Annals of Botany **103**: 477–484, 2009
doi:10.1093/aob/mcn231, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

Highly efficient uptake of phosphorus in epiphytic bromeliads

Uwe Winkler[1,]* and Gerhard Zotz[1,2]

[1]*Functional Ecology Group, Universität Oldenburg, D-26111 Oldenburg, Germany and* [2]*Smithsonian Tropical Research Institute, Balboa, Panama*

Received: 29 August 2008 Returned for revision: 22 September 2008 Accepted: 17 October 2008 Published electronically: 25 November 2008

• *Background and Aims* Vascular epiphytes which can be abundant in tree crowns of tropical forests have to cope with low and highly intermittent water and nutrient supply from rainwater, throughfall and stem flow. Phosphorus rather than nitrogen has been suggested as the most limiting nutrient element, but, unlike nitrogen, this element has received little attention in physiological studies. This motivated the present report, in which phosphate uptake kinetics by leaves and roots, the subsequent distribution within plants and the metabolic fate of phosphate were studied as a step towards an improved understanding of physiological adaptations to the conditions of tree canopies.

• *Methods* Radioactively labelled [^{32}P]phosphate was used to study uptake kinetics and plant distribution of phosphorus absorbed from bromeliad tanks. The metabolism of low molecular phosphorus metabolites was analysed by thin-layer chromatography followed by autoradiography.

• *Key Results* Uptake of phosphate from tanks is an ATP-dependent process. The kinetics of phosphorus uptake suggest that epiphytes possess effective phosphate transporters. The K_m value of 1·05 μM determined for leaves of the bromeliad *Aechmea fasciata* is comparable with values obtained for the high affinity phosphate transporters in roots of terrestrial plants. In this species, young leaves are the main sink for phosphate absorbed from tank water. Within these leaves, phosphate is then allocated from the basal uptake zone into distal sections of the leaves. More than 80 % of the phosphate incorporated into leaves is not used in metabolism but stored as phytin.

• *Conclusions* Tank epiphytes are adapted to low and intermittent nutrient supply by different mechanisms. They possess an effective mechanism to take up phosphate, minimizing dilution and loss of phosphorus captured in the tank. Available phosphorus is taken up from the tank solution almost quantitatively, and the surplus not needed for current metabolism is accumulated in reserves, i.e. plants show luxury consumption. Young, developing leaves are preferentially supplied with this nutrient element. Taken together, these features allow epiphytes the efficient use of scarce and variable nutrient supplies.

Key words: Epiphytic bromeliads, phosphorus uptake, forest canopies, luxury consumption, phytotelms, plant nutrition, *Aechmea fasciata*.

INTRODUCTION

Vascular epiphytes form a highly diverse group of plants which are especially common in humid tropical forests (Benzing, 1990). Even in these habitats, which are characterized by abundant absolute precipitation, growth in tree crowns lacking soil is equivalent to highly intermittent water and nutrient supply. A suite of anatomical, morphological and physiological adaptations allows epiphytes to cope with this irregular resource supply, a particularly remarkable adaptation being the ‘tank’ found in many bromeliads, an impounding structure formed by overlapping leaf bases. On the one hand, such an impoundment improves the supply of nutrients by acting as a catchment area for water and organic debris; on the other hand, catchment fluids prolong the time available to take up the very same water and nutrient resources. In the extreme case, this uptake is achieved exclusively by specialized leaf trichomes, whereas the function of roots is reduced to that of non-absorbing holdfasts (Benzing, 2000).

Although alleviating the resource limitations in the epiphytic habitat, available evidence indicates that *in situ* many if not most individual tanks may fall dry for extended periods of time (Zotz and Thomas, 1999). Moreover, tank bromeliads generally show low contents of nutrient elements (Stuntz and Zotz, 2001) and grow very slowly even under near-optimal conditions (Hietz *et al.*, 2002; Schmidt and Zotz, 2002). These are all typical features of stress-tolerant plants associated with nutrient-poor habitats (Grime, 2001). In this group of plants, nutrient uptake capacities are normally tuned towards the capture of short pulses, and such a combination of high uptake of nutrient elements and slow potential growth frequently leads to an accumulation of reserves (termed ‘luxury consumption’, Chapin, 1980).

Most of the information on the nutrient ecology of epiphytic plants is related to nitrogen. A number of studies suggested that organic nitrogen forms such as amino acids or urea are more important for the nutrition of tank bromeliads than for terrestrial plants (Benzing, 1970; Nyman *et al.*, 1987; Endres and Mercier, 2001; Endres and Mercier, 2003), and a recent study by Inselsbacher *et al.* (2007) reported detailed uptake kinetics of various nitrogen compounds. For example, tanks of *Vriesea gigantea* showed a marked preference for the uptake of NH_4^+ compared with NO_3^-, the uptake rates of glycine being intermediate. Uptake of most nitrogen compounds followed Michaelis–Menten kinetics, and the

* For correspondence. E-mail u.winkler@uni-oldenburg.de

© The Author 2008. Published by Oxford University Press on behalf of the Annals of Botany Company. All rights reserved.
For Permissions, please email: journals.permissions@oxfordjournals.org

estimates of the Michaelis–Menten constants (K_m) were in the range of those of roots of terrestrial plants, indicating an effective uptake system for NH_4^+.

This relative wealth of information on nitrogen uptake in vascular epiphytes contrasts with the few data on the uptake of other important nutrient elements such as phosphorus (see Benzing, 1970; Benzing and Renfrow, 1980). However, in many tropical forests, phosphorus rather than nitrogen limits productivity, and this seems also to be true for many vascular epiphytes. For example, there is evidence that phosphorus is most limiting for reproduction in some bromeliads (Benzing, 1990; Zotz and Richter, 2006). Similarly, a major role for phosphorus in vegetative function is also indicated by higher resorption efficiencies and proficiencies (*sensu* Killingbeck, 1996) for phosphorus than for nitrogen during leaf senescence in a large suite of epiphytes (Zotz, 2004), or by a sharp, 10-fold, decrease in the N : P ratio of field-grown bromeliads when fertilized in the laboratory (Benzing and Renfrow, 1974). Hence, the present study had a 2-fold goal. First, the hypothesis that epiphytic bromeliads show highly efficient uptake of phosphorus was tested with a detailed analysis of the uptake kinetics of phosphorus in leaves and roots. Secondly, the metabolic fate of phosphorus within the plant was investigated as an additional step towards a more mechanistic understanding of phosphorus metabolism.

MATERIALS AND METHODS

Plant material

The main part of this study was carried out with *Aechmea fasciata* 'Primera' plants (Bromeliaceae) that were kindly supplied by a commercial nursery (Corn. Bak B.V., Asseldelft, The Netherlands). An additional experiment was conducted with other bromeliads, *Vriesea splenriet* and *Vriesea duvaliana* (also from Bak B.V.), and two species collected in tropical lowland forests in Panama: *Tillandsia elongata* and *Werauhia sanguinolenta*. All plants had 6–8 leaves, were 8–10 cm high and had a tank volume of approx. 1 mL. In *Aechmea*, only the youngest fully developed leaf has immediate contact with the tank water by its completely rounded and overlapping leaf bases. Older leaves are less connected to the tank solution.

Plants were kept in the greenhouse at 25 °C, at a relative humidity of approx. 40 % and a light dark regime of 12 : 12 h, being illuminated by natural sunlight, supplemented with artificial light (400 W metal halide lamps, master HPI-T plus; Philips, The Netherlands) when necessary to achieve a photosynthetic photon flux density of at least 150–180 µmol m^{-2} s^{-1} at the level of the plants.

Plants received a nutrient solution containing 28 mg L^{-1} NO_3^-, 65 mg L^{-1} NH_4^+, 63 mg L^{-1} PO_4^{3-}, 63 mg L^{-1} K^+ and 13 mg L^{-1} Mg^{2+} once a week, and were otherwise irrigated daily with water. Before the onset of experiments, plants were not fertilized for 14 d.

Phosphorus uptake

Experiments were performed in the radionuclide laboratories of the University of Oldenburg. During experiments, plants were kept in Plexiglas boxes under conditions comparable with those in the greenhouse. In experiments lasting up to 24 h, plants were illuminated during the complete experiment. Before starting the uptake studies, tanks were thoroughly rinsed with the phosphate buffer used later in the experiments. For the experiments, carrier-free [^{32}P]phosphoric acid (Hartmann Analytic, Germany), containing 37 MBq in 100 µL of water (initial specific activity: 5·5 MBq $nmol^{-1}$), was used. Phosphate (P_i) uptake was measured as ^{32}P depletion from tank solutions. The activity of the ^{32}P in the tank solution was adjusted to 25×10^6 dpm by addition of 0·1–1·0 µL from the radioactive preparation to 0·5 mL of unlabelled phosphate buffer, pH 6·1. In all mixtures, buffer capacity was sufficient to maintain the pH value. Final concentrations of 0·1–50 µM P_i were used in experiments to measure the concentration dependence of P_i uptake in *Aechmea*, and 100 µM P_i in experiments to analyse the distribution of P_i in the plant tissue. We compared the uptake rates of five different bromeliads at a concentration of 10 µM P_i. This concentration is regarded as an average value of naturally occurring P_i concentrations available to bromeliads from rainwater; throughfall and stem flow waters, and in tanks of epiphytes, ranging from 2·6 to 24 µM P_i (Benzing, 2000; Richardson *et al.*, 2000). For kinetic measurements, samples were taken every 30 min and uptake rates were calculated from the initial linear phase of ^{32}P depletion. Experiments were performed at a constant tank volume of 0·5 mL. Before sampling, plants were weighed, and weight loss was compensated by adding water. After correcting the tank volume, tank fluids were mixed with Pasteur pipettes and aliquots of 5 µL taken at regular intervals, put into liquid scintillation vials and mixed with 8 mL of scintillation cocktail (Lume gel save, Lumac, Germany). Measurements were done in a Wallac 1415 liquid scintillation counter, supplied with an external standard to calculate dpm rates. Furthermore, in one set of experiments, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) at a concentration of 20 µM was used as an ATPase inhibitor to separate active and passive uptake (Dahlman *et al.*, 2004). In this experiment, tanks were pre-incubated with inhibitor and phosphate buffer for 1 h before starting the measurement of phosphate uptake.

In experiments lasting for >2 d, counting rates were corrected for the half-life of ^{32}P. In one set of experiments, uptake rates of the roots were measured in a similar way, except that incubation solutions were placed into an Erlenmeyer flasks. Plants were tied above the flask in such a way that only the roots came into contact with the radioactive incubation solution. At a wide range of P_i concentrations applied to tanks, the uptake rates were not dependent on the ratio of labelled/unlabelled phosphate.

Cumulative uptake of phosphate from the tanks was calculated according to eqn (1):

$$\text{uptake (nmol)} = PS - (C \times Vol_{Tank} \times PS/V_C/A_{total}) \quad (1)$$

where PS is nmol phosphate in the tank, C is the counting rate in dpm, Vol_{Tank} is the tank volume in µL, V_C is the volume used for measuring the counting rate in µL and A_{total} is the sum of activity in the incubation solution in dpm.

Rates were calculated on a whole-plant basis for time kinetics of P_i uptake or on a dry weight (d. wt) basis for

substrate-dependent P_i uptake kinetics and comparison of different bromeliad species. Dry matter was determined after 24 h at 95 °C in a drying oven.

Distribution within the plant

Pulse–chase experiments were performed to determine the distribution of incorporated P_i within the plant. The incubation solution of 0·5 mL, containing 50 nmol P_i and 25 × 10^6 dpm of ^{32}P actitivity, was completely taken up within 12–15 h. Subsequently, tanks were watered daily for up to 14 d. Thereafter, leaf discs (4 mm diameter) were cut. In further experiments, complete plants were sectioned and cut into pieces (<8 mm). Leaf discs or plant segments were transferred to scintillation vials half filled with cotton to prevent samples from dropping to the bottom of the vial, which would decrease counting rates. After addition of scintillation cocktail, ^{32}P activity was measured without quenching. Before extraction of labelled compounds, suitable plant sections were selected by measuring ^{32}P activities non-destructively by Cerenkov counting without scintillation cocktail.

Phosphate metabolism

Low molecular weight acid-soluble molecules were extracted from ^{32}P-incubated leaf discs or from leaf segments after homogenization in liquid nitrogen. Extractions were performed for 1 h at 40 °C in a solution with 0·25 M Na_2SO_4 and 0·1 N HCl. Large molecules such as nucleic acids or membrane lipids are not extractable with this solvent (Organ *et al.*, 1988). Negatively charged metabolites in the extract were separated by one-dimensional thin-layer chromatography (TLC) on PEI-cellulose (Macherey & Nagel, Düren, Germany) according to Sun *et al.* (2007). Sample aliquots as spots of 1 µL were applied to the plate and separated in 1 N HCl. Developed plates were oven-dried at 60 °C. Labelled compounds were visualized by autoradiography using Biomax MR films (Kodak, USA). The following standards for the identification of labelled compounds were used: P_i (1 µL of incubation medium), phytin (30 mg mL^{-1}), and ATP and GTP (2 mg mL^{-1}). Phytin and the nucleotides were stained with molybdate reagent (Wood, 1968).

Data analysis

All statistical analyses were done with R 2·6·0. (R Development Core Team, 2007). Error terms are standard deviations. The Michaelis constant (K_m) was determined via non-linear regression for three replicate runs.

RESULTS

The nutrient-starved *Aechmea* plants took up P_i very rapidly: approx. 90 % of the ^{32}P activity was withdrawn from the tanks within 12 h (Fig. 1). After complete removal of ^{32}P from tanks, plant tissue was analysed for recovery of activity. About 88 ± 9 % ($n = 4$) of the ^{32}P removed from the tanks reappeared in the plant tissue. Loss of activity was low, which allows estimation of the incorporation of P_i into the plant tissue from the decrease of radioactivity in the tanks

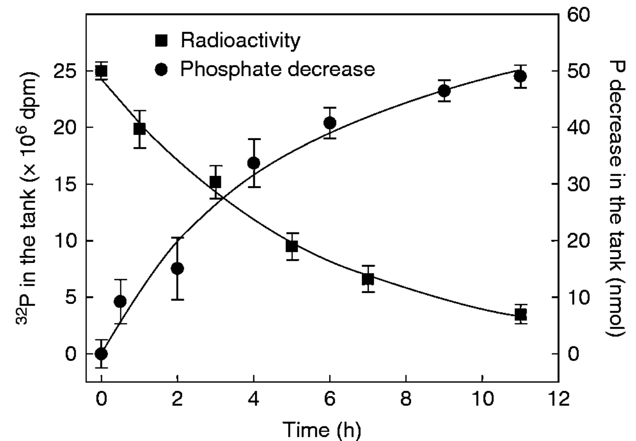

FIG. 1. Time course of phosphate depletion from tanks of *Aechmea* plants. Tanks were filled with a mixture of labelled ^{32}P (total activity of 25 × 10^6 dpm) and 50 nmol unlabelled phosphate buffer, pH 6·1 in a total volume of 0·5 mL. Phosphate (P) decrease in the tank was calculated from the decrease of radioactivity. Data are means ± s.d., $n = 4$. The regression equations are $y = 24{\cdot}46 \times e^{-0{\cdot}18x}$; $r^2 = 0{\cdot}99$, for decrease of radioactivity and $y = (75{\cdot}8\, x)/(5{\cdot}6 + x)$; $r^2 = 0{\cdot}99$, for phosphate uptake (x = time in h).

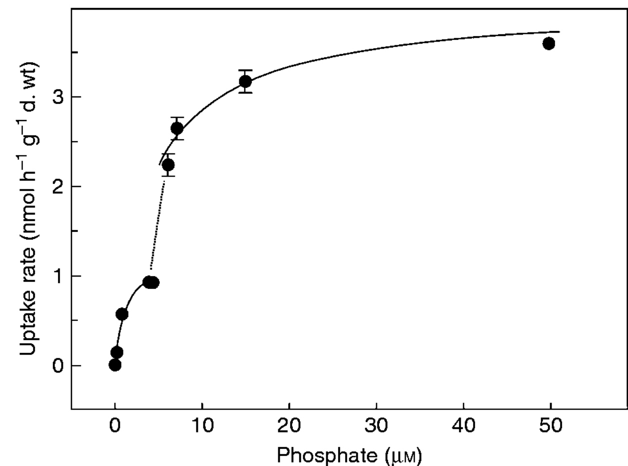

FIG. 2. Biphasic kinetics of phosphate uptake from tanks of *Aechmea* in the presence of 0·1–50 µM substrate. The transition is not well resolved by the data, and is indicated by a dotted region between the regression lines. Uptake rates (R) were calculated from the decrease of ^{32}P radioactivity during the first 1–2 h of uptake. Data are means ± s.d., $n = 4$. The regression equations are $R = 1{\cdot}23 \times x/(1{\cdot}14 + x)$ for the putative high affinity system and $R = 4{\cdot}02x/(4{\cdot}02 + x)$ for the low affinity system, $r^2 > 0{\cdot}98$; x = phosphate concentration in µM. K_m values at low (P_i < 5 µM) and high phosphate concentrations (P_i = 5–50 µM) were calculated using Eadie–Hofstee plots.

(Fig. 1). *Aechmea* plants were capable of taking up approx. 40 nmol P_i from a tank volume of 0·5 mL within 12–15 h, depending on the size of the plants used.

The concentration dependence of P_i uptake was measured in *Aechmea* at 0·1–50 µM P_i (Fig. 2). A K_m value of 1·05 ± 0·35 µM was calculated using the Michaelis–Menten plot. However, uptake rates increased much faster in the low micromolar range of P_i than at concentrations >10 µM. Therefore, concentration dependencies were calculated separately from 0·1 to 5 µM and from 5 to 50 µM P_i applying the Eadie–Hofstee

plot. Different K_m values of $1{\cdot}07 \pm 0{\cdot}25$ μM P_i for the low micromolar range and $4{\cdot}43 \pm 1{\cdot}4$ μM for P_i concentrations >5 μM indicate a biphasic uptake system, which allows rapid uptake of P_i at concentrations that are naturally available to bromeliads. Comparison of the uptake rates of *A. fasciata* at 10 μM P_i with those of four other species of tank bromeliads revealed quite similar values in *V. duvaliana* and *T. elongata*, and significantly lower values in *W. sanguinolenta* and *V. splenriet* (Table 1). Subsequently, the nature of the uptake mechanism in *Aechmea* was studied further. Applying the ATPase inhibitor CCCP resulted in a 93 % inhibition of cumulative P_i uptake compared with controls (Fig. 3).

Roots of *Aechmea* only removed about 7 % of the P_i from the incubation medium compared with tanks (Fig. 4). After thoroughly rinsing the root surfaces, no ^{32}P activity was detectable in root tissue, indicating that label removed from the incubation medium was only adsorbed at the root surface and not taken up into living root tissue.

In experiments intended to determine the fate of nutrients within a plant, tanks were only watered after the initial ^{32}P pulse. Thirty hours after labelling, the highest uptake of P_i was found in the tissues in direct contact with the tank (Fig. 5), i.e. especially the leaf surrounding the tank and the stem fraction containing meristematic tissue and other leaf bases, but also the leaf immediately adjacent to the tank-forming leaf (fifth leaf in Fig. 5). Differences in ^{32}P among organs were highly significant (two-way ANOVA; $F_{7,64} = 65{\cdot}3$; $P < 0{\cdot}001$), but relative distributions changed with time (Table 2, significant interaction term), with a pronounced increase in P_i in leaves that were slightly older yet still actively

TABLE 1. *Uptake of phosphate from tanks of different bromeliads*

Species	Uptake rate (nmol phosphate h^{-1} g^{-1} d. wt)
Aechmea fasciata	$3{\cdot}52 \pm 1{\cdot}66^a$
Vriesea duvaliana	$3{\cdot}69 \pm 1{\cdot}01^a$
Vriesea splenriet	$0{\cdot}72 \pm 0{\cdot}27^b$
Werauhia sanguinolenta	$1{\cdot}25 \pm 0{\cdot}40^b$
Tillandsia elongata	$3{\cdot}73 \pm 0{\cdot}91^a$

Tanks were filled with 10 μM phosphate at the beginning of the experiments. Data are means ± s.d. ($n = 4$). Different superscripts indicate significant differences (one-way ANOVA, *post hoc* Tukey-test, $P < 0{\cdot}05$).

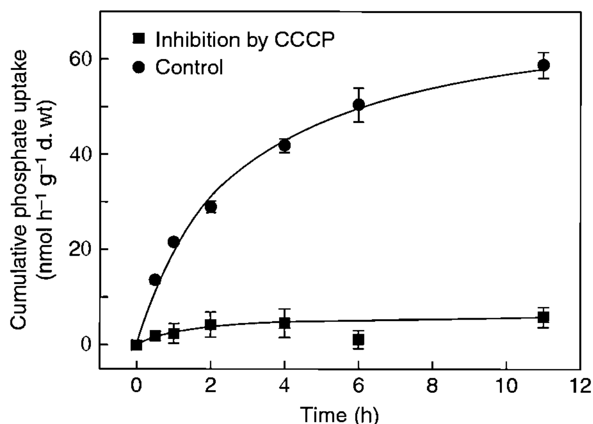

FIG. 3. Inhibition of phosphate uptake from tanks of *Aechmea* by carbonyl cyanide *m*-chlorophenylhydrazone (CCCP); uptake was inhibited by 93 %. Experimental details are as described in Fig. 1. Data are means ± s.d., $n = 4$. The regression equations are $y = 71{\cdot}61x/(2{\cdot}64 + x)$, $r^2 = 0{\cdot}99$, for the control and $y = 6{\cdot}46x/(1{\cdot}47 + x)$, $r^2 = 0{\cdot}99$, for inhibition by CCCP (x = time in h).

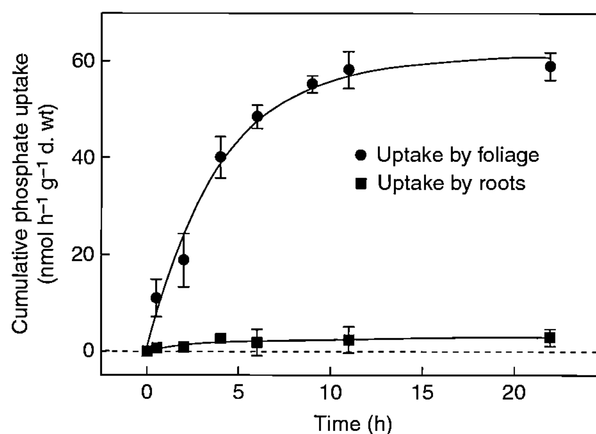

FIG. 4. Uptake of phosphate by roots of *Aechmea* compared with uptake by foliage. Experimental details for the uptake by tanks are as described in Fig. 1. Roots were incubated in 10 mL of medium containing the same concentration of labelled and unlabelled phosphate used for uptake from tanks. In thoroughly rinsed root tissue, no ^{32}P activity was measured. Data are means ± s.d., $n = 4$. The regression equations are $y = 61{\cdot}0 - 60{\cdot}4\ e^{-x/4{\cdot}05}$, $r^2 = 0{\cdot}98$ for uptake by foliage and $y = 3{\cdot}20x/(2{\cdot}82 + x)$, $r^2 = 0{\cdot}81$, $P < 0{\cdot}05$ for uptake by roots (x = time in h).

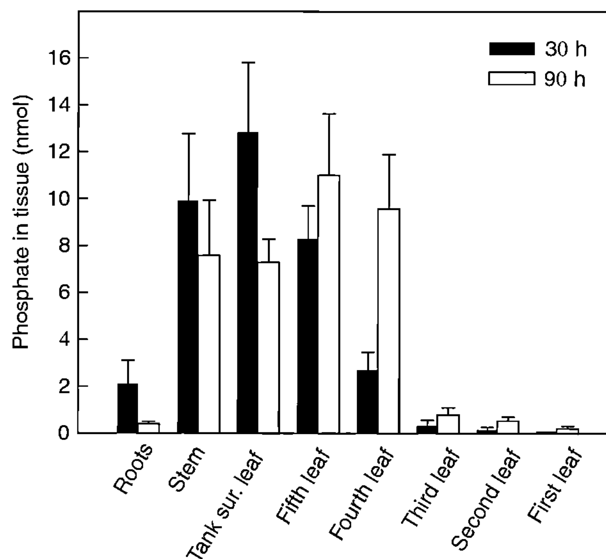

FIG. 5. Distribution of phosphate in roots, stems and leaves of *Achmea*. Experimental details for the uptake by tanks are as described in Fig 1. After complete uptake of phosphate, tanks were watered without P_i. After 30 h and 90 h, plants were harvested and cut into small sections. Amounts of phosphate were calculated from the label incorporated. Leaves are numbered according to their age. The youngest leaf surrounds the tank (sixth leaf) and the oldest leaf (first leaf) is the outer leaf of the rosette; 'Tank sur. leaf' = sections of the tank surrounding the leaf. Data are means ± s.d., $n = 5$. For statistical analysis, see Table 2.

TABLE 2. *Results of a two-way ANOVA on the effects of organ type and time on plant P concentration*

Factor	d.f.	SS	*F*-value	*P*-value
Organ (O)	7	1404·9	65·3	$\ll$ 0·001
Time (T)	1	0·41	0·13	0·71
O × T	7	236·2	10·98	$\ll$ 0·001
Residuals	64	196·7		

The data are from Fig. 5.

TABLE 3. *Results of a three-way ANOVA on the effects of leaf number, time and leaf section on plant P concentration*

Factor	d.f.	SS	*F*-value	*P*-value
Leaf (L)	2	21·8	23·7	$\ll$ 0·001
Time (T)	1	0·87	1·9	0·17
Leaf section (S)	4	88·6	48	$\ll$ 0·001
L × T	2	23·6	25·6	$\ll$ 0·001
L × S	8	4·5	1·2	0·3
T × S	4	5·2	2·8	<0·05
L × T × S	8	11·4	3·1	<0·01
Residuals	90	41·5		

The data are from Fig. 6.

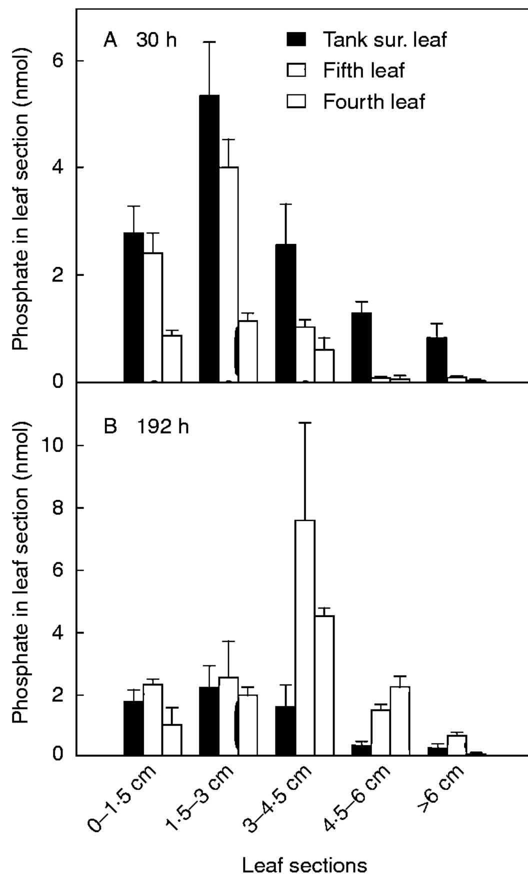

FIG. 6. Distribution of phosphate in sections of the tank surrounding the leaf (Tank sur. leaf) and two consecutive leaves (fifth and fourth leaf of the rosette) of *Aechmea* plants. (A) Plants harvested after 30 h, (B) plants harvested after 192 h. Experimental details are as described in Fig. 5. Data are means ± s.d., $n = 4$. For statistical analysis, see Table 3.

growing (leaves 2 and 3). Independently of incubation time, the outer leaves and the roots received only a minor portion of the incorporated P_i. The considerable variation in P_i contents within plant tissues may not only reflect translocation of P_i, but may be the consequence of differentially developed leaves. The youngest leaf surrounding the tank and the consecutive leaves are actively growing parts of the plant and therefore leaves of different plants used in the experiments may have differed in size and developmental stage.

At a yet finer scale, i.e. within a leaf, there were also differences in the distribution of ^{32}P (Fig. 6). After 30 h of incubation, the majority of ^{32}P was not detected in the basal leaf segments with direct contact with the tank water, but slightly higher, at 1·5–3·0 cm from the leaf base (Fig. 6A). The P_i had obviously been translocated from the actual uptake zone. This translocation process continued during prolonged incubation periods. After 192 h, the highest amounts of ^{32}P were found 3·0–4·5 cm above the leaf base (Fig. 6B). Consistent with the results of the previous experiment (Fig. 5, Table 2), this represents a significant transfer of P_i from the more proximal portions of the youngest leaf to slightly more distal portions of older, but still actively growing leaves (Table 3). Notably, this apparent translocation cannot be explained by basal elongation of these leaves during the experiment, which amounted to only 0·3 ± 0·1 cm week^{-1} ($n = 15$ leaves).

The varying accumulation of ^{32}P in different parts of the leaves raises the question of its utilization. The ^{32}P activity in leaf sections was tested by Cerenkov counting. From leaf discs with a high accumulation of activity, acidic extracts were prepared. In plants harvested after 30 h and 14 d incubation with ^{32}P, 93·7 ± 2·4 and 81·3 ± 5·7 %, respectively, ($n = 10$) of the activity was extractable, indicating that most of the absorbed P_i was present in an unaltered form or bound to small molecules, but not used for synthesis of large biomolecules. On autoradiograms obtained from plants that were harvested 30 h after the incubation of tanks with labelled ^{32}P, the P_i taken up was still present in the free soluble inorganic form (Fig. 7). No other labelled substances were detectable. However, after an incubation period of 14 d, the P_i label disappeared and the major labelled compound detected in the chromatogram was phytin.

DISCUSSION

Epiphytic tank bromeliads have to cope with water and nutrient deficiency. A number of morphological and anatomical structures allow epiphytes to cope with this ecological condition. The bases of leaves form a tank to retain water and nutrients. Leaves possess trichomes that function as uptake organs, as indicated by the positive correlation between uptake rates for labelled ^{45}Ca, ^{32}P, ^{35}S and ^{65}Zn, and the density of trichomes in different bromeliads (Benzing and Pridgeon, 1983; Benzing, 2000).

A low and irregular supply of nutrients demands an effective uptake mechanism. The uptake rates for P_i in five different tank bromeliads ranged from 0·7 to 3·7 nmol P_i h^{-1} g^{-1}

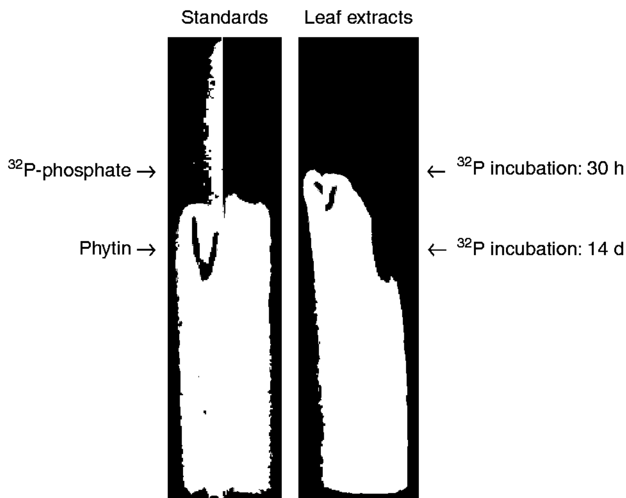

FIG. 7. Autoradiogram of ^{32}P-labelled substances extracted from *Aechmea* plants that were incubated with 50 nmol P_i and watered without nutrients for 30 h or 14 d. Experimental details of plant incubation are as described in Fig 5. After the incubation period, acid-soluble substances were extracted from those sections of the third leaf that showed the highest accumulation of ^{32}P. Aliquots of 1 μL from the extracts were separated by ion-exchange thin-layer chromatography on PEI-cellulose. Labelled substances were visualized by autoradiography. After incubation for 30 h, the phosphate incorporated was still present. After an incubation period of 14 d, all labelled phosphate was found in phytin. ^{32}P was used as a standard to detect labelled phosphate. The phytin standard was visualized by a colouring reaction with ammonium heptamolybdate.

d. wt, when tanks were filled with 10 μM P_i.. The naturally occurring P_i concentration available to bromeliads from rainwater, throughfall, stem flow and tank water of epiphytes *in situ* was estimated to range from 2·6 to 24 μM Pi (Benzing, 2000; Richardson *et al.*, 2000). The highly effective P_i uptake mechanism should thus allow bromeliads *in situ* to absorb small amounts of naturally occurring P_i within a few hours or days.

Initial uptake rates obtained in experiments (Figs 3 and 4) were also related to the watered surface area of tanks filled with 0·5 mL of incubation solution (calculated from the diameter and height of filling). The value of 1·4 pmol min^{-1} mm^{-2} obtained for *Aechmea* is comparable with the value of 1·0 pmol min^{-1} mm^{-2} recalculated from the uptake rates by root hairs of *Arabidospsis* (Bates and Lynch, 2000). This rapid uptake is achieved by phosphate transporters with high affinity for their substrate. The kinetic properties of the phosphate transporters were analysed in *Aechmea.* Different calculation methods resulted in comparable K_m values of 1·05 μM (Michaelis–Menten plot for all P_i concentrations) and 1·07 μM (Eadie–Hofstee plot for P_i <5 μM) for P_i uptake. The substrate affinity of the phosphate transporter from the bromeliad agrees with the K_m values of 1–7 μM which were obtained for high affinity phosphate transporters in roots and protoplasts from terrestrial plants (Furihata *et al.*, 1992; Poirier and Bucher, 2002; Bucher, 2007; Sousa *et al.*, 2007). The phosphate transporters of terrestrial plants appear to be highly regulated with regard to the plant P_i status. If P_i is withheld from roots of tomato and barley for a few days, P_i influx is markedly stimulated in these plants. When P_i is re-supplied to the roots, the influx of P_i declines within a few hours (Raghothama, 1999; Bucher, 2007). In *Aechmea*, a K_m value of 4·4 μM for P_i uptake was calculated for P_i concentrations of 5–50 μM, indicating a biphasic uptake system, possibly comparable with the low affinity phosphate transporters present in roots that are sufficiently supplied with P_i.

The irregular supply of nutrients and their dilution in tanks during heavy rainfall represents a physiological challenge for epiphytes. Consistent with the present results for P_i uptake, the K_m values for uptake of NH_4^+ by tanks of *V. gigantea* were also in the range of those from roots of terrestrial plants (Inselsbacher *et al.*, 2007). Thus, available evidence suggests that tank epiphytes are able to take up both nitrogen and phosphorus in a highly efficient manner.

The uptake of P_i from *Aechmea* tanks was ATP dependent (inhibited by CCCP, an inhibitor of ATP-dependent transport across cellular membranes; Dahlmann, 2004), as was the uptake of NH_4^+ by *Vriesea* (Inselsbacher *et al.*, 2007). It is believed that the ATP-dependent secondary active transport is the most common uptake mechanisms for nutrient ions in root hairs of terrestrial plants (Raghothama, 1999; Smith *et al.*, 2000). Although different in morphology and anatomy, biochemical properties and mechanisms of nutrient uptake seem to be comparable in root hairs and trichomes.

Roots of the greenhouse-grown *Aechmea* plants used in the present experiments did not take up P_i. Other nutrients such as Mn, Se, Zn and Cs are also taken up by foliage of greenhouse-cultured *Guzmania lingulata*, but not by their roots (Nadkarni and Primack, 1989). In contrast, naturally occurring epiphytes rooted in mosses and canopy humus show phosphatase activity, indicating that P_i uptake via roots is possible (Antibus and Lescic, 1990). ^{32}P uptake by roots was also detected in small juveniles of *W. sanguinolenta* (K. Wilhelm and U. Winkler, unpubl. res.). In summary, this suggests that roots of epiphytic bromeliads are not completely incapable of taking up nutrients, but nutrient acquisition capacity by roots may rather depend upon ontogenetic stage and environmental conditions.

Application of radioisotopes such as ^{32}P is an effective tool to study allocation and utilization of nutrients. The present results indicate that P_i is not distributed uniformly within *Aechmea* plants: young leaves are the main sink for P_i, while roots and older leaves benefit only to a small extent. Even 1 week after a ^{32}P pulse, allocation of P_i to these plant parts was low. Experiments to measure ^{32}P distribution in entire plants by autoradiography provided no reliable results, because overlapping tissue resulted in complete darkening of the X-ray films. Therefore, radioactivity in plant tissues was measured by liquid scintillation counting. Application of different methods may explain the uniform distribution of ^{32}P in tissue of two other *Aechmea* species obtained by autoradiography (Benzing, 1970).

The varying accumulation of P_i in different parts of the leaves raises the question of its utilization. Nucleic acids and phospholipids of membranes are the most important sinks for P_i. Nucleic acids should accumulate in meristematic zones of leaves at their bases and in the meristem itself. In the present experiments, the basal leaf meristems were treated as part of the 'stem' section. This compartment is well supplied with P_i taken up from the tank, but most of the nutrient element was transported into distal leaf segments. Phospholipids should accumulate in the

greening zone of leaves, where chloroplast membranes are synthesized. However, most of the P_i was located in segments above the greening zone of the leaves. P_i used for growth should be incorporated in cellular macromolecules. However, 14 d after the ^{32}P pulse, <20 % of P_i was found in high molecular weight molecules, but most of the P_i taken up was attached to *myo*-inositol, thus forming phytin, the most widespread storage molecule for phosphorus in plants (Lott *et al.*, 2000).

The data therefore indicate that epiphytes which are generally slow-growing plants (Schmidt and Zotz, 2002) take up nutrients in excess of current needs to sustain later growth, confirm earlier findings of luxury consumption (Benzing and Renfrow, 1980; Benzing, 2000) and are in line with general ecological theory of luxury consumption in nutrient-poor habitats (Chapin, 1980).

In *Aechmea* the excess P_i consumed is stored in the form of phytin. Hence, analysis of phytin content of plants *in situ* could be used to estimate the importance of P_i nutrition in limiting vegetative growth and/or reproduction in bromeliads. Absence or low phytin concentrations is an indicator of insufficient supply of P_i. High phytin concentrations, in contrast, would indicate that more P_i is present than currently needed in metabolism. The wide range of N : P ratios measured in bromeliads (Zotz, 2004) could also be explained by different amounts of P_i stored in phytin.

In the present experiments only about 3 % of the P_i taken up was allocated to older leaves and roots of *Aechmea*. These organs are anatomically connected via the 'stem'. Stems were supplied with a large amount of P_i, which could have been mobilized and translocated to older leaves and roots during the experimental period. Additional experiments are needed to clarify if the small amounts of P_i transferred to older leaves and roots were sufficient to maintain synthesis of phosphorus-containing biomolecules, or if nutrient deprivation is a tool to induce senescence in leaves. Starvation of older leaves in favour of supplying young, developing leaves with nutrients would be an additional mechanism to adapt to a low supply of nutrients. This would mean that phosphorus is stored in younger leaves rather than transferred to older leaves to maintain their function.

With regard to P_i, the results of the present experiments can be interpreted in terms of a 3-fold adaptation of tank epiphytes to the low supply of nutrients *in situ*. First, the possession of absorbing trichomes is the morphological prerequisite to replace root hairs as the site for capturing nutrients derived from atmospheric and other sources. Secondly, an effective physiological uptake mechanism permits the use of a highly fluctuating resource, avoiding the regular dilution and loss of phosphorus captured in the tank by very fast uptake. Thirdly, the capacity to store phosphorus not immediately needed for current growth ('luxury consumption') leads to an additional independence from a highly fluctuating external supply (Chapin, 1980). All three features allow these plants to cope with poor nutrient supply and to thrive in this nutrient-poor habitat.

ACKNOWLEDGEMENTS

We gratefully acknowledge the skilful help and excellent assistance of Peter Kanje in designing and performing the labelling experiments.

LITERATURE CITED

Antibus K, Lescic P. 1990. Root surface acid phosphatase activities of vascular epiphytes of a Costa Rican rain forest. *Plant and Soil* **128**: 233–240.

Bates TR, Lynch JP. 2000. The efficiency of *Arabidopsis thaliana* (Brassicaceae) root hairs in phosphate acquisition. *American Journal of Botany* **78**: 964–970.

Benzing DH. 1970. Availability of exogenously supplied nitrogen to seedling of Bromeliaceae. *Bulletin of the Torrey Botanical Club* **97**: 154–159.

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

Benzing DH. 2000. *Bromeliaceae. Profile of an adaptive radiation.* Cambridge, UK: Cambridge University Press.

Benzing DH, Pridgeon A. 1983. Foliar trichomes of Pleurothallidinae (Orchidaceae): functional significance. *American Journal of Botany* **70**: 173–180.

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

Benzing DH, Renfrow A. 1980. The nutritional dynamics of *Tillandsia circinnata* in southern florida and the origin of air plant strategy. *Botanical Gazette* **141**: 165–172.

Bucher M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytologist* **173**: 11–26.

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

Dahlmann L, Persson J, Palmquist K, Näsholm T. 2004. Organic and inorganic nitrogen uptake in lichens. *Planta* **129**: 459–467.

Endres I, Mercier H. 2001. Influence of nitrogen forms on the growth and nitrogen metabolism of bromeliads. *Journal of Plant Nutrition* **24**: 29–42.

Endres I, Mercier H. 2003. Amino acid uptake and profile in Bromeliads with different habits cultivated *in vitro*. *Plant Physiology and Biochemistry* **41**: 181–187.

Furihata T, Suzuki M, Sakurai H. 1992. Kinetic characterization of two phosphate uptake systems with different affinities in suspension-cultured *Catharanthus roseus* protoplasts. *Plant and Cell Physiology* **33**: 1151–1157.

Grime JP. 2001. *Plant strategies, vegetative processes and ecosystem properties.* Chichester, UK: Wiley-Liss., Inc.

Hietz P, Ausserer J, Schindler G. 2002. Growth, maturation and survival of epiphytic bromeliads in a Mexican humid montane forest. *Journal of Tropical Ecology* **18**: 177–191.

Inselsbacher E, Cambui CA, Richter A, Stange CF, Mercier H, Wanek W. 2007. Microbial activities and foliar uptake of nitrogen in the epiphytic bromeliad *Vriesea gigantea*. *New Phytologist* **175**: 311–320.

Killingbeck KT. 1996. Nutrients in senescent leaves: keys to the search for potential resorption proficiency. *Ecology* **77**: 1716–1726.

Lott NA, Ockenden J, Rasboy V, Batte D. 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. *Seed Science Research* **10**: 11–33.

Nadkarni NM, Primack RB. 1989. The use of gamma spectrometry to measure within plant nutrient allocation of a tank bromeliad, *Guzmania lingulata*. *Selbyana* **11**: 22–25.

Organ MG, Greenwood JS, Bewley JD. 1988. Phytin is synthesized in cotyledons of germinating castor-bean seeds in response to exogenously supplied phosphate. *Planta* **174**: 513–517.

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

Poirier Y, Bucher M. 2002. Phosphate transport and homeostasis in *Arabidopsis*. In: *The Arabidopsis book.* Rockville, MD: American Society of Plant Biologists.

R Development Core Team. 2007. *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical computing.

Raghothama K. 1999. Phosphate acquisitation. *Annual Review of Plant Physiology* **50**: 665-693.

Richardson BA, Rogers C, Richardson MJ. 2000. Nutrients, diversity, and community structure of two phytotelm systems in a lower montane forest, Puerto Rico. *Ecological Entomology* **25**: 348–356.

Schmidt G, Zotz G. 2002. Inherently slow growth in two carribbean epiphytic species: a demographic approach. *Journal of Vegetation Science* **13**: 527–534.

Smith FW, Rae AL, Hawkesford MJ. 2000. Molecular mechanisms of phosphate and sulphate transport in plants. *Biochimica et Biophysica Acta* **1465**: 236–245.

Sousa FM, Facanha AR, Tavares RM, Lino-Neto T, Geros H. 2007. Phosphate transport by proteoid roots of *Hakea sericea. Plant Science* **173**: 550–558.

Stuntz S, Zotz G. 2001. Photosynthesis in vascular epiphytes – a survey of diverse taxonomical origin. *Flora* **196**: 132–141.

Sun Y, Thompson M, Lin GH, *et al.* 2007.. Inositol 1,3,4,5,6-pentakisphosphate 2-kinase from maize. Molecular and biochemical characterization. *Plant Physiology* **144**: 133–138.

Wood T. 1968. The detection and identification of intermediates of the pentose phosphate cycle and related compounds. *Journal of Chromatography* **35**: 352–361.

Zotz G. 2004. The resorption of phosphorus is greater than of nitrogen in senescing leaves of vascular epiphytes from lowland Panama. *Journal of Tropical Ecology* **20**: 693-696.

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

Zotz G, Richter A. 2006. Changes in the carbohydrate and nutrient contents throughout a reproductive cycle indicate that phosphorus is a limiting nutrient in the epiphytic bromeliad, *Werauhia sanguinolenta. Annals of Botany* **97**: 45–754.