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Authors

Nyman, LP
Davis, JP
O'dell, SJ
et al.

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Active Uptake of Amino Acids by Leaves of an Epiphytic Vascular Plant, *Tillandsia paucifolia* (Bromeliaceae)[1]

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LESLIE PAUL NYMAN*, JAMES P. DAVIS, STEPHEN J. O'DELL, JOSEPH ARDITTI, GROVER C. STEPHENS, AND DAVID H. BENZING
Department of Developmental and Cell Biology, University of California, Irvine, California, 92717 (L.P.N., J.P.D., J.A., G.C.S.); *and Department of Biology, Oberlin College, Oberlin, Ohio, 44047* (D.H.B.)

ABSTRACT

Specialized epidermal trichomes on the leaves of the epiphyte, *Tillandsia paucifolia* (Bromeliaceae) accumulate amino acids from solution. Simultaneous net uptake of 17 amino acids was determined using high performance liquid chromatography. Uptake occurs against concentration gradients at least as high as 10^4.

Analyses of tropical forest canopy leachates reveal that atmospheric bromeliads (subfamily Tillandsioideae), those species lacking absorption roots and foliar impoundment, occupy oligotrophic habitats (2, 3). These most specialized of all bromeliads counter episodic availability of water and essential nutrients through (a) reduction in total root and shoot biomass, (b) slow growth, (c) consolidation of vegetative functions in the shoot, and (d) the possession of foliar trichomes capable of absorbing both ions and moisture (3, 7).

Trichomes of the Tillandsioideae have also been implicated in the uptake of organic solutes (3). The research described in this paper was undertaken for the purpose of (a) definitively demonstrating net uptake of amino acids by trichomes and (b) to determine whether or not such uptake involved trichome-mediated active transport of amino acids. Most previous work on amino acid uptake in the Tillandsioideae and in plants generally has been carried out utilizing excised tissues (10). This study differs in that attention is focused on net uptake of amino acids by living cells of an intact leaf epidermis.

MATERIALS AND METHODS

Plant Material. *Tillandsia paucifolia* was used because its trichomes are relatively large and numerous. The plants were collected at the Everglades National Park. They were allowed to acclimatize to greenhouse conditions at the University of California, Irvine for 8 weeks prior to the experiment.

Amino Acid Uptake. Detached leaves of *T. paucifolia* were placed in 25 ml of continuously mixed aqueous solutions consisting of 18 amino acids, each at an initial concentration of 1 μM. Leaves were oriented so that their cut surfaces did not contact the solution. All leaves were washed thoroughly in distilled H_2O for 30 min before each experiment to minimize bacterial contamination and potential interference by exogenous organic compounds. The relationship between influx (uptake) and net entry (influx minus efflux) of amino acids was established by measuring the disappearance of [^{14}C]leucine, [^{14}C]arginine, or [^{14}C]lysine, as determined by liquid scintillation counting while net disappearance was simultaneously monitored using HPLC. These experiments were carried out in a mixture of 18 amino acids (aspartic acid, glutamine, asparagine, serine, histidine, glutamine, glycine, threonine, arginine, taurine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, lysine) each at an initial concentration of 1 μM plus 1 μCi [^{14}C]leucine, [^{14}C]arginine or [^{14}C]lysine. Changes in the concentration of specific amino acids in the medium were observed using HPLC (5, 8).

Amino Acid Content of Leaves. Outer leaf tissues (*i.e.* epidermis plus a few layers of outer mesophyll cells) were homogenized and extracted with 80% ethanol. Concentrations of leucine, arginine, lysine, and asparagine were determined using HPLC (5, 8) and calculated as a function of total leaf water.

Autoradiography. A drop of tritiated leucine (10 μCi ml^{-1}) with a specific activity of 47.6 Ci mmol^{-1}) in 10 μM $CaCl_2$ and buffered with 10 μM KH_2PO_4 (pH 5.8) was placed on the surface of a leaf for 30 min and rinsed with distilled H_2O. Leaf sections exposed to the radioactive leucine were fixed in formalin acetic acid, dehydrated in a standard ethanol series, embedded in paraffin, sectioned at 10 μm and mounted on glass slides. The slides were coated with photographic emulsion KTB2 (Eastman Kodak Co.) diluted 1:1 with distilled H_2O heated to 45°C, and incubated for 1 week in the dark at room temperature. After the incubation the slides were developed with full strength Kodak D19 developer for 1 min, washed and fixed in Kodak Rapid Fixer for 10 min (2). The developed slides were dehydrated with standard series of ethanol followed by xylene. Permount was used as the mounting medium.

Scanning Electron Microscopy. Leaf sections (1 mm^2) were fixed overnight in 4% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 6.8) at 0°C. The sections were dehydrated in a standard acetone series, infiltrated with freon, dried in a Bomar 900 Ex critical point drier, mounted on aluminum stubs, and coated with gold for 3 min in a Hummer 2 sputter coater.

RESULTS AND DISCUSSION

Tillandsia paucifolia trichomes are too small to be resolved with the naked eye, and appear as a white powdery covering over the leaf epidermis. Under the scanning electron microscope, they appear to be complex multicellular structures consisting of an outer wing, 200 μm in diameter, and composed of dead cells (2, 3). This wing is subtended by a basal stalk, 62 μm in diameter, consisting of living cells (2) which penetrate the epidermis (Figs. 1 and 2). Trichomes occur at a density of 4000 to 5000 cm^{-2}. Unwashed trichomes support a few bacteria. However, specimens observed under the scanning electron microscope (Fig. 2)

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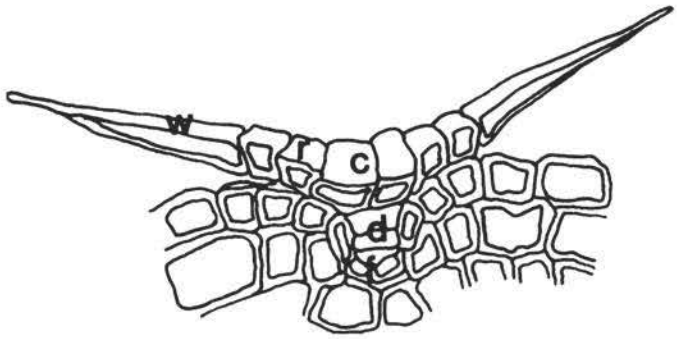

FIG. 1. Line drawing of a typical foliar trichome of the Tillandsioideae as viewed in cross section under dry conditions showing dead surface cells of the trichome, through which water is initially absorbed including those of the central disk (c), ring (r), and wing (w). The dome cells (d) and foot cells (f) are alive in functioning trichomes and are the sites of accumulated radiolabel in autoradiograms.

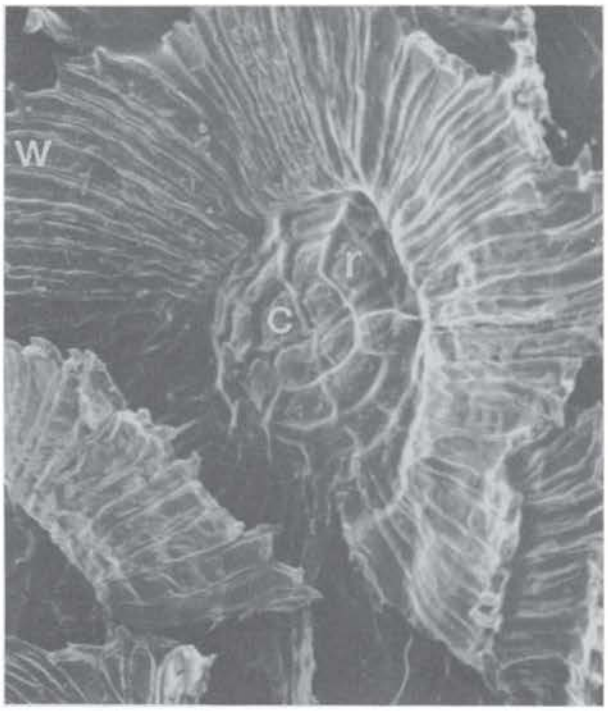

FIG. 2. Scanning electron micrograph of a foliar trichome of *T. paucifolia*. Four central disc cells (c), two rows of outer ring cells (r), and an outer wing (w) are visible in surface view. These cells in contrast to the dome and foot cells below the plane of the photograph are dead at maturity.

were virtually microbe-free. This fact, coupled with the short time course of our experiments, ensures that epifauna affected our results very little if at all.

It has long been recognized that trichomes are the major organ of moisture absorption in most species belonging to the Tillandsioideae (7). In part, evidence for this is based on the density and morphology of trichomes on the leaf surface (2). Under dry conditions, the upper dead cells of the trichome form a cup-like shape with the rim composed of slightly curled wing cells (Fig. 1). When wetted, these cells rapidly absorb water and the wing cells flatten against the surface of surrounding epidermal cells. All water entry into the plant follows a route from the wing cells through the ring and central disc cells to the living dome and foot cells. Although this pathway of water movement is well understood, net entry of dissolved solutes has not been previously demonstrated.

Histo-autoradiographic studies using labeled amino acids indicate that living stalk cells of *T. paucifolia* trichomes may be capable of taking up dissolved free amino acids from extrafoliar solutions. Similar results were obtained with other *Tillandsia* species (2). When solutions containing [^{3}H]leucine were placed on the surface of leaves, the tissues accumulated a considerable amount of radiolabel within 30 min, most of which was concentrated inside the stalk cells of the trichome (Fig. 3). The uniform distribution of label throughout the interior of specific stalk cells indicates absorption of leucine into these cells. If the leucine had been merely adsorbed onto the external cell wall, grain densities in our autoradiograms would have been higher at the periphery of the cells. This is clearly not the case (Fig. 3). Radiolabel did not appear in epidermal cells which were not part of the trichome structures. Similar autoradigraphic evidence has been reported by others (2). However, autoradiograms provide no information about the mechanism of absorption.

Uptake experiments demonstrate the simultaneous net absorption of amino acids (Fig. 4). After a 2 to 3 h exposure, concentrations of 17 of the amino acids were reduced (Fig. 4). Arginine and lysine were taken up most rapidly, at an average rate of 115.2 ± 0.5 nmol g^{-1} dry weight h^{-1}. All other amino acids were absorbed at least an order of magnitude slower. Neutral amino acids were removed from solution at an average rate of 27.9 ± 5.2 nmol g^{-1} dry weight h^{-1}, whereas acidic ones were taken up at 9.6 ± 1.0 nmol g^{-1} dry weight h^{-1}.

Asparagine was the only amino acid showing net efflux into the medium. However, in all cases the observed loss was much less than the combined uptake of the other 17 amino acids. The combined rate of uptake over a 2 h period was 650 nmol g^{-1} dry weight h^{-1} for 17 amino acids (Fig. 4) and the corresponding net efflux of asparagine was 79 nmol g^{-1} dry weight. Thus, the net accumulation of amino acids from the medium during a 2 h period was 571 nmol g^{-1} dry weight. The physiological significance of the asparagine loss is unclear, but there is evidence that the general amino acid transport system in plants has a low affinity for asparagine (9).

Influx of radiolabeled leucine and lysine was equal to the net entry of these amino acids (Fig. 5). Net entry of arginine appeared to proceed more rapidly than influx of radiolabel. This suggests that arginine is being metabolized during the course of these experiments and that some of the radiolabeled metabolic product(s) are released into the medium. The identity of possible

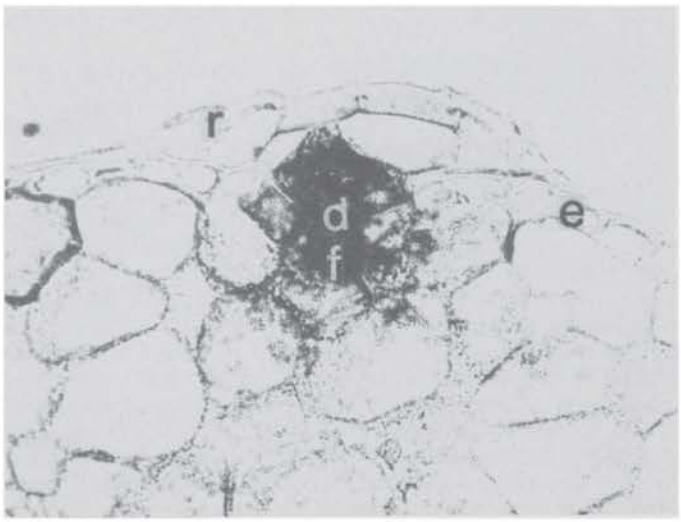

FIG. 3. Autoradiogram of a foliar trichome treated for 30 min with tritiated leucine. The dome cells (d) and the foot cells (f) of the trichome are clearly labeled. Epidermis (e) and ring cells (r) are also shown.

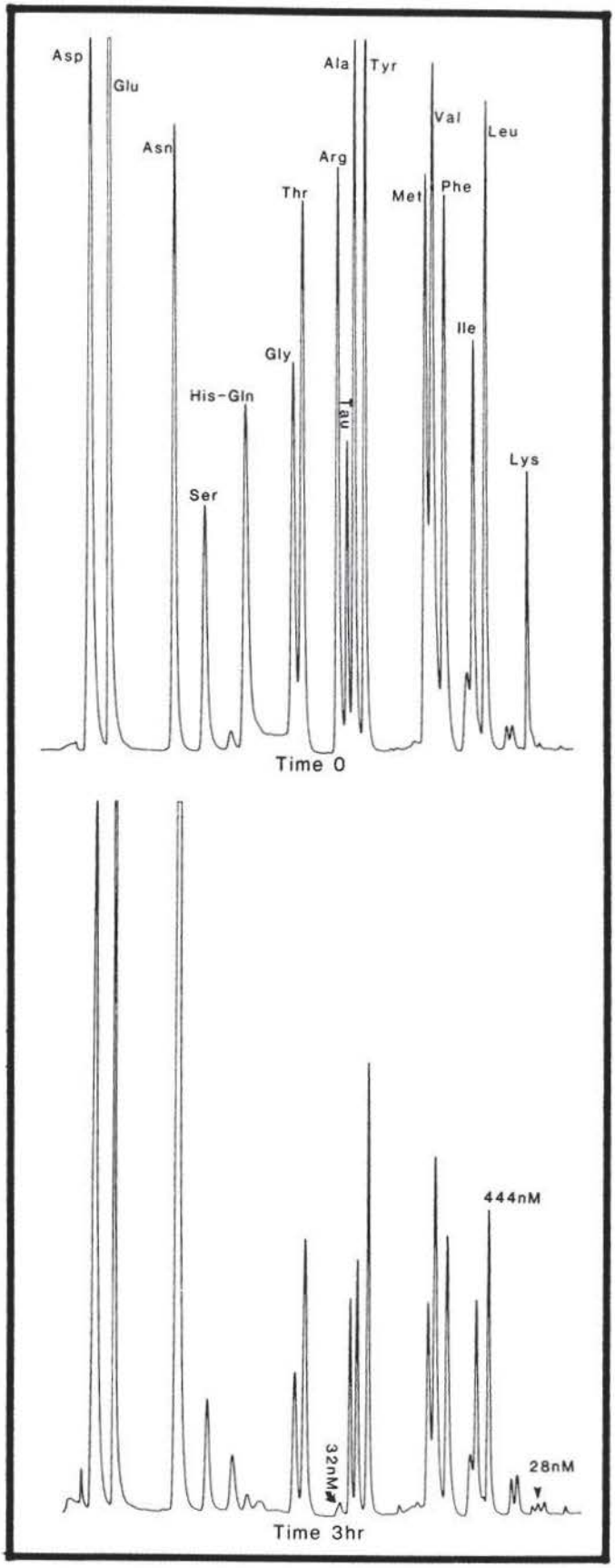

FIG. 4. HPLC chromatograms of the incubation medium. Each of the 18 amino acids is present at an initial concentration of 1 μM (time 0, upper chromatogram). Substantial removal occurs during 3 h (lower chromatogram). The concentrations of arginine, leucine, and lysine are indicated.

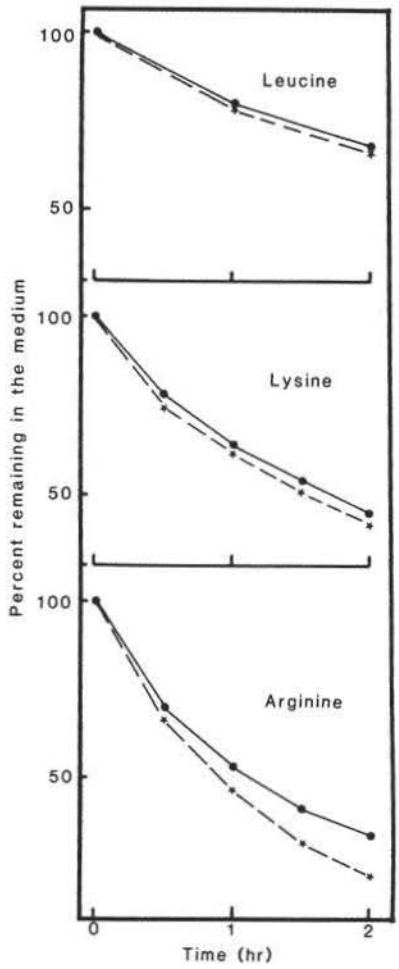

FIG. 5. Influx (dashed lines) and net entry as determined by HPLC (solid lines) of labeled leucine, lysine, and arginine. The influx data are determined on the basis of loss of label from the external medium. Net entry is calculated on the basis of loss of amino acid from the external medium.

Table I. *Internal and External Amino Acid Concentrations*

Amino Acid	Internal Tissue Concentrations	External Concentrations		Rate of net uptake
	μM	μM		μM h^{-1}
		T_0	T_3	
Leucine	17.2	1.0	0.45	26.6
Lysine	500.0	1.0	0.02	115.7
Arginine	8.6	1.0	0.03	114.9
Asparagine	5680.0	1.0	2.90	−79.0[a]

[a] The negative value represents a net efflux of asparagine over the 3 h duration of the experiment.

metabolic intermediate(s) was not determined but it is certain the radiolabeled compounds released did not include free $^{14}CO_2$. The latter was excluded from samples prepared for scintillation counting by acidification before the addition of fluor. Samples were counted 24 h after acidification with 0.5 N HCl.

Arginine, lysine, and leucine concentrations in leaves were 8.6 μM, 0.5 mM, and 17.2 μM, respectively (Table I). Total free amino acid concentration of the internal pool was 8.2 mM of which asparagine accounts for 70% or 5.7 mM. The extraction technique utilized in this study does not allow us to differentiate between cytoplasmic and vacuolar pools. However, in the case of lysine

net entry continues in external concentrations as low as 28 nM (Fig. 4; Table I), or against a concentration ratio of 1:17,850. Net entry of leucine, and arginine also proceedes against significant concentration gradients (Table I). Accumulation of these solutes against the observed concentration gradients strongly suggests active transport (4).

Most angiosperms are exclusively autotrophic. Therefore, despite the large proportion of soil nitrogen which is bound in the proteins and amino acids of organic components, research has been focused on the acquisition of inorganic nitrogen (11, 12). However, amino acid uptake by plant cells, tissues, and excised organs *in vitro* is well known (10, 11). It is also possible that germinating orchid seeds and developing seedlings take up amino acids (1). Furthermore, some authors have suggested that amino acids and amides may be taken up directly from soil by roots (6).

Our findings provide definitive evidence of net uptake of dissolved free amino acids by trichomes on an intact epidermal surface of an epiphytic angiosperm. The nutritional significance of amino acid uptake by *T. paucifolia* in the field is unclear. Little is known about the nitrogen budget of bromeliads and we do not know what proportion of the metabolic needs of *T. paucifolia* is met by uptake of amino acids. However, it should be noted that organic nitrogen in the form of all the common amino acids is available to plants perched in tree crowns (12, 13).

Hundreds of epiphytic ferns, orchids, bromeliads, and other plants occupy habitats similar to those of *Tillandsia* species. Many of these species lack absorbing roots (during their entire life or during certain developmental stages only) and some possess epidermal trichomes. More work is required to assess the nutritional significance of amino acid uptake in canopy habitats and the extent to which this process occurs in soil rooted angiosperms.

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