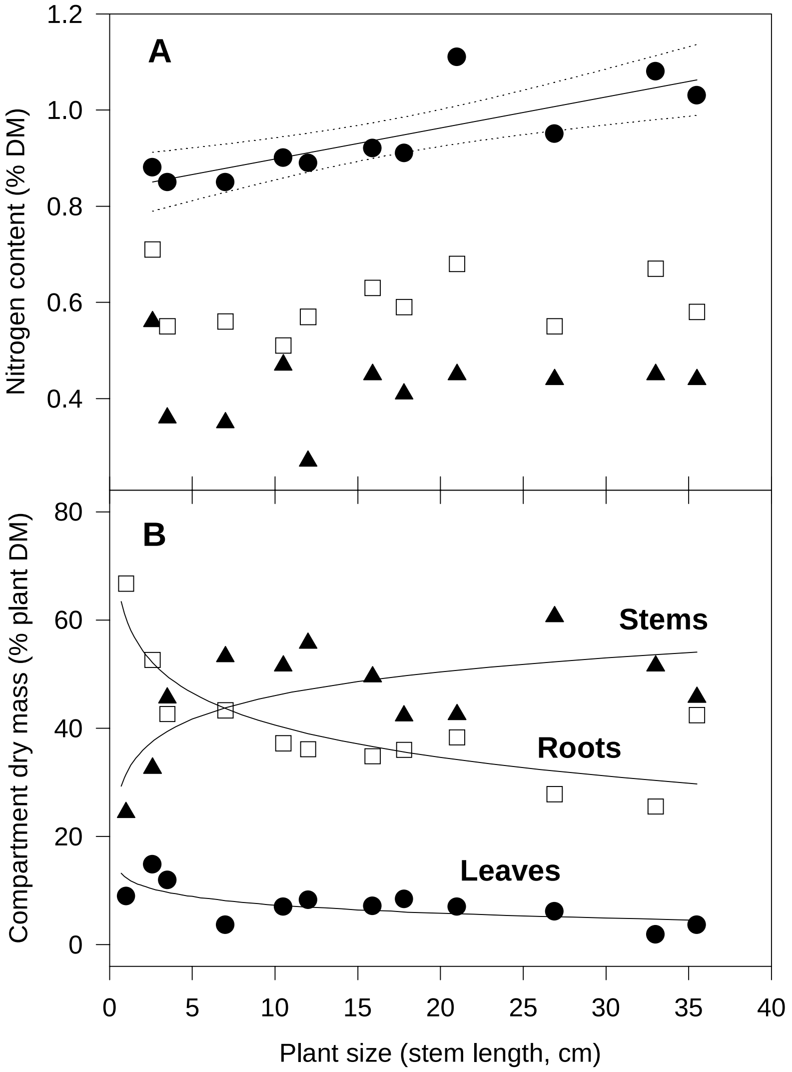

**Figure 7.3A** Nitrogen content (N) and **Figure 7.3B** dry mass (DM) of different organs as a function of plant size for *D. emarginata.* Symbols represent averaged values for all leaves of the most recent stem (closed circles), all stems of a plant (closed triangles), and roots (open squares). Regressions and means ± SD are: N (leaves) = 0.01 × SL + 0.83, $r^2$ = 0.63, p = 0.003 , n = 11 ; N (roots) = 0.60 ± 0.06, n = 11; N (stems) = 0.42 ± 0.08, n = 11; DM (leaves) = -2.2 × ln SL + 12.4, $r^2$ = 0.45, n = 12; DM (roots) = -8.6 × ln SL + 60.3, $r^2$ = 0.75, n = 12; DM (stems) = 6.3 × ln SL + 31.5, $r^2$ = 0.49, n = 12.

*Controlled environment*

After one year under the respective treatment, well watered greenhouse plants (W+) showed no differences in leaf N, PC or $RGR_{SL}$ (Table 7.3) irrespective of nutrient availability. Likewise, poorly watered plants (W-) differing in fertilization regimes were also indistinguishable (Table 7.3). As a major trend, however, well watered individuals achieved significantly higher values of all investigated parameters compared to poorly watered conspecifics. For PC this difference was independent of the fertilization regime (Table 7.3) while leaf N and $RGR_{SL}$ of poorly watered specimens were only significantly different from well watered individuals when nutrient supply was high (Table 7.3). For individuals with even higher frequencies of watering (W++) and higher levels of nutrient supply (F++; compare Table 7.3), harvested prior to the start of the experiment, $PC_{LA}$ was significantly elevated compared to all treatment categories at the end of the experiment, while dry mass related PC was indistinguishable from well-watered plants (Table 7.3).

**Table 7.3** Treatments and results in the 'controlled environment' – experiment with *D. emarginata*. Categories refer to watering (W) and fertilizing (F) of initially 13 plants per treatment. Individuals that died or exhibited irregular branching patterns were excluded from analyses. Treatment prior to the start of the experiment is represented by W++F++. For all treatments 50 ml of fertilizer solution (POLY CRESCAL, Aglukon Spezialdünger GmbH, Düsseldorf, Germany) were applied three times in two weeks. Stem lengths are means ± SD determined at the start of the experiment. Values for area and dry mass related photosynthetic capacity (PC$_{LA}$, PC$_{DM}$), leaf nitrogen content (leaf N) and relative growth rate (RGR$_{SL}$) are means ± SD. Significant differences between treatments are indicated by different letters ($p < 0.05$, ANOVA, Duncan posthoc test). Plants were cultivated for about one year under the respective treatment conditions.

| **Parameter \ Treatment** | **W++F++** | **W+F+** | **W+F-** | **W-F+** | **W-F-** |
|---|---|---|---|---|---|
| Irrigation (de-ionized water) | Twice daily | Daily | Daily | Twice weekly | Twice weekly |
| Fertilizer solution (N, µg g$^{-1}$) | 5 | 3 | 0.3 | 3 | 0.3 |
| Number of plants | 8 | 9 | 11 | 8 | 8 |
| Stem length (cm) | 14.4 ± 3.1 | 15.7 ± 2.4 | 15.5 ± 2.7 | 15.4 ± 2.1 | 15.5 ± 2.3 |
| PC$_{LA}$ (µmol m$^{-2}$ s$^{-1}$) | 17.9 ± 2.4 $^{a}$ | 13.4 ± 5.1 $^{b}$ | 13.9 ± 4.3 $^{b}$ | 8.5 ± 4.3 $^{c}$ | 9.2 ± 3.1 $^{c}$ |
| PC$_{DM}$ (nmol g$^{-1}$ s$^{-1}$) | 198 ± 25 $^{a}$ | 198 ± 136 $^{a}$ | 229 ± 136 $^{a}$ | 86 ± 55 $^{b}$ | 89 ± 31 $^{b}$ |
| Leaf N (%) | 1.43 ± 0.11 $^{a}$ | 1.40 ± 0.15 $^{a}$ | 1.30 ± 0.15 $^{ab}$ | 1.25 ± 0.10 $^{b}$ | 1.18 ± 0.18 $^{b}$ |
| RGR$_{SL}$ (cm cm$^{-1}$ a$^{-1}$) | | 1.30 ± 1.36 $^{a}$ | 0.55 ± 0.70 $^{ab}$ | - 0.12 ± 0.38 $^{b}$ | - 0.02 ± 0.39 $^{b}$ |

## Discussion

The goal of this paper was to identify the driving force(s) behind the observed size-related physiological variability in vascular epiphytes, which could potentially be (1) age or developmental stage, (2) nutrient availability and (3) water availability or a combination thereof. The first possibility can clearly be ruled out: *D. emarginata* showed a very plastic response of leaf N and PC subsequent to changes in climate conditions and experimental water and nutrient supply. Using the regression equations of Figure 7.2, in situ PC and leaf N in *D. emarginata* (SL = 12 - 19 cm) ranged from 5.2 to 5.9 µmol $O_2$ m$^{-2}$ s$^{-1}$ (or 59 to 64 nmol g$^{-1}$ s$^{-1}$) and 1.25 to 1.34% DM, respectively. Transferred to the greenhouse and treated after a standard protocol of orchid growers (Table 7.3), PC more than tripled, and leaf N increased by about 10% within four months. Trends were reversed under experimental conditions: values for poorly watered individuals in the experiment (W-) were in or close to the range of individuals from the field (Table 7.3; compare also Zotz 1997a). This indicates that the greenhouse treatment was probably quite successful in imitating natural conditions. More important, this finding demonstrates the highly plastic response of PC and leaf N to the availability of resources. Further evidence against age or developmental stage as an explanation of the observed variation in physiological parameters comes from a demographic study: in situ growth rates of *D. emarginata* exhibit considerable variability, indicating a weak correlation between plant size and age (Zotz 1998).

The distinction between the effect of water and nutrient availability is much more difficult, because nitrogen as well as other macro- and micro-nutrients are taken up as ions and their availability is thus naturally coupled to the availability of water (Chapin 1980). To overcome this problem, a set of experiments were conducted, each with certain limitations, but conclusive as a whole. Photosynthetic capacity and leaf N increased in small plants of both *D. emarginata* and *P. foliosa* in the shade (Figures 7.1 & 7.2; Table 7.2). This suggests that improved water availability due to shading overrode the counteracting effects of reduced PPFD in smaller plants. In larger plants, on the other hand, the higher internal water storage capacities (Zotz 1997a; Schmidt et al. 2001) increasingly ameliorated the effects of the highly fluctuating external water supply, hence showing the typical difference of PC in plants from exposed and shaded sites (Boardman 1977). Irrespective of irradiation regime, however, PC and leaf N scaled with plant size. According to the functional convergence hypothesis (Mooney & Gulmon 1979 & 1982) and the optimal partitioning theory (Bloom et al. 1985) investment in any plant function should cease when the return on that investment falls below the return on alternative investments. In our case lower water availability at the exposed site seems to impose a strong limitation on higher returns in small plants, i.e. in terms of photosynthetic potential and growth. Conversely, at shaded sites PPFD seems to increasingly limit investment into the photosynthetic apparatus among larger plants.

The comparison of shaded and exposed individuals of *P. foliosa* was conducted by comparing two populations in two different host tree species (Figure 7.1; Table 7.2). Although the bark of the two species looks similar macroscopically, it cannot be excluded that the observations made, were influenced by the phorophytes. Even the experimental shading of *D. emarginata* on the same host tree species (Figure 7.2; Table 7.2) does not rule out possible differences in nutrient availability between exposed and shaded sites. Moister conditions in the shade may also lead to better nutrient supply, simply by increasing the time roots are moist and can take up nutrients. However, Benzing (1990) pointed out that nutrient uptake is maximal immediately after the onset of precipitation. Thus, prolonged wetness of the substrate should primarily increase the uptake of water, and only to a lesser extent, of nutrients.

Other observations, e.g. the pattern of dry mass and nitrogen partitioning in *D. emarginata* (Table 7.3), support the notion that water, not nutrient, availability is limiting further investment of resources in the photosynthetic apparatus in the natural environment. Recalculations of the data of Figure 7.3 reveal that translocation of only a low proportion of the nitrogen stored in stems would allow, e.g. the smallest plants of the study to reach leaf N levels of large conspecifics, without any uptake of external nitrogen (Table 7.4). While the sum of leaf plus stem N was assumed to remain constant such a translocation would lead to stem N concentrations well within the range observed in situ (Table 7.4). Differences in leaf N from exposed and shaded small *D. emarginata* (Table 7.2) require even lower nitrogen input and may thus also be explained by N translocation from the stems. This indicates that nutrient uptake is not limiting under field conditions. In general, plants preferentially allocate resources to organs and functions that most strongly limit growth (Bloom et al. 1985; Chapin et al. 1987). It is thus suggested that the continuously decreasing root-shoot ratio in *D. emarginata* with plant size may be primarily a response to differences in water rather than in nutrient availability, although a strict distinction is impossible with these results alone. Such a distinction is achieved with the greenhouse experiment, where plant size was controlled (Table 7.3): equally sized *D. emarginata* responded to a better water supply with increased PC, leaf N and $RGR_{SL}$, compared to poorly watered individuals. Differences in nutrient supply had hardly any effect.

**Table 7.4** Evaluation of nitrogen partitioning among two different organs in *D. emarginata*. Leaf and stem nitrogen content (leaf N, stem N) were estimated for the smallest (SL = 2.6 cm) and the largest (SL = 36 cm) plant with regressions of Figure 7.3. For a hypothetical scenario (italics) in situ leaf N concentration of a large plant were presumed for the small plant and vice versa. In both cases, i.e. realistic and hypothetical scenario, nitrogen is supposed to be exclusively translocated between the two organs "leaves" and "stems" and total leaf plus stem N was taken to remain constant. Calculated stem N concentrations of the hypothetical scenario (italics) were within the range of in situ stem N.

| **Plant size** | | **Leaf N (%)** | **Leaf N (mg)** | **Stem N (mg)** | **Stem N (%)** |
|---|---|---|---|---|---|
| Small | with leaf N (%) of small plants | 0.84 | 0.29 | 0.52 | 0.42 ± 0.08 |
| | *with leaf N (%) of large plants* | *1.06* | *0.36* | *0.45* | *0.36* |
| Large | with leaf N (%) of large plants | 1.06 | 4.94 | 23.29 | 0.42 ± 0.08 |
| | *with leaf N (%) of small plants* | *0.84* | *3.91* | *24.32* | *0.46* |

Taken together, the results allow a distinction between the different scenarios outlined above: neither age or developmental stage nor insufficient time for nutrient uptake in younger plants can explain the results. Instead, findings are consistent with the concept of differential water availability as the driving force behind size-related physiological differences in vascular epiphytes.

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# SUMMARY

A central objective of many ecophysiological investigations is the establishment of mechanistic explanations for plant distributions in time and space. The important, albeit mostly ignored, question arises as to the nature of the organisms that should be used as representative in pertinent experiments. I suggest that it is essential to use a “demographic approach” in physiological ecology, because physiological parameters such as photosynthetic capacity (PC, determined under non-limiting conditions with the oxygen electrode) may change considerably with plant size. Moreover, as shown for nine epiphyte species covering the most important taxonomic groups, the *intraspecific* variability in PC was almost always higher than the *interspecific* variability when comparing only large individuals. In situ studies with the epiphytic bromeliad *V. sanguinolenta* revealed that besides physiological parameters (such as PC) almost all morphological, anatomical and other physiological leaf parameters studied changed with plant size as well. Likewise, important processes proved to be size-dependent on whole-plant level. For example, long-term water availability was clearly improved in large specimens compared to smaller conspecifics due to the increased efficiency of the tanks to bridge rainless periods. As model calculations on whole-plant level for *V. sanguinolenta* under natural conditions have shown photosynthetic leaf carbon gain as well as respiratory losses of heterotrophic plant parts scaled with plant size. The resulting area related annual carbon balances were similar for plants of varying size, which corresponded to observations of size-independent (and low) relative growth rates in situ. Under favorable conditions in the greenhouse, however, small *V. sanguinolenta* exhibited surprisingly high relative growth rates, similar to annuals, which clearly contradicts the prevalent, but barely tested notion of epiphytes as inherently slow growing plants and simultaneously illustrates the profound resource limitations that epiphytes are subjected to in the canopy of a seasonal rain forest. From habitat conditions it seems that size-related differences in water availability are the driving force behind the observed size-dependent ecophysiological changes: the larger an epiphyte grows the more independent it is with regard to precipitation patterns.

In conclusion, the results strongly emphasize the need to treat plant size as an important source of intraspecific variability and thus urge researchers to consider plant size in the design of ecophysiological experiments with vascular epiphytes.

# ZUSAMMENFASSUNG

Eines der Hauptziele zahlreicher ökophysiologischer Studien ist eine mechanistische Erklärungen für Pflanzenverteilungen in Raum und Zeit. Eine für diese Zielsetzung zentrale Frage, nämlich nach den Pflanzen, die in entsprechenden Experimenten als Repräsentanten einer Art verwendet werden sollen, wurde bisher allerdings meist vernachlässigt. Den Resultaten dieser Dissertation folgend, ist bei Arbeiten mit vaskulären Epiphyten eine „demographische Herangehensweise" auch für Belange der Ökophysiologie notwendig, da sich z.B. physiologische Parameter wie Photosynthesekapazität (PC, unter nicht limitierenden Bedingungen in der Sauerstoffelektrode gemessen) regelhaft mit der Pflanzengröße änderten. Darüber hinaus war die intraspezifische Variabilität von PC meist höher als zwischenartliche Unterschiede, wie für neun Epiphyten aus den wichtigsten taxonomischen Gruppen gezeigt wurde. In situ Studien mit der epiphytischen Tankbromelie *Vriesea sanguinolenta* ergaben, dass sich neben physiologischen Parametern wie PC fast alle untersuchten morphologischen, anatomischen und anderen physiologischen Blattparameter mit der Pflanzengröße ändern. Auch auf der Ebene gesamter Pflanzen erwiesen sich wichtige Prozesse als stark größenabhängig. Zum Beispiel war die Wasserverfügbarkeit aufgrund einer steigenden Effizienz der Wassertanks für große Individuen deutlich verbessert gegenüber kleineren Artgenossen. Desweiteren ergaben Modellberechnungen für *V. sanguinolenta* unter natürlichen Bedingungen, dass der photosynthetischen Kohlenstoffgewinn der Blätter, ebenso wie Respirationsverluste heterotropher Organe regelhaft mit der Pflanzengröße anstiegen. Die resultierenden Blattflächen bezogenen Jahreskohlenstoffbilanzen waren für Pflanzen verschiedener Größen ungefähr gleich, was Beobachtungen von größenunabhängigen (und niedrigen) relativen Wachstumsraten in situ entsprach. Unter günstigen Bedingungen im Gewächshaus stiegen die relativen Wachstumsraten kleiner *V. sanguinolenta* auf ein überraschend hohes Niveau, vergleichbar dem von terrestrischen, anuellen Pflanzen. Dies widerspricht klar der gängigen, aber kaum belegten Vorstellung von Epiphyten als inherent langsam wachsende Organismen und zeigen gleichzeitig auf, dass Epiphyten im Kronenraum eines saisonalen Regenwaldes aufgrund erheblicher Ressourcenlimitierung ihr Wachstumspotential bei weitem nicht ausschöpfen können. Von den Habitatsbedingungen scheint der systematische Unterschied in der Wasserverfügbarkeit bei verschieden großen Artgenossen die treibende Kraft für die beobachteten größenabhängigen Veränderungen zu sein: Je größer eine Pflanze wird, desto weniger wird sie von unterschiedlichen Niederschlagsmustern beeinflusst.

Die Ergebnisse verdeutlichen den nachhaltigen Einfluss von Pflanzengröße auf die intraspezifische Variabilität ökophysiologischer Parameter und unterstreichen somit die Dringlichkeit Pflanzengröße in experimentellen Designs von ökophysiologischen Studien mit vaskulären Epiphyten zu integrieren.

# REFERENCE LIST

**Adams III WW, Martin CE** 1986a. Morphological changes accompanying the transition from juvenile (atmospheric) to adult (tank) forms in the Mexican epiphyte *Tillandsia deppeana* (Bromeliaceae). American Journal of Botany 73: 1207-1214.

**Adams III WW, Martin CE** 1986b. Physiological consequences of changes in life form of the Mexican epiphyte *Tillandsia deppeana* (Bromeliaceae). Oecologia 70: 298-304.

**Andrade J-L, Nobel PS** 1996. Habitat, $CO_2$ uptake, and growth for the CAM epiphytic cactus *Epiphyllum phyllanthus* in a Panamanian tropical forest. Journal of Tropical Ecology 12: 291-306.

**Arnon DI** 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Physiology 24: 1-15.

**Ball E, Hann J, Kluge M, Lee HSJ, Lüttge U, Orthen B, Popp M, Schmitt A, Ting IP** 1991a. Ecophysiological comportment of the tropical CAM-tree *Clusia* in the field. I. Growth of *Clusia rosea* Jacq. on St. John, US. Virgin Islands, Lesser Antilles. New Phytologist 117: 473-481.

**Ball E, Hann J, Kluge M, Lee HSJ, Lüttge U, Orthen B, Popp M, Schmitt A, Ting IP** 1991b. Ecophysiological comportment of the tropical CAM-tree *Clusia* in the field. II. Modes of photosynthesis in trees and seedlings. New Phytologist 117: 483-491.

**Barbosa P, Schultz JC** (eds) 1987. Insect outbreaks. San Diego. Academic Press.

**Barthlott W, Porembski S, Kluge M, Hopke J, Schmidt L** 1997. *Selenicereus wittii* (Cactaceae): an epiphyte adapted to Amazonian Igapó inundation forests. Plant Systematics and Evolution 206: 175-185.

**Benzing DH** 1970. Roots in certain species of *Tillandsia* and *Vriesea* and their role in the epiphytic environment. Bromeliad Society Bulletin 20: 79-84.

**Benzing DH** 1978. The nutritional status of three *Encyclia tampensis* (Orchidaceae) populations in Southern Florida as compared with that of *Tillandsia circinnata* (Bromeliaceae). Selbyana 2: 224-229.

**Benzing DH** 1980. The biology of bromeliads. Eureka. Mad River Press.

**Benzing DH** 1981. The population dynamics of *Tillandsia circinnata* (Bromeliaceae): cypress crown colonies in Southern Florida. Selbyana 5: 256-263.

**Benzing DH** 1990. Vascular epiphytes. General biology and related biota. Cambridge. Cambridge University Press.

**Benzing DH** 2000. Bromeliaceae - Profile of an Adaptive Radiation. Cambridge. Cambridge University Press.

**Benzing DH, Bent A, Moscow D, Peterson G, Renfrow A** 1982. Functional correlates of deciduousness in *Catasetum integerrimum* (Orchidaceae). Selbyana 7: 1-9.

**Benzing DH, Burt KM** 1970. Foliar permeability among twenty species of the Bromeliaceae. Bulletin of the Torrey Botanical Club 97: 269-279.

**Benzing DH, Dahle CE** 1971. The vegetative morphology, habitat preference and water balance mechanisms of the Bromeliad *Tillandsia ionantha* Planch. American Midland Naturalist 85: 11-21.

**Benzing DH, Davidson EA** 1979. Oligotrophic *Tillandsia circinnata* Schlecht (Bromeliaceae): an assessment of its patterns of mineral allocation and reproduction. American Journal of Botany 66: 386-397.

**Benzing DH, Friedman WE** 1981. Patterns of foliar pigmentation in Bromeliaceae and their adaptive significance. Selbyana 5: 224-240.

**Benzing DH, Pockman WT** 1989. Why do nonfoliar green organs of leafy orchids fail to exhibit net photosynthesis? Lindleyana 4: 53-60.

**Benzing DH, Renfrow A** 1971a. The biology of the atmospheric bromeliad *Tillandsia circinnata* Schlecht. I. The nutrient status of populations in South Florida. American Journal of Botany 58: 867-873.

**Benzing DH, Renfrow A** 1971b. The significance of photosynthetic efficiency to habitat preference and phylogeny among Tillandsioid Bromeliads. Botanical Gazette 132: 19-30.

**Benzing DH, Renfrow A** 1971c. Significance of the patterns of $CO_2$ exchange to the ecology and phylogeny of the Tillandsioideae (Bromeliaceae). Bulletin of the Torrey Botanical Club 98: 322-327.

**Benzing DH, Renfrow A** 1974a. The mineral nutrition of Bromeliaceae. Botanical Gazette 135: 281-288.

**Benzing DH, Renfrow A** 1974b. The nutritional status of *Encyclia tampense* and *Tillandsia circinnata* on *Taxodium ascendens* and the availability of nutrients to epiphytes on this host in South Florida. Bulletin of the Torrey Botanical Club 101: 191-197.

**Bergstrom DM, Tweedie CE** 1998. A conceptual model for integrative studies of epiphytes: nitrogen utilisation, a case study. Australian Journal of Botany 46: 273-280.

**Bermudes D, Benzing DH** 1991. Nitrogen fixation in association with Ecuadorian bromeliads. Journal of Tropical Ecology 7: 531-538.

**Berryman AA** 1987. The theory and classification of outbreaks. In: Barbosa P, Schultz JC (eds), Insect outbreaks. San Diego. Academic Press: 3-30.

**Beßler B, Schmitgen S, Kühnemann F, Gabler R, Urban W** 1998. Light-dependent production of ethylene in *Tillandsia usneoides* L. Planta 205: 140-144.

**Beyschlag W, Lange OL, Tenhunen JD** 1987. Photosynthese und Wasserhaushalt der immergrünen mediterranen Hartlaubpflanze *Arbutus unedo* L. im Jahreslauf am Freilandstandort in Portugal. II. Charakteristische Gaswechselparameter von $CO_2$-Aufnahme und Transpiration. Flora 179: 399-420.

**Biebl R** 1964. Zum Wasserhaushalt von *Tillandsia recurvata* L. und *Tillandsia usneoides* L. auf Puerto Rico. Protoplasma 58: 345-368.

**Björkman O** 1981. Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds), Physiological Plant Ecology I. Responses to the Physical Environment. Berlin. Springer Verlag: 57-107.

**Björkman O, Holmgren P** 1966. Photosynthetic adaption to light intensity in plans native to shaded and exposed habitats. Physiologia Plantarum 19: 854-859.

**Bloom AJ, Chapin III FS, Mooney HA** 1985. Resource limitation in plants - an economic analogy. Annual Review of Ecology and Systematics 16: 363-392.

**Boardman NK** 1977. Comparative photosynthesis of sun and shade plants. Annual Review of Plant Physiology 28: 355-377.

**Bonates LCdM** 1993. Estudios ecofiosiologicos de Orchidaceae da Amazonia. II - Anatomia ecologica foliar. Acta Amazonica 23: 315-348.

**Bonner JT** 1965. Size and Cycle. Princton. Princton University Press.

**Borland AM, Griffiths H** 1990. The regulation of CAM and respiratory recycling by water supply and light regime in the $C_3$-CAM intermediate *Sedum telephium*. Functional Ecology 4: 33-39.

**Borland AM, Griffiths H, Maxwell C, Broadmeadow MSJ, Griffiths NM, Barnes JD** 1992. On the ecophysiology of the Clusiaceae in Trinidad: expression of CAM in *Clusia minor* L. during the transition from wet to dry season and characterization of three endemic species. New Phytologist 122: 349-357.

**Borland AM, Griffiths H, Maxwell C, Fordham MC, Broadmeadow MSJ** 1996. CAM induction in *Clusia minor* L. during the transition from wet to dry season in Trinidad: the role of organic acid speciation and decarboxylation. Plant, Cell and Environment 19: 655-664.

**Boyer Y** 1964. Contribution a l'étude de l'écophysiologie de deux fougères épiphytes: *Platycerum stemari*a (Beauv.) Desv. et *P. angolense* Welch. Annales des Sciences Naturelles, Botanique, Paris 5: 87-228.

**Brown KW, Jordan, W. R., Thomas JC** 1976. Water stress induced alterations of the stomatal response to decreases in leaf water potential. Physiologica Plantarum 37: 1-5.

**Burt KM, Benzing DH** 1969. The absorption of nutrients by leaves and roots in *Billbergia chlorosticta*. Bromeliad Society Bulletin 19: 5-10.

**Cape JN, Percy KE** 1996. The interpretation of leaf-drying curves. Plant, Cell and Environment 19: 356-361.

**Carter JP, Martin CE** 1994. The occurrence of crassulacean acid metabolism among epiphytes in a high-rainfall region of Costa Rica. Selbyana 15: 104-106.

**Chapin III FS** 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11: 233-260.

**Chapin III FS, Bloom AJ, Field CB, Waring RH** 1987. Plant responses so multiple environmental factors. BioScience 37: 49-57.

**Chiariello NR, Gulmon SL** 1991. Stress effects on plant reproduction. In: Mooney HA, Winner WE, Pell EJ (eds), Responses of plants to Multiple Stresses. San Diego. Academic Press.

**Clarkson DT, Kuiper PJC, Lüttge U** 1986. Sources of nutrients for land plants from outside the pedosphere. Progress in Botany 48: 80-96.

**Clifford PE, Neo HH, Hew CS** 1995. Regulation of assimilate partitioning in flowering plants of the monopodial orchid *Aranda* Noorah Alsagoff. New Phytologist 130: 381-389.

**Cockburn W, Goh CJ, Avadhani PN** 1985. Photosynthetic carbon assimilation in a shootless orchid, *Chiloschista usneoides* (DON) LDL: a variant on crassulacean acid metabolism. Plant Physiology 77: 83-86.

**Coley PD, Bryant JP, Chapin FSI** 1985. Resource availability and plant antiherbivore defense. Science 230: 895-899.

**Coutinho LM** 1965. Algumas informações sôbre a capacidade rítmica diária da fixação e acumulação de $CO_2$ no escuro em epífitas e erbáceas terrestres da mata pluvial. Botanica 21: 395-408.

**Coutinho LM** 1969. Novas observações sôbre a ocorrência do "efeito de de Saussure" e suas relações com a suculência, a temperatura folhear e os movimentos estomáticos. Botanica 24: 77-102.

**Croat T** 1978. Flora of Barro Colorado Island. Stanford. Stanford University Press.

**D'Arcy WG** 1987. Flora of Panama: Checklist and Index. St. Louis. Miss. Bot. Gardens.

**De Soyza AG, Franco AC, Virginia RA, Reynolds JF, Whitford WG** 1996. Effects of plant size on photosynthesis and water relations in the desert shrub *Prosopis glandulosa* (Fabaceae). American Journal of Botany 83: 99-105.

**Dejean A, Olmsted IC, Camal JF** 1992. Interaction between *Atta cephalotes* and arboreal ants in the Biosphere Reserve Sian Ka'an (Quintana Roo, Mexico): efficient protection of the trees (Hymenoptera: Formicidae). Sociobiology 20: 57-76.

**Demmig-Adams B, Adams WWI, Winter K, Meyer A, Schreiber U, Pereira JS, Krüger A, Czygan FC, Lange OL** 1989. Photochemical efficiency of photosystem II, photon yield of $O_2$ evolution, photosynthetic capacity, and carotenoid composition during the midday depression of the net $CO_2$ uptake in *Arbutus unedo* growing in Portugal. Planta 177: 377-387.

**DeVries PJ** 1997. The butterflies of Costa Rica and their natural history. Princeton. Princeton University Press.

**Diaz M, Haag-Kerwer A, Wingfield R, Ball E, Olivares E, Grams TEE, Ziegler H, Lüttge U** 1996. Relationships between carbon and hydrogen isotope ratios and nitrogen levels in leaves of *Clusia* species and two other Clusiaceae genera at various sites and different altitudes in Venezuela. Trees 10: 351-358.

**Donovan LA, Ehleringer JR** 1991. Ecophysiological differences among juvenil and reproductive plants of several woody species. Oecologia 86: 594-597.

**Donovan RD, Arditti J, Ting IP** 1984. Carbon fixation by *Paphiopedilum insigne* and *Paphiopedilum parishii* (Orchidaceae). Annals of Botany 54: 583-586.

**Dycus AM, Knudson L** 1957. The role of the velamen of the aerial roots of Orchids. Botanical Gazette 119: 78-87.

**Earnshaw MJ, Winter K, Ziegler H, Stichler W, Cruttwell NEG, Kerenga K, Cribb PJ, Wood J, Croft JR, Carver KA, Gunn TC** 1987. Altitudinal changes in the incidence of crassulacean acid metabolism in vascular epiphytes and related life forms in Papua New Guinea. Oecologia 73: 566-572.

**Edwards PJ, Grubb PJ** 1977. Studies of mineral cycling in a montane rainforest in New Guinea I. The distribution of organic matter in the vegetation and soil. Journal of Ecology 65: 943-969.

**Erickson LC** 1957. Respiration and photosynthesis in *Cattleya* roots. American Orchid Society Bulletin 26: 401-402.

**Evans JR** 1989. Photosynthesis and nitrogen relationships in leaves of $C_3$ plants. Oecologia 78: 9-19.

**Farrar JF** 1988. Temperature and the partitioning and the translocation of carbon. In: Long SP, Woodward FI (eds), Plants and Temperature. Cambridge. Company of Biologists: 203-235.

**Farrar JF, Gunn S** 1996. Effects of temperature and atmospheric carbon dioxide concentrations on source sink relations in the context of climate change. In: Zamske E, Scheffer A (eds), Photoassimilate Distribution in Plants and Crops. New York. Dekker: 389-406.

**Farrar JF, Williams JHH** 1991. Control of the rate of respiration in roots: compartmentation, demand and the supply of substrate. In: Emes M (ed), Compartmentation of Plant Metabolism in Non-photosynthetic Tissue. Cambridge. Cambridge University Press: 167-188.

**Feild TS, Lawton RO, Dawson TE** 1997. Comparative nutrient relations in canopy-rooted and ground-rooted *Didymopanax pittieri* Marchal (Araliaceae) hemiepiphytes in a wind-exposed tropical montane forest. Biotropica 28: 774-776.

**Field C, Mooney HA** 1986. The photosynthesis-nitrogen relationship in wild plants. In: Givnish TJ (ed), On the economy of plant form and function. Cambridge. Cambridge University Press: 25-55.

**Fisher BL, Sternberg LdSL, Price D** 1990. Variation in the use of extra-floral nectar by ants. Oecologia 83: 263-266.

**Fisher BL, Zimmerman JK** 1988. Ant/orchid associations in the Barro Colorado National Monument, Panama. Lindleyana 3: 12-16.

**Frank JH, Curtis GA** 1981. Bionomics of the bromeliad-inhabiting mosquito *Wyeomyia vanduzeei* and its nursery plant *Tillandsia utriculata*. Florida Entomologist 64: 491-506.

**Fu CF, Hew CS** 1982. Crassulacean acid metabolism in orchids under water stress. Botanical Gazette 143: 294-297.

**Galilei G** 1638. Discorsi e dimostrazioni mathematiche, intorno a due nuove scienze (Dialogues concerning two new sciences). New York, 1914.

**García-Franco JG, Rico-Gray V** 1992. Frequency of galls in the roots of *Tillandsia ionantha* (Bromeliaceae) in a tropical dry forest in the state of Veracruz, Mexico. Selbyana 13: 57-61.

**Gay H** 1993. Animal-fed plants: an investigation into the uptake of ant-derived nutrients by the far-eastern epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). Biological Journal of the Linnean Society 50: 221-233.

**Gentry AH, Dodson CH** 1987. Diversity and biogeography of neotropical vascular epiphytes. Annals of the Missouri Botanical Garden 74: 205-233.

**Goh CJ** 1983. Rhythms of acidity and $CO_2$ production in orchid flowers. New Phytologist 93: 25-32.

**Goh CJ, Arditti J, Avadhani PN** 1983. Carbon fixation in orchid aerial roots. New Phytologist 95: 367-374.

**Goh CJ, Avadhani PN, Loh CS, Hanegraaf C, Arditti J** 1977. Diurnal stomatal and acidity rhythms in orchid leaves. New Phytologist 78: 365-372.

**Grant L** 1995. Bromelienstudien. Tropische und Subtropische Pflanzenwelt 91: 1-57.

**Gregg KB** 1982. Sunlight-enhanced ethylene evolution by developing inflorescences of *Catasetum* and *Cycnoches* and its relation to female flower production. Botanical Gazette 143: 466-475.

**Griffiths H** 1988. Carbon balance during CAM: an assessment of respiratory $CO_2$ recycling in the epiphytic bromeliads *Aechmea nudicaulis* and *Aechmea fendleri*. Plant, Cell and Environment 11: 603-611.

**Griffiths H** 1989. Carbon dioxide concentrating mechanisms and the evolution of CAM in vascular epiphytes. In: Lüttge U (ed), Vascular Plants as Epiphytes: Evolution and Ecophysiology. Heidelberg. Springer Verlag: 42-86.

**Griffiths H, Lüttge U, Stimmel KH, Crook CE, Griffiths NM, Smith JAC** 1986. Comparative ecophysiology of CAM and $C_3$ bromeliads. III. Environmental influences on $CO_2$ assimilation and transpiration. Plant, Cell and Environment 9: 385-393.

**Griffiths H, Smith JAC** 1983. Photosynthetic pathways in the Bromeliaceae of Trinidad: relations between life-forms, habitat preference and the occurrence of CAM. Oecologia 60: 176-184.

**Griffiths H, Smith JAC, Bassett ME, Griffiths NM** 1984. Ecophysiology and habitat preference of Trinidad epiphytic bromeliads. In: Chadwick AC, Sutton SL (eds), Tropical Rain-Forest: The Leeds Symposium. Leeds, U.K. Leeds Philosophical and Literary Society: 117-122.

**Griffiths H, Smith JAC, Lüttge U, Popp M, Cram WJ, Diaz M, Lee HSL, Medina E, Schäfer C, Stimmel KH** 1989. Ecophysiology of xerophytic and halophytic vegetation of a coastal alluvial plain in northern Venezuela. IV. *Tillandsia flexuosa* Sw. and *Schomburgkia humboldtiana* Reichb., epiphytic CAM plants. New Phytologist 111: 273-282.

**Grime JP, Hunt R** 1975. Relative growth rate: its range and adaptive significance in a local flora. Journal of Ecology 63: 393-422.

**Guehl JM, Aussenac G, Boucachrine J, Zimmerman R, Pennes JM, Ferhi A, Grieu P** 1991. Sensitivity of leaf gas exchange to atmospheric drought, soil drought, and water use efficiency in some Mediteranean *Abies* species. Canadian Journal of Forestry Research 21: 1507-1515.

**Guralnick LJ, Ting IP, Lord EM** 1986. Crassulacean acid metabolism in the Gesneriaceae. American Journal of Botany 73: 336-345.

**Harper RM** 1919. Water and mineral content of an epiphytic fern. American Fern Journal 9: 99-103.

**Harris JA** 1918. On the osmotic concentration of the tissue fluids of phanerogamic epiphytes. American Journal of Botany 5: 490-506.

**Helbsing S, Riederer M, Zotz G** 2000. Cuticles of vascular epiphytes: an efficient barrier for water after stomatal closure? Annals of Botany, in press

**Hertl H** 1963. Structure, form, movement. New York. Reinhold Publishing Co.

**Hew CS** 1984. *Drymoglossum* under water stress. American Fern Journal 74: 37-39.

**Hew CS, Hin SE, Yong JWH, Gouk SS, Tanaka M** 1995. *In vitro* $CO_2$ enrichment of CAM orchid plantlets. Journal of Horticultural Science 70: 721-736.

**Hew CS, Lim LY** 1989. Mineral uptake by orchid plantlets grown on an agar culture medium. Soilless Culture 5: 23-34.

**Hew CS, Lim LY, Low CM** 1993. Nitrogen uptake by tropical orchids. Environmental and Experimental Botany 33: 273-281.

**Hew CS, Ng CKY** 1996. Changes in mineral and carbohydrate content in pseudobulbs of the $C_3$ epiphytic orchid hybrid *Oncidium* Goldiana at different growth stages. Lindleyana 11: 125-134.

**Hew CS, Ng CKY, Gouk SS, Yong JWH, Wong SC** 1996. Variation in d13C values for different plant parts of an *Oncidium* orchid. Photosynthetica 32: 135-139.

**Hew CS, Ng YW, Wong SC, Yeoh HH, Ho KK** 1984. Carbon dioxide fixation in orchid aerial roots. Physiologia Plantarum 60: 154-158.

**Hew CS, Soh WP, Ng CKY** 1998. Variation in photosynthetic characteristics along the leaf blade of *Oncidium* Goldiana, a $C_3$ tropical epiphytic orchid hybrid. International Journal of Plant Sciences 159: 116-120.

**Hew CS, Ye QS, Pan RC** 1989. Pathway of carbon fixation in some thin-leaved orchids. Lindleyana 4: 154-157.

**Hew CS, Ye QS, Pan RC** 1991. Relation of respiration to $CO_2$ fixation by Aranda orchid roots. Environmental and Experimental Botany 31: 327-331.

**Hietz P** 1997. Population dynamics of epiphytes in a Mexican humid montane forest. Journal of Ecology 85: 767-777.

**Hietz P, Ausserer J** 1998. Population dynamics and growth of epiphytes in a humid montane forest in Mexico. Second International Forest Canopy Conference, Forest Canopies 1998: Global Perspectives. Sarasota, Florida, USA.

**Hietz P, Briones O** 1998. Correlation between water relations and within-canopy distribution of epiphytic ferns in a Mexican cloud forest. Oecologia 114: 305-316.

**Ho KK, Yeoh HH, Hew CS** 1983. The presence of photosynthetic machinery in in aerial roots of leafy orchids. Plant and Cell Physiology 24: 1317-1321.

**Holbrook NM, Putz FE** 1996. From epiphyte to tree: differences in leaf structure and leaf water relations associated with the transition in growth form in eight species of hemiepiphytes. Plant, Cell and Environment 19: 631-642.

**Holdridge LR, Grenke WC, Hatheway WH, Liang T, Tosi JA** 1971. Forest environments in tropical life zones: a pilot study. Oxford. Pergamon Press.

**Howe HH, Westley LC** 1988. Ecological relationships of plants and animals. New York. Oxford University Press.

**Huxley CR** 1978. The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. New Phytologist 80: 231-268.

**Ipson DC** 1960. Units, dimensions and dimensionless numbers. New York. McGraw-Hill.

**Janzen H** 1974. Epiphytic myrmecophytes in Sarawak: mutualism through the feeding of plants by ants. Biotropica 6: 237-259.

**Jones HG** 1973. Moderate-term water stresses and associated changes in some photosynthetic parameters in cotton. New Phytologist 72: 1095-1105.

**Jones HG** 1992. Plants and microclimate: a quantitive approach to environmental plant physiology. Cambridge. Cambridge University Press.

**Jordan CF** 1984. Nutrient regime in the wet tropics: physical factors. In: Medina E, Mooney HA, Vásquez-Yánes C (eds), Physiological Ecology of Plants of the Wet Tropics. The Hague. Junk Publisher: 3-12.

**Jordan PW, Nobel PS** 1979. Infrequent establishment of seedlings of *Agave deserti* (Agavaceae) in the northwestern Sonoran desert. American Journal of Botany 66: 1079-1084.

**Kerstiens G** 1996. Cuticular water permeability and its physiological significance. Journal of Experimental Botany 47: 1813-1832.

**Kleiber M** 1932. Body size and metabolism. Hilgardia 6: 315-353.

**Kluge M, Avadhani PN, Goh CJ** 1989. Gas exchange and water relations in epiphytic tropical ferns. In: Lüttge U (ed), Vascular Plants as Epiphytes: Evolution and Ecophysiology. Heidelberg. Springer Verlag: 87-108.

**Kluge M, Brulfert J, Rauh W, Ravelomanana D, Ziegler H** 1995. Ecophysiological studies on the vegetation of Madagascar: a d13C and dD survey for incidence of crassulacean acid metabolism (CAM) among orchids from montane forests and succulents from the xerophytic thorn-bush. Isotopes Environment Health Studies 31: 191-210.

**Kluge M, Lange OL, Eichmann Mv, Schmid R** 1973. Diurnaler Säurerhythmus bei *Tillandsia usneoides*: Untersuchungen über den Weg des Kohlenstoffs sowie die Abhängigkeit des $CO_2$-Gaswechsels von Lichtintensität, Temperatur und Wassergehalt der Pflanze. Planta 112: 357-372.

**Kluge M, Ting IP** 1978. Crassulacean Acid Metabolism: Analysis of an Ecological Adaptation. Berlin. Springer Verlag.

**Kluge M, Vinson B, Ziegler H** 1997. Ecophysiological studies on orchids of Madagascar: incidence and plasticity of crassulacean acid metabolism in species of the genus *Angraecum* Bory. Plant Ecology 135: 43-57.

**Koptur S, Rico-Gray V, Palacios-Rios M** 1998. Ant protection of the nectaried fern *Polypodium plebeium* in central Mexico. American Journal of Botany 85: 736-739.

**Kress WJ** 1986. A symposium: the biology of tropical epiphytes. Selbyana 9: 1-22.

**Lambers H, Chapin III FS, Pons TL** 1998. Plant Physiological Ecology. New York. Springer.

**Lambers H, Poorter H** 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Advances in Ecological Research 23: 187-261.

**Larcher W** 1994. Ökologie der Pflanzen. Stuttgart. Ulmer.

**Larcher W, Eggarter H** 1959. Anwendung des Triphenyltetrazoliumchlorids zur Beurteilung von Frostschäden in verschiedenen Achsengeweben bei *Pirus*-Arten, und Jahresgang der Resistenz. Protoplasma 4: 595-619.

**Larson RJ** 1992. Population dynamics of *Encyclia tampensis* in Florida. Selbyana 13: 50-56.

**Leigh EG, Jr., Rand AS, Windsor DM** (eds) 1982. The ecology of a tropical forest. Seasonal rhythms and long-term changes. Washington, D.C. Smithsonian Institution Press.

**Leigh EG, Jr., Wright SJ** 1990. Barro Colorado Island and tropical biology. In: Gentry AH (ed), Four Neotropical Forests. New Haven and New York. Yale University Press: 28-47.

**Lesica P, Antibus RK** 1991. Canopy soil variability - potential significance to epiphyte species richness in wet tropical. Res. Explor. 7: 156-165.

**Lieske R** 1915. Beiträge zur Kenntnis der Ernährungsphysiologie extrem atmosphärischer Epiphyten. Jahrbücher für wissenschaftliche Botanik 56: 112-122.

**Loeschen VS, Martin CE, Smith M, Eder SL** 1993. Leaf anatomy and $CO_2$ recycling during crassulacean acid metabolism in twelve epiphytic species of *Tillandsia* (Bromeliaceae). International Journal of Plant Sciences 154: 100-106.

**Lowman MD, Brown M, Desrosiers A, Randle DC** 1999. Temporal variation in herbivory of a Peruvian bromeliad. Journal of the Bromeliad Society 49: 81-83.

**Lowman MD, Wittman PK, Murray D** 1996. Herbivory in a bromeliad of the Peruvian rain forest canopy. Journal of the Bromeliad Society 46: 52-55.

**Lüttge U** (ed) 1989. Vascular plants as epiphytes: evolution and ecophysiology. Ecological Studies. Heidelberg. Springer Verlag.

**Lüttge U, Ball E, Kluge M, Ong BL** 1986. Photosynthetic light requirements of various tropical vascular epiphytes. Physiologie Végetale 24: 315-331.

**Martin CE, Adams III WW** 1987. Crassulacean acid metabolism, $CO_2$-recycling, and tissue desiccation in the Mexican epiphyte *Tillandsia schiedeana* Steud. (Bromeliaceae). Photosynthesis Research 11: 237-244.

**Martin CE, Eades CA, Pitner RA** 1986. Effects of irradiance on crassulacean acid metabolism in the epiphyte *Tillandsia usneoides* L. (Bromeliaceae). Plant Physiology 80: 23-26.

**Martin CE, McLeod KW, Eades CA, Pitzer AF** 1985. Morphological and physiological responses to irradiance in the CAM epiphyte *Tillandsia usneoides* L. (Bromeliaceae). Botanical Gazette 146: 489-494.

**Martin CE, Schmitt AK** 1989. Unusual water relations in the CAM atmospheric epiphyte *Tillandsia usneoides* L. (Bromeliaceae). Botanical Gazette 150: 1-8.

**Martin CE, Siedow JN** 1981. Crassulacean acid metabolism in the epiphyte *Tillandsia usneoides* L. (Spanish moss). Plant Physiology 68: 335-339.

**Maxwell C, Griffiths H, Borland AM, Broadmeadow MSJ, McDavid CR** 1992. Photoinhibitory responses of the epiphytic bromeliad *Guzmania monostachia* during the dry season in Trinidad maintain photochemical integrity under adverse conditions. Plant, Cell and Environment 15: 37-47.

**Maxwell C, Griffiths H, Borland AM, Young AJ, Broadmeadow MSJ, Fordham MC** 1995. Short-term photosynthetic responses of the $C_3$-CAM epiphyte *Guzmania monostachia*: var. *monostachia* to tropical seasonal transitions under field conditions. Australian Journal of Plant Physiology 22: 771-781.

**Maxwell C, Griffiths H, Young AJ** 1994. Photosynthetic acclimation to light regime and water stress by the $C_3$-CAM epiphyte *Guzmania monostachia*: gas-exchange characteristics, photochemical efficiency and the xanthophyll cycle. Functional Ecology 8: 746-754.

**McMahon TA** 1983. On size and life. New York. Scientific American Books.

**McWilliams EL** 1970. Comparative rates of dark $CO_2$ uptake and acidification in the Bromeliaceae, Orchidaceae and Euphorbiaceae. Botanical Gazette 131: 285-290.

**Medina E** 1974. Dark $CO_2$ fixation, habitat preference and evolution within the Bromeliaceae. Evolution 28: 677-686.

**Medina E, Delgado M, Garcia V** 1989. Cation accumulation and leaf succulence in *Codonanthe macradenia* J. D. Smith (Gesneriaceae) under field conditions. Amazoniana 11: 13-22.

**Medina E, Delgado M, Troughton JH, Medina JD** 1977. Physiological ecology of $CO_2$ fixation in *Bromeliaceae*. Flora 166: 137-152.

**Medina E, Minchin P** 1980. Stratification of d13C values in Amazonian rain forest. Oecologia 45: 377-378.

**Medina E, Troughton JH** 1974. Dark $CO_2$ fixation and the carbon isotope ratio in Bromeliaceae. Plant Science Letters 2: 357-362.

**Mercier H, Kerbauy GB, Sotta B, Miginiac E** 1997. Effects of $NO_3^-$, $NH_4^+$ and urea nutrition on endogenous levels of IAA and four cytokinins in two epiphytic bromeliads. Plant, Cell and Environment 20: 387-392.

**Milburn TR, Pearson DJ, Ndegwe NA** 1968. Crassulacean acid metabolism under natural tropical conditions. New Phytologist 67: 883-897.

**Mooney HA** 1972. The carbon balance of plants. Annual Review of Ecology and Systematics 3: 315-346.

**Mooney HA, Bullock SH, Ehleringer JR** 1989. Carbon isotope ratios of plants of a tropical forest in Mexico. Functional Ecology 3: 137-142.

**Mooney HA, Gulmon SL** 1979. Environmental and evolutionary constraints on photosynthetic characteristics of higher plants. In: Solbrig OT, Jain S, Jonson GB, Raven PH (eds), Topics in Plant Population Biology. New York. Columbia University Press: 316-337.

**Mooney HA, Gulmon SL** 1982. Constraints on leaf structure and function in relation to herbivory. BioScience 32: 198-206.

**Muchow RC, Sinclair TR** 1989. Epidermal conductance, stomatal density and stomatal size among genotypes of *Sorghum bicolor* (L.) Moench. Plant, Cell and Environment 12: 425-431.

**Neales TF, Hew CS** 1975. Two types of carbon fixation in tropical Orchids. Planta 123: 303-306.

**Ng CKY, Hew CS** 1996. Pathway of phloem loading in the C3 tropical orchid hybrid *Oncidium* Goldiana. Journal of Experimental Botany 47: 1935-1939.

**Niklas K** 1994. Plant allometry: the scaling of form and process. Chicago. Chicago University Press.

**Nobel PS, Calkin HW, Gibson AC** 1984. Influences of PAR, temperature and water vapor concentration on gas exchange by ferns. Physiologia Plantarum 62: 527-534.

**Nowak EJ, Martin CE** 1997. Physiological and anatomical responses to water deficits in the CAM epiphyte *Tillandsia ionantha* (Bromeliaceae). International Journal of Plant Science 158: 818-826.

**Nyman LP, Davis JP, O'Dell SJ, Arditti J, Stephens GC, Benzing DH** 1987. Active uptake of amino acids by leaves of an epiphytic vascular plant, *Tillandsia paucifolia* (Bromeliaceae). Plant Physiology 83: 681-684.

**Ögren E, Evans JR** 1993. Photosynthetic light-response curves, I. The influence of $CO_2$ partial pressure and leaf inversion. Planta 189: 182-190.

**Ong BL, Kluge M, Friemert V** 1986. Crassulacean acid metabolism in the epiphytic ferns *Drymoglossum piloselloides* and *Pyrrosia longifolia*: studies on the responses to environmental signals. Plant, Cell and Environment 9: 547-557.

**Pedley TJ** (ed) 1977. Scale effects in animal locomotion. New York/London. Academic Press.

**Penning de Vries FWT** 1975. Use of assimilates in higher plants. In: Cooper JP (ed), Photosynthesis and productivity in different environments. London. Cambridge University Press: 459-480.

**Pessin LJ** 1924. A physiological and anatomical study of the leaves of *Polypodium polypodioides*. American Journal of Botany 11: 370-381.

**Pessin LJ** 1925. An ecological study of the polypody fern *Polypodium polypodioides* as an epiphyte in Mississippi. Ecology 6: 17-38.

**Picon C, Guehl JM, Ferhi A** 1996. Leaf gas exchange and carbon isotope composition responses to drought in a drought-avoiding (*Pinus pinaster*) and a drought-tolerant (*Quercus petraea*) species under present and elevated $CO_2$ concentrations. Plant, Cell and Environment 19: 182-190.

**Pisek A, Berger E** 1938. Kutikuläre Transpiration und Trockenresistenz isolierter Blätter und Sprosse. Planta 28: 124-155.

**Pittendrigh CS** 1948. The Bromeliad-Anopheles-Malaria complex in Trinidad. I-The Bromeliad flora. Evolution 2: 58-89.

**Poorter H, Remkes C, Lambers H** 1990. Carbon and Nitrogen Economy of 24 Wild Species Differing in Relative Growth Rate. Plant Physiology 94: 621-627.

**Popp M, Kramer D, Lee H, Diaz M, Ziegler H, Lüttge U** 1987. Crassulacean acid metabolism in tropical dicotyledonous trees of the genus *Clusia*. Trees 1: 238-247.

**Putz FE, Holbrook NM** 1989. Strangler fig rooting habits and nutrient relations in the Llanos of Venezuela. American Journal of Botany 76: 781-788.

**Rada F, Jaimez R** 1992. Comparative ecophysiology and anatomy of terrestrial and epiphytic *Anthurium bredemeyeri* Schott in a tropical Andean cloud forest. Journal of Experimental Botany 43: 723-727.

**Rauh W** 1990. Bromelien. Stuttgart. Ulmer.

**Reinert F, Meirelles ST** 1993. Water acquisition strategy shifts in the heterophyllous saxicolous bromeliad, *Vriesea geniculata* (Wawra) Wawra. Selbyana 14: 80-88.

**Renner O** 1933. Zur Kenntnis des Wasserhaushalts javanischer Kleinepiphyten. Planta 18: 215-287.

**Rickson FR** 1979. Absorption of animal tissue breakdown products into a plant stem - the feeding of a plant by ants. American Journal of Botany 66: 87-90.

**Röbbelen G** 1957. Untersuchungen an strahleninduzierten Blattmutanten von *Arabidopsis thaliana* (L.) Heynh. Zeitschrift für Induktive Abstammungs-Vererbungslehre 88: 189-252.

**Roberts A, Griffiths H, Borland AM, Reinert F** 1996. Is crassulacean acid metabolism activity in sympatric species of hemi-epiphytic stranglers such as *Clusia* related to carbon cycling as a photoprotective process? Oecologia 106: 28-38.

**Sarrus PF, Rameaux JF** 1838. Rapport sur un mémoir adressé a l'Académie Royale de Médecine. Commissaires Robiquet et Thillaye, rapprteurs. Bull. Acad. Roy. Med. (Paris) 3: 1094-1100.

**Savé R, Marfà JO, Rabella R** 1985. Water relations and photosynthetic rate of an epiphytic fern during a cycle of drying/rewatering. HortScience 20: 113-115.

**Schäfer C, Lüttge U** 1986. Effects of water stress on gas exchange and water relations of a succulent epiphyte, *Kalanchoë uniflora*. Oecologia 71: 127-132.

**Schlesinger WJ, Marks PL** 1977. Mineral cycling and the niche of Spanish moss, *Tillandsia usneoides* L. American Journal of Botany 64: 1254-1262.

**Schmidt G, Stuntz S, Zotz G** 2001. Plant size - an ignored parameter in epiphyte ecophysiology? Plant Ecology 153: 65-72.

**Schmidt G, Zotz G** 2001. Ecophysiological consequences of differences in plant size - *in situ* carbon gain and water relations of the epiphytic bromeliad, *Vriesea sanguinolenta* Cogn. & Marchal. Plant, Cell and Environment 21: 101-112.

**Schmidt G, Zotz G** 2000. Herbivory in the epiphyte *Vriesea sanguinolenta* Cogn. & Marchal (Bromeliaceae). Journal of Tropical Biology 16: 829-839.

**Schmidt-Nielsen K** 1984. Scaling. Why is animal size so important? Cambridge. Cambridge University Press.

**Schmitt AK, Lee HSJ, Lüttge U** 1988. The response of the $C_3$-CAM tree, *Clusia rosea*, to light and water stress. I. Gas exchange characteristics. Journal of Experimental Botany 39: 1581-1590.

**Schoonhoven LM, Jermy T, van Loon JJA** 1998. Insect-plant biology, from physiology to evolution. London. Chapman & Hall.

**Schulze E-D, Hall AE** 1982. Stomatal responses, water loss and $CO_2$ assimilation rates of plants in contrasting environments. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds), Water Relations and Carbon Assimilation. Berlin. Springer-Verlag: 181-230.

**Sengupta B, Nandi AS, Samanta RK, Pal D, Sengupta DN, Sen SP** 1981. Nitrogen fixation in the phyllosphere of tropical plants: occurrence of phyllosphere nitrogen-fixing micro-organisms in Eastern India and their utility for the growth and nitrogen nutrition of host plants. Annals of Botany 48: 705-716.

**Shreve F** 1908. Transpiration and water storage in *Stelis ophioglossoides* Sw. Plant World 2: 165-172.

**Silvertown JW, Doust JL** 1993. Introduction to plant population biology. Oxford. Blackwell.

**Sinclair R** 1983a. Water relations of tropical epiphytes. I. Relationships between stomatal resistance, relative water content and the components of water potential. Journal of Experimental Botany 34: 1652-1663.

**Sinclair R** 1983b. Water relations of tropical epiphytes. II. Performance during droughting. Journal of Experimental Botany 34: 1664-1675.

**Sinclair R** 1984. Water relations of tropical epiphytes. III. Evidence for CAM. Journal of Experimental Botany 35: 1-7.

**Sipes DL, Ting IP** 1985. Crassulacean acid metabolism and crassulacean acid metabolism modifications in *Peperomia camptotricha*. Plant Physiology 77: 59-63.

**Smith EL** 1937. The influence of light and carbon dioxide on photosynthesis. Journal of General Physiology 20: 807-830.

**Smith JAC, Griffiths H, Bassett M, Griffiths NM** 1985. Day-night changes in the leaf water relations of epiphytic bromeliads in the rain forest of Trinidad. Oecologia 067: 475-485.

**Smith JAC, Griffiths H, Lüttge U, Crook CE, Griffiths NM, Stimmel KH** 1986. Comparative ecophysiology of CAM and C3 bromeliads. IV. Plant water relations. Plant, Cell and Environment 9: 395-410.

**Solbrig OT** (ed) 1980. Demography and evolution in plant populations. Botanical Monographs. Oxford. Blackwell Scientific.

**Starnecker G, Winkler S** 1982. Zur Ökologie epiphytischer Farne aus Südbrasilien. II. Anatomische und physiologische Anpassungen. Flora 172: 57-68.

**Stewart GR, Schmidt S, Handley LL, Turnbull MH, Erskine PD, Joly CA** 1995. $^{15}$N natural abundance of vascular rainforest epiphytes: implications for nitrogen source and acquisition. Plant, Cell and Environment 18: 85-90.

**Stiles KC, Martin CE** 1996. Effects of drought stress on $CO_2$ exchange and water relations in the CAM epiphyte *Tillandsia utriculata* (Bromeliaceae). Journal of Plant Physiology 149: 721-728.

**Stuart TS** 1968. Revival of Respiration and Photosynthesis in Dried Leaves of *Polypodium polypodioides*. Planta 83: 185-206.

**Stuntz S, Zotz G** 2000. Photosynthesis in vascular epiphytes - a survey of 27 species of diverse taxonomic origin. Flora, in press.

**Swarbrick JT, Skarratt DB** 1994. The ecological requirements and potential Australian distribution of pond apple (*Annona glabra*), Wet Tropics Management Agency.

**Tanner EVJ** 1977. Four montane rain forests of Jamaica: a quantitative characterization of the floristics, the soils and the foliar mineral levels, and a discussion of the interrelations. Journal of Ecology 65: 883-918.

**Tenhunen JD, Lange OL, Gebel J, Beyschlag W, Weber JA** 1984. Changes in photosynthetic capacity, carboxylation efficiency, and $CO_2$ compensation point associated with midday stomatal closure and midday depression of net $CO_2$ exchange of leaves of *Quercus suber*. Planta 162: 193-203.

**Tenhunen JD, Weber JA, Yocum CS, Gates DM** 1976. Development of a photosynthesis model with an emphasis on ecological application. II. Analysis of a data set describing the $P_M$ surface. Oecologia 26: 101-119.

**Thompson DAW** 1917. On growth and form. Cambridge. Cambridge University Press.

**Tichá I** 1982. Photosynthetic characteristics during ontogenesis of leaves. 7. Stomata density and sizes. Photosynthetica 16: 375-471.

**Ting IP** 1989. Photosynthesis of arid and subtropical succulent plants. Aliso 12: 387-406.

**Ting IP, Hann J, Holbrook NM, Putz FE, Sternberg LdSL, Price D, Goldstein G** 1987. Photosynthesis in hemiepiphytic species of *Clusia* and *Ficus*. Oecologia 74: 339-346.

**Treseder KK, Davidson DW, R. EJ** 1995. Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. Nature 375: 137-139.

**Vandermeer J** 1978. Choosing category size in a stage projection matrix. Oecologia 32: 79-84.

**Von Caemmerer S, Farquhar GD** 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153: 376-387.

**Wadasinghe G, Hew CS** 1995. The importance of back shoots as a source of photoassimilates for growth and flower production in *Dendrobium* cv. Jashika Pink (Orchidaceae). Journal of Horticultural Science 70: 207-214.

**Walker D** 1992. Excited leaves. New Phytologist 121: 325-345.

**Windsor DM** 1990. Climate and moisture variability in a tropical forest: long-term records from Barro Colorado Island, Panamá. Washington. Smithsonian Institution.

**Winter K, Medina E, Garcia V, Mayoral ML, Muniz R** 1985. Crassulacean acid metabolism in roots of the leafless orchid, *Campylocentrum tyrridion* Garay & Dunsterv. Journal of Plant Physiology 118: 73-78.

**Winter K, Osmond CB, Hubick KT** 1986. Crassulacean acid metabolism in the shade. Studies on an epiphytic fern, *Pyrrosia longifolia*, and other rainforest species from Australia. Oecologia 68: 224-230.

**Winter K, Wallace BJ, Stocker GC, Roksandic Z** 1983. Crassulacean acid metabolism in Australian vascular epiphytes and some related species. Oecologia 57: 129-141.

**Winter K, Zotz G, Baur B, Dietz K-J** 1992. Light and dark $CO_2$ fixation in *Clusia uvitana* and the effect of plant water status and $CO_2$ availability. Oecologia 91: 47-51.

**Wong SC, Hew CS** 1976. Diffusive resistance, titratable acidity, and $CO_2$ fixation in two tropical epiphytic ferns. American Fern Journal 66: 121-124.

**Wong S-C, Hew C-S** 1975. Do Orchid leaves photorespire? American Orchid Society Bulletin 44: 902-906.

**Yong JWH, Hew CS** 1995a. The importance of photoassimilate contribution from the current shoot and connected back shoots to inflorescence size in the thin-leaved sympodial orchid *Oncidium* Goldiana. International Journal of Plant Sciences 156: 450-459.

**Yong JWH, Hew CS** 1995b. Partitioning of $^{14}C$ assimilates between sources and sinks during different growth stages in the sympodial thin-leaved orchid *Oncidium* Goldiana. International Journal of Plant Sciences 156: 188-196.

**Yong JWH, Hew CS** 1995c. The patterns of photoassimilate partitioning within connected shoots for the thin-leaved sympodial orchid *Oncidium* Goldiana during different growth stages. Lindleyana 10: 92-108.

**Zimmerman JK** 1990. Role of pseudobulbs in growth and flowering of *Catasetum viridiflavum* (Orchidaceae). American Journal of Botany 77: 533-542.

**Zimmerman JK** 1991. Ecological correlates of labile sex expression in the orchid *Catasetum viridiflavum*. Ecology 72: 597-608.

**Zimmerman JK, Aide TM** 1989. Patterns of fruit production in a neotropical orchid: pollinator vs. resource limitation. American Journal of Botany 76: 67-73.

**Zimmerman JK, Ehleringer JR** 1990. Carbon isotope ratios are correlated with irradiance levels in the Panamanian Orchid *Catasetum viridiflavum*. Oecologia 83: 247-249.

**Zotz G** 1995. How fast does an epiphyte grow? Selbyana 16: 150-154.

**Zotz G** 1997a. Photosynthetic capacity increases with plant size. Botanica Acta 110: 306-308.

**Zotz G** 1997b. Substrate use of three epiphytic bromeliads. Ecography 20: 264-270.

**Zotz G** 1998. Demography of the epiphytic orchid, *Dimerandra emarginata*. Journal of Tropical Ecology 14: 725-741.

**Zotz G** 1999. What are backshoots good for? Seasonal changes in mineral, carbohydrate, and water content of different organs of the epiphytic orchid, *Dimerandra emarginata*. Annals of Botany 84: 791-798.

**Zotz G, Andrade J-L** 1998. Water relations of two co-occurring epiphytic bromeliads. Journal of Plant Physiology 152: 545-554.

**Zotz G, Andrade J-L** 2000. Ecología de epífitas y hemiepífitas. In: Guariguata M, Kattan G (eds), Ecología de bosques lluviosos neotropicales. San José, Costa Rica. IICA, in press.

**Zotz G, Bermejo P, Dietz H** 1999a. The epiphyte vegetation of *Annona glabra* on Barro Colorado Island, Panama. Journal of Biogeography 26: 761-776.

**Zotz G, Harris G, Königer M, Winter K** 1995. High rates of photosynthesis in the tropical pioneer tree, *Ficus insipida* Willd. Flora 190: 265-272.

**Zotz G, Patiño S, Tyree MT** 1997. $CO_2$ gas exchange and the occurrence of CAM in tropical woody hemiepiphytes. Flora 192: 143-150.

**Zotz G, Reichling P, Krack S** 1999b. Another woody hemiepiphyte with CAM: *Havetiopsis flexilis* Spruce ex Planch. & Tr. (Clusiaceae). Flora 194: 215-220.

**Zotz G, Thomas V** 1999. How much water is in the tank? Model calculations for two epiphytic bromeliads. Annals of Botany 83: 183-192.

**Zotz G, Tyree MT** 1996. Water stress in the epiphytic orchid, *Dimerandra emarginata* (G. Meyer) Hoehne. Oecologia 107: 151-159.

**Zotz G, Tyree MT, Cochard H** 1994. Hydraulic architecture, water relations and vulnerability to cavitation of *Clusia uvitana*: a $C_3$-CAM tropical hemiepiphyte. New Phytologist 127: 287-295.

**Zotz G, Winter K** 1993. Short-term photosynthesis measurements predict leaf carbon balance in tropical rain-forest canopy plants. Planta 191: 409-412.

**Zotz G, Winter K** 1994a. Annual carbon balance and nitrogen use efficiency in tropical $C_3$ and CAM epiphytes. New Phytologist 126: 481-492.

**Zotz G, Winter K** 1994b. A one-year study on carbon, water and nutrient relationships in a tropical $C_3$-CAM hemiepiphyte, *Clusia uvitana*. New Phytologist 127: 45-60.

**Zotz G, Winter K** 1994c. Predicting annual carbon balance from leaf nitrogen. Naturwissenschaften 81: 449.

**Zotz G, Winter K** 1996. Diel patterns of $CO_2$ exchange in rainforest canopy plants. In: Mulky SS, Chazdon RL, Smith AP (eds), Tropical forest plant ecophysiology. New York. Chapman & Hall: 89-113.

**Zotz G, Ziegler H** 1997. The occurrence of crassulacean acid metabolism among vascular epiphytes from Central Panama. New Phytologist 137: 223-229.

**Zotz G, Ziegler H** 1999. Size-related differences in carbon isotope discrimination in the epiphytic orchid, *Dimerandra emarginata*. Naturwissenschaften 86: 39-40.

# ABBREVIATION LIST

| | |
|---|---|
| **Index** $^{sec}$ | Indexed variable refers to all of the three sections, i.e. apical, central, basal |
| **Indices** $^{ap,\ ce,\ ba}$ | Indexed variable refers to the apical, central or basal section |
| **Index** $^{Sh}$ | Indexed variable refers to the shoot |
| **Index** $^{Lb}$ | Indexed variable refers to leaf bases |
| **Index** $^{plant}$ | Indexed variable refers to the entire plant |
| **Index** $^{shed}$ | Indexed variable refers to shed leaves |
| **Subscript** $_{2m}$ | Value of variable after two months |
| **Subscript** $_{0}$ | Value of variable at the beginning (of calculations) |
| **c + unit** | Centi |
| **k + unit** | Kilo |
| **M + unit** | Mega |
| **m + unit** | Milli |
| **n + unit** | Nano |
| **µ + unit** | Micro |

| | |
|---|---|
| $\alpha$ | Photosynthetic yield |
| **a** | Year |
| **ANCOVA** | Analysis of co-variance |
| **ANOVA** | Analysis of variance |
| $A_{proj}$ | Projected plant area |
| **BCI** | Barro Colorado Island |
| **BCNM** | Barro Colorado Nature Monument |
| $\Delta c$ | Gradient of water vapour concentration inside and outside of the leaf |
| **°C** | Degree Celsius |
| ***c*** | Conversion factor for incorporated $CO_2$ to DM gain after Penning de Vries (1975) |
| **c.** | Approximately |
| $C_3$ | $C_3$ pathway |
| **CAM** | Crassulacean acid metabolism |
| $C_{act}$ | Actual tank water content |
| $C_{max}$ | Maximal tank water content |
| $CB_{24h}$ | Daily carbon balance |
| $c_i$ | $CO_2$ concentration inside the leaf |
| $CO_2$ | Carbon dioxide |
| **comm.** | Communication |
| $\Delta$ | Difference |
| **d** | Day |
| $d_{empty\ n}$ | Correction factor accounting for the relative reduction of gas exchange for day n after the tank is empty |
| **df** | Degree of freedom |
| **DM** | Dry mass |
| $DMB_a$ | Annual DM balance |
| **DML** | Dry mass loss |
| **e.g.** | For example |
| **F** with “+” or “-“ | Fertilized |
| **F** | Rain throughfall through the canopy after Frank & Curtis (1981) |
| $FM_{SAT}$ | Fresh mass of fully hydrated (= water saturated) organs or plants |
| **g** | Gram |
| **gR** | Growth respiration |
| $g_w$ | Leaf conductance to water vapor transfer |
| $\Delta H^+$ | Nocturnal acidification |
| **h** | Hour |
| $H^+$ | Titrable acidity |
| $H_2O$ | Water |
| **ha** | Hectare |
| **i.e.** | That is |
| **IRGA** | Infrared gas analyzer |
| **K** | Potassium |

| | |
|---|---|
| ***k*** | Correction factor accounting for the reduction of evaporation in the crown of *A. glabra* compared to evaporation above the canopy |
| **l** | Liter |
| **LA** | Leaf area |
| **LAI** | Leaf area index |
| **LL** | Leaf length |
| **$LL_{max}$** | Length of the longest leaf of a plant |
| **ln** | Logarithm, basis e |
| **log** | Logarithm, basis 10 |
| **µeq** | Micro equivalents |
| **m** | Meter |
| **max** | Maximal |
| **min** | Minute |
| **Min** | Minimum |
| **mR** | Maintenance respiration |
| **N** | Nitrogen |
| **n** | Sample size |
| **n.d.** | Not determined |
| **n.s.** | Not significant |
| **$nL^{shed}$** | Number of leaves shed within one year |
| **NP** | Net photosynthesis |
| **$NP_{24h}$** | Net photosynthesis per 24h |
| **$NP_{max}$** | Maximal net photosynthesis |
| **$NP_{pot}^{sec}$** | Potential $NP_{max}$ of a section under saturating PPFD |
| **$O_2$** | Oxygen |
| **obs.** | Observation |
| **p** | Significance value |
| **P** | Permeance |
| **Pa** | Pascal |
| **PC** | Photosynthetic capacity |
| **pers.** | Personal |
| **PLA** | Plant leaf area |
| **PPFD** | Photosynthetic photon flux density |
| **PPLA** | Projected plant leaf area |
| **PsbL** | Length of the most recent pseudobulb |
| **$r^2$** | Coefficient of determination |
| **R** | Respiration |
| **$R_{24h}$** | Respiration per 24h |
| **$R_d$** | Dark respiration |
| **rDM** | Relative DM |
| **$rDM^{dSh}$** | Relative DM of dead shoot tissue (related to shoot DM) |
| **RGR** | Relative growth rate after Kluge & Ting (1978) |
| **rLC** | Relative light response curve (standardized to $NP_{max}$) |
| **rLI** | Relative light interception of a plant part (related to PPFD above the plant) |
| **rLL** | Relative leaf length (related to total length of the leaf) |
| **RT** | Residual transpiration |
| **RWD** | Relative water deficit |
| **$RWD_{STC}$** | Relative water deficit at stomata closure |
| **s** | Second |
| **SC** | Size category |
| **SI** | Succulence index |
| **SL** | Shoot length |
| **SLW** | Specific leaf weight |
| **$S_{max}$** | Maximal tank water surface of a plant |
| **SWC** | Shoot water content |
| **syn.** | Synonym |
| **$\Delta t$** | Time interval |
| **T** | Transpiration |
| ***t*** | Correction factor that accounts for temperature dependent changes in respiration |
| **$t_d$** | By adjusting respiration to average day time temperature |
| **$t_n$** | By adjusting respiration to average night time temperature |
| **TM** | Mean hourly transmission through the tree crown |

| | |
|---|---|
| **Tp** | Temperature |
| **UK** | United Kingdom |
| **unpubl.** | Unpublished |
| **USA** | United States of America |
| **v/v** | Volume per volume |
| **vs.** | Versus |
| **W** with “+” or “-“ | Watered |
| **Ψ** | Water potential |

*Comment to time and date formats*

Although the dissertation is in ‘American’ English I used the European way of expressing time and date, which facilitated the electronic data processing of time series.

# PUBLICATIONS

## Journal articles

**Schmidt G, Zotz G** 2000. Herbivory in the epiphyte *Vriesea sanguinolenta* Cogn. & Marchal (Bromeliaceae). Journal of Tropical Biology 16: 829-839.

**Schmidt G, Stuntz S, Zotz G** 2001. Plant size - an ignored parameter in epiphyte ecophysiology? Plant Ecology 153: 65-72.

**Schmidt G, Zotz G** 2001. Ecophysiological consequences of differences in plant size - in situ carbon gain and water relations of the epiphytic bromeliad, *Vriesea sanguinolenta* Cogn. & Marchal. Plant, Cell and Environment 24: 101-112.

**Zotz G, Hietz P, Schmidt G** 2001. Small plants, large plants - the importance of plant size for the physiological ecology of vascular epiphytes. Journal of Experimental Botany, accepted.

**Schmidt G, Zotz G** 2001. Growth in two epiphyte species - a demographic approach. Journal of Vegetation Science, submitted.

**Schmidt G, Zotz G** 2001. Water availability -the proximate cause for size-dependent ecophysiological differences in vascular epiphytes? in prep.

## Meeting Participation

**Schmidt G, Zotz G** 1999. Carbon balance of an epiphytic tank bromeliad - a model links leaf physiology and plant growth. Annual meeting of the British Ecological Society, Leeds, UK.

**Schmidt G, Zotz G** 1999. Carbon balance of an epiphytic tank Bromeliad – a model links leaf physiology and plant growth. Internationales Graduiertenkolleg, Retzbach, Germany.

**Schmidt G, Zotz G** 1998. An increase of photosynthetic capacity with plant size - a general principle in tropical vascular epiphytes? Annual meeting of the British Ecological Society, York, UK.

**Schmidt G, Zotz G** 1998. Size: the neglected parameter in ecophysiological studies with vascular epiphytes. European Science Foundation, Oxford, UK.

**Schmidt G, Stuntz S, Zotz G** 1998. Plant size – an ignored parameter in epiphyte ecophysiology? Deutsche Botanikertagung, Bremen, Germany.