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**Cation accumulation and leaf succulence in *Codonanthe macradenia*
J. D. SMITH (Gesneriaceae) under field conditions**

by

Ernesto Medina, Milena Delgado & Victor Garcia

Dr. Ernesto Medina, Victor Garcia, Centro de Ecología, Instituto Venezolano de Investigaciones Científicas, Aptdo. 21827, Caracas 1020-A, Venezuela.

Milena Delgado, Facultad de Farmacia, Universidad Central de Venezuela, Caracas, Venezuela.

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Abstract

The development of leaf succulence and the content of K, Ca, Mg, P, and N were measured in the gesneriad *Codonanthe macradenia* J. D. SMITH, which grows as epiphyte in open sclerophyll forests on white sands in the Amazon basin (upper Rio Negro basin), under a wide range of light exposures. Leaves show a multiseriate adaxial epidermis occupying 35 % - 68 % of the total leaf thickness. Succulence and epidermis thickness are linearly correlated with the amount of K per unit leaf area, resulting in a nearly constant K concentration per unit tissue water. Both K and P are more concentrated in the epidermis than in the underlying photosynthetic tissue. Succulence and K accumulation appears to be associated to N deficiency during leaf development. Succulence in this species may play an ecological role as a drought endurance mechanism.

Keywords: epiphytes, leaf succulence, cation accumulation, nitrogen deficiency.

Resumen

El desarrollo de la succulencia foliar y el contenido de K, Ca, Mg, P y N se midieron en la gesneriacea *Codonanthe macradenia* J.D. SMITH, especie que crece como epifita en bosques esclerófilos abiertos de arenas blancas en la cuenca Amazónica (cuenca alta del Rio Negro), bajo una variedad de regímenes lumínicos. Las hojas muestran una epidermis adaxial que ocupa entre 35 - 68 % del espesor foliar total. Succulencia y espesor de epidermis están linealmente correlacionados con la cantidad de K por unidad de área foliar, lo que resulta en una concentración casi constante de K por unidad de agua tisular. Tanto el K como el P se encuentran más concentrados en la epidermis que en el parénquima fotosintético subyacente. Succulencia y acumulación de K parecen estar asociados a la deficiencia de N durante el desarrollo foliar. La succulencia foliar en esta especie puede jugar un papel ecológico como mecanismo de resistencia a la sequía.

Dedicated to Dr. Hans Klinge, Max-Planck-Institut Plön, in occasion of his 60th birthday.

Introduction

Epiphytes with succulent leaves are well represented in humid and seasonal neotropical forests and are found among families such as the Bromeliaceae, Orchidaceae, Piperaceae, and Gesneriaceae. Epiphytic habitats are characterized by a low nutrient supply and frequent dry spells, even in areas with high rainfall values (MEDINA 1987).

Leaf succulence is a result of development of "water tissues" (STÄLFELT 1956) which can be epidermal or hypodermal, or a water-storing parenchyma within two photosynthetic parenchyma layers (GESSNER 1956; NAPP-ZINN 1973). Leaf succulence is associated with the accumulation of inorganic cations such as K^+ in Commelinaceae (DELGADO & MEDINA 1978), Ca^{2+} in Crassulaceae (PHILLIPS & JENNINGS 1976), and Na^+ in succulent halophytes (ADRANI 1958). In some Commelinaceae species leaf succulence is induced by cultivation under N deficiency (MOTHES 1932; GESSNER & SCHUMANN-PETERSEN 1948; LÖTSCH 1971). In several species it has been shown that increased leaf water content nearly compensates for increased content of inorganic cations, therefore cation concentration per unit tissue water tends to remain constant (BIEBL & KINZEL 1965; DELGADO & MEDINA 1978).

The genus *Codonanthe* has a few epiphytic species widely distributed in the american humid tropics, frequently found associated with ant nests (KLEINFELDT 1978). Some species show a high degree of leaf succulence, with stomata only in the abaxial side, and with a clear capacity for accumulation of organic acids during the night (GURALNICK et al. 1986). In this paper we report the variability in leaf succulence observed in *Codonanthe macradenia* J. D. SMITH growing in a sclerophyllous low caatinga forest near San Carlos de Rio Negro (KLINGE & MEDINA 1979) and analyze the correlation between the development of succulent tissues and the accumulation and distribution of inorganic ions in leaf tissues.

Materials and methods

Specimens of *Codonanthe macradenia* were collected in a sclerophyllous vegetation typical of white sands in the upper Rio Negro basin (MEDINA, SOBRADO & HERRERA 1978; KLINGE & MEDINA 1979) near the town of San Carlos in southern Venezuela. Specimens grew epiphytically under a wide range of light conditions (from 10 - 15 % day light within the forest to full exposure in the open scrub). Their roots were generally associated with ant nests, a phenomenon commonly observed in epiphytes of the Amazon caatinga forest. Specimens with marked variations in leaf size, color and degree of succulence were collected at the beginning of the dry season (February) and during the first half of the rainy season (June). Leaves were weighed in the field and immersed in liquid nitrogen for transportation to the laboratory.

To evaluate the variability of epidermis thickness and succulence a separate set of fresh leaves were drawn on graphic paper to determine leaf area, and subsequently cut transversally and the maximal leaf and epidermis thicknesses were measured under a dissecting microscope.

For anatomical studies leaves were fixed in a standard mixture of formaline-acetic acid-alcohol (FAA), dehydrated in a tert-butanol series and embedded in paraffin. Sections were cut between 8 - 11 μm and stained with safranin-fast green.

Water content was calculated as the difference between fresh and dry weight. Concentration of K, Ca and Mg was determined through atomic absorption spectrophotometry (ALLEN et al. 1976).

Cell sap extracted by crushing fresh leaves in a mortar was measured directly previous dilution to adjust concentration to the maximal instrument sensitivity. Dry leaves were ground and digested in a mixture of HClO₄ and H₂SO₄ in the presence of vanadium pentoxide. Soluble P was measured by the molybdenum blue colorimetric method (JACKSON 1964). Total N in dried leaves was analyzed with a standard micro-Kjeldahl procedure (JACKSON 1964). Chlorophyll was extracted with 80 % acetone and measured spectrophotometrically according to ARNON (1948).

Results

Anatomical features

Cross sections of leaves show a very well developed multiseriate adaxial epidermis as in other Gesneriaceae genera (METCALFE & CHALK 1950; NAPP-ZINN 1973). The origin of this type of epidermis from periclinal divisions of the protoderm was established for *Codonanthe crassifolia* by GURALNICK et al. (1986). The number of epidermal cell layers vary from 3 to 6. Its size increases towards the adaxial side. While the palisade mesophyll is 1 cell thick, the spongy tissue consists of about 11 layers of isodiametric cells with inter-cellular spaces. The uniseriate abaxial epidermis bears the stomata (Fig. 1).

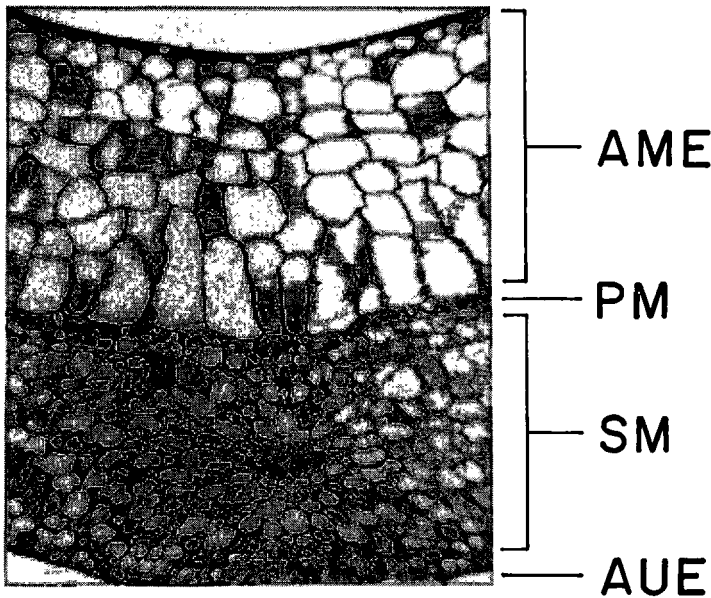


Fig. 1:
Cross section of a leaf of *Codonanthe macradenia*. AME: adaxial multiseriate epidermis;
PM: palisade mesophyll; SM: spongy mesophyll; AUE: uniseriate abaxial epidermis.

A linear relationship between total leaf thickness and epidermis thickness was established (epidermis thickness = $-0.40 + 0.73$ leaf thickness; $r = 0.88$; $n = 74$) (Fig. 2), indicating that variation in leaf thickness is essentially determined by an increase in epidermis

thickness, while the thickness of photosynthetic parenchyma is less variable. The proportion of leaf thickness occupied by epidermis ranges from 35 % in the thinnest leaves to 72 % in the thickest ones. As expected, the degree of succulence expressed as water content per unit of leaf area is also linearly correlated with leaf thickness, although the correlation coefficient is a little lower than in the previous case ($r = 0.79$; $n = 38$).

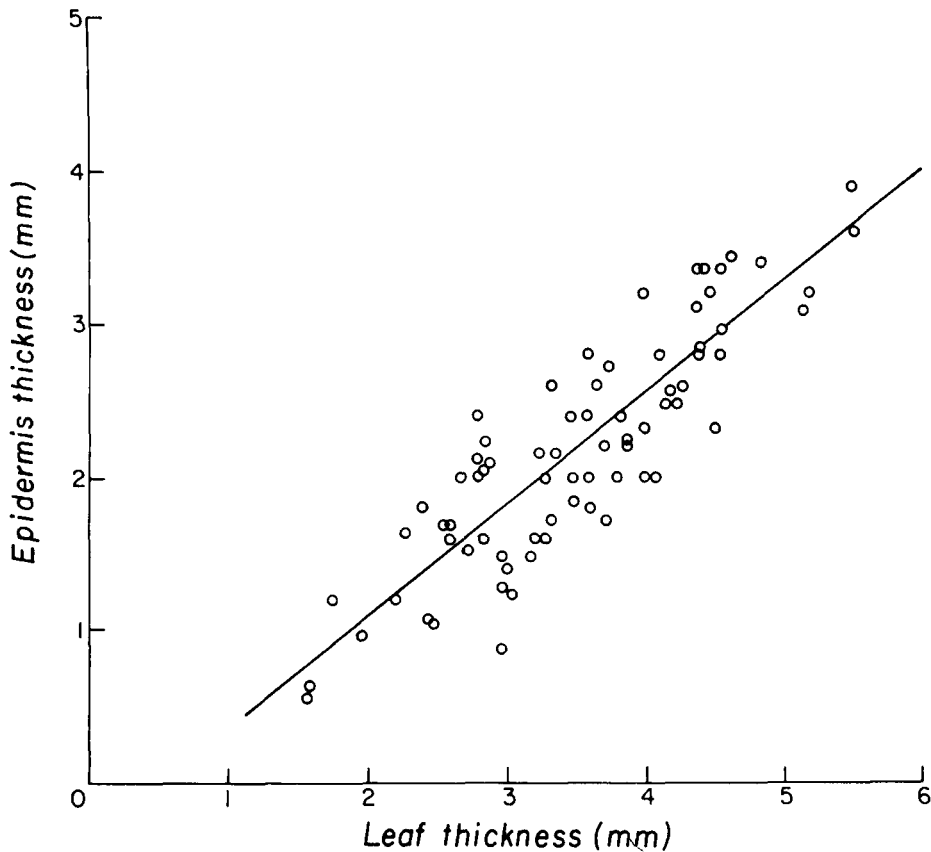


Fig. 2:
Regression between multiseriate epidermis thickness and total leaf thickness. Measurements were made on leaf cross sections under a dissection microscope.

Cation content and degree of succulence

Leaf area, leaf color (chlorophyll content), succulence (mol water per unit of leaf area) and cation content per unit of leaf area was very variable within the population sampled (Table 1). The large range of variation in leaf properties appeared to be associated with nutrient availability and degree of sun exposure. Plants growing under fully exposed conditions, had less chlorophyll and N, but higher K content than those growing in partially shaded habitats. It was apparent that the degree of succulence was associated with the

accumulation of cations, in particular with K and Mg (Fig. 3), and that N content per unit dry weight was inversely correlated to K content per unit of leaf area. The strongest correlation was found between succulence and K concentration per unit of leaf area (the 3rd degree polynomial regression for Fig. 3 give an $r^2 = 0.847$). Further sampling confirmed the relationships between K, N and succulence.

Table 1: Variability of leaf characteristics of a *Codonanthe macradenia* population growing as epiphyte in a low Caatinga forest near San Carlos de Rio Negro, Venezuela.

Leaf characteristic	Mean	Standard error	Range
Leaf area (m ²)	2.96 E-4	0.50 E-4	1 - 10 E-4
Chlorophyll (g m ⁻²)	94.4 E-3	20.0 E-3	20 - 128 E-3
Nitrogen (mmol kg ⁻¹ dry wt.)	511	161	250 - 1100
Succulence (mol water m ⁻²)	98.0	11.0	25.6 - 142.3
Soluble K (mmol m ⁻²)	161.7	25.2	48.1 - 314.4
Soluble Ca (mmol m ⁻²)	41.7	11.2	17.5 - 76
Soluble Mg (mmol m ⁻²)	61.8	14.2	15.2 - 84.4

Leaf nitrogen content per unit of dry weight is inversely correlated with leaf dry weight/area ratio, leaf fresh weight/area ratio and also succulence (Table 2), showing that in this species variations of leaf area in the natural habitat is associated with N availability, and apparently N deficiency is in some way related to succulence development. This relationship between N content per unit of dry weight and succulence apparently is mediated by the increase in K content per unit leaf area, because these parameters are strongly correlated (Table 2). As in the previous sampling, leaf succulence was strongly and positively correlated with K content per unit area (Table 2), appearing that increase in K accumulation in the leaf is accompanied by a similar increase in water content. The consequence is that the actual soluble K concentration per unit of leaf tissue water tended to remain within narrow limits, being maintained between 1 - 2 mmol/mol with an almost 6-fold variation in succulence (Fig. 4).

The contribution of epidermis to leaf thickness and the strong correlation of K content per unit of leaf area with succulence suggested that there might be also an asymmetric distribution of this cation within the leaf tissues. Analyses of isolated epidermis and photosynthetic parenchyma showed that the contents of water, K and P per unit area were higher in the epidermis. However, this was not the case for Ca and Mg (Table 3). Actual epidermis/parenchyma concentration ratios of ions per unit of tissue water were 1.86 for K, 1 for P, 0.09 for Ca, and 0.34 for Mg. This result indicates that K is preferentially accumulated in the epidermis, while Ca and Mg are somewhat excluded. Phosphorus appears to be distributed uniformly within the leaf tissues.

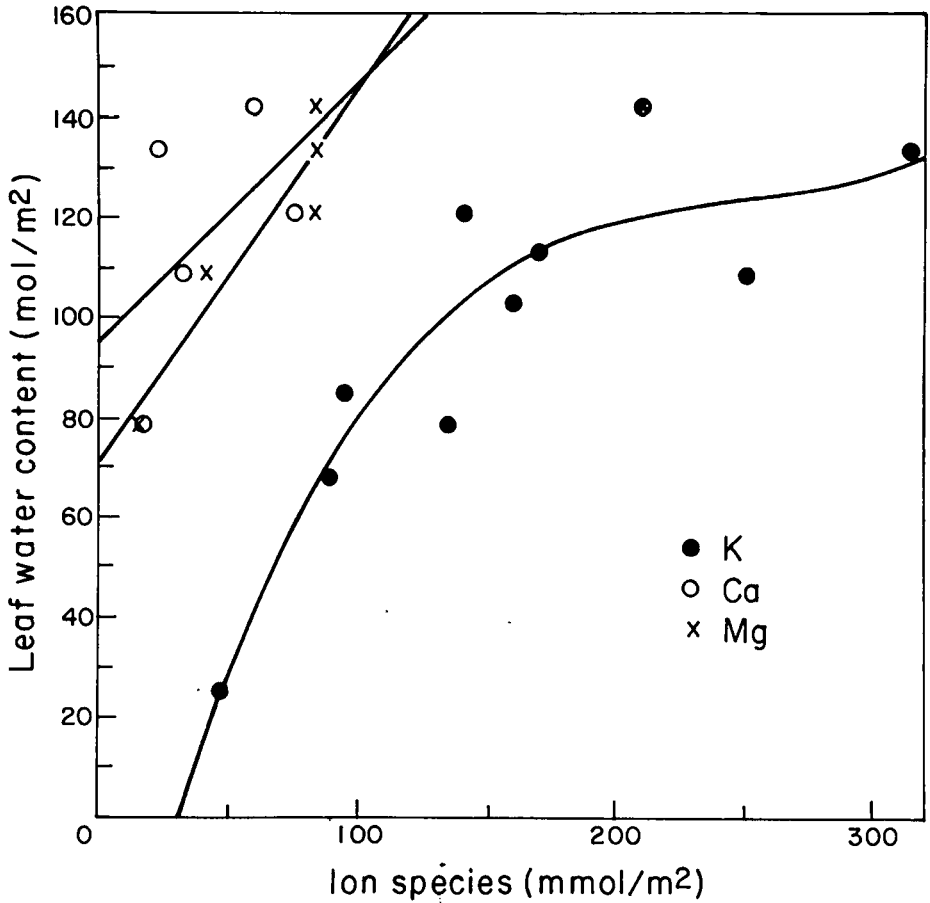


Fig. 3:
Relationships between leaf ion content and water content per unit of leaf area.

Table 2: Regression equations between succulence, N and K contents obtained for samples including the whole range of leaf variation indicated in Table 1.

N content: mol kg⁻¹ dry weight

K content: mmol m⁻² leaf area

Leaf dry weight/area ratio (g m⁻²) = 115.49 - 83.378 N content
R² = 0.702 n = 23

Leaf fresh weight/area ratio (g m⁻²) = 235.9 - 1582.7 N content
R² = 0.625 n = 23

Leaf succulence (mol water m⁻²) = 130.17 - 81.190 N content
R² = 0.702 n = 23

K content = 63.378 N - 0.89185
R² = 0.730 n = 23

Leaf succulence (mol water m⁻²) = - 15.266 + 1.158 K content - 2.5497 E-3 K content²
R² = 0.875 n = 24

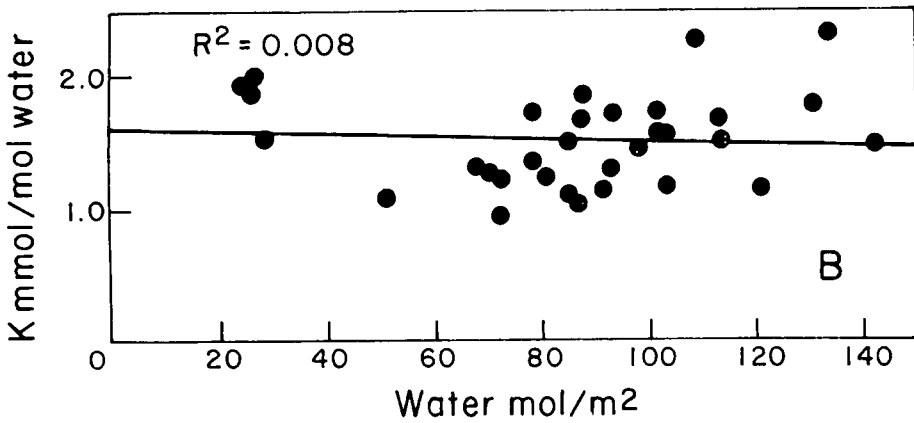
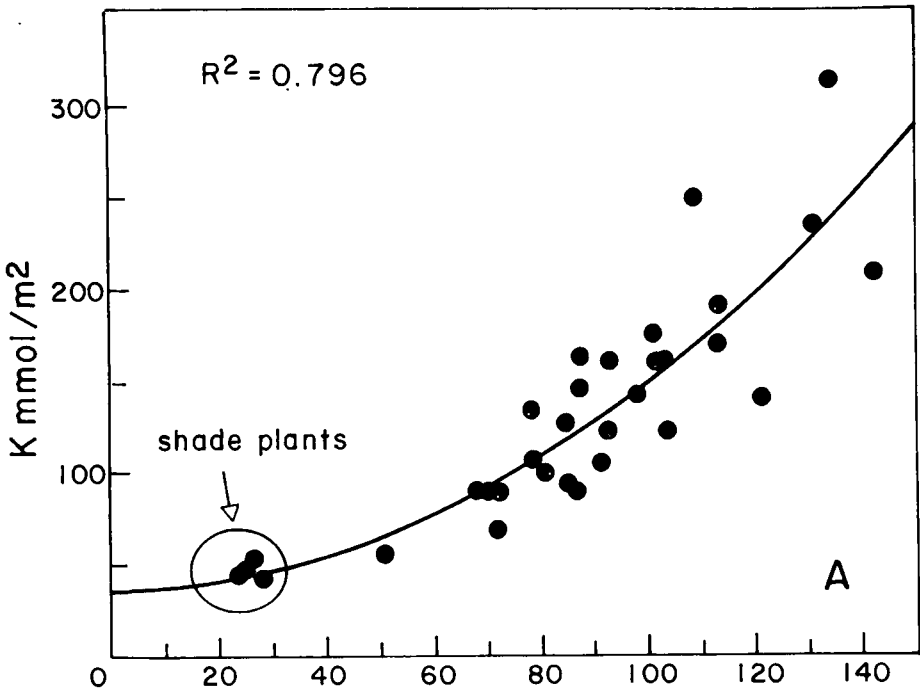


Fig. 4:
 Concentrations of leaf K content per unit of leaf area (A) and per unit of leaf water content (B) associated with variations in leaf water content per unit of leaf area.

Table 3: Soluble phosphorus and inorganic cation content of leaf disks of *Codonanthe macradenia*. Values are averages of 5 whole disks, and 5 disks separated in epidermis and parenchyma. In parenthesis standard deviation.

	Water content mol m ⁻²	K	Ca mmol m ⁻²	Mg	P
Whole disk	132.2 (12.8)	234.1 (33.9)	62.5 (5.3)	138.7 (30.9)	71.4 (8.4)
Epidermis	95.0 (13.9)	180.5 (29.7)	9.8 (1.5)	51.4 (7.8)	49.7 (16.8)
Parenchyma	50.6 (1.2)	51.8 (15.9)	56.3 (7.5)	81.1 (12.8)	26.2 (8.4)
ion concentration per unit tissue water (mmol mol ⁻¹ tissue water)					
epidermis		1.90	0.10	0.54	0.52
Parenchyma		1.02	1.11	1.60	0.52

Discussion

As epiphyte, *C. macradenia* inhabits an environment with limited supply of nutrients and is subjected to frequent periods of drought. The epidermis, which constitutes between 35 % - 68 % of the whole leaf thickness, represents a water storage tissue which can reversibly shrink during periods of water stress. This type of tissue has been described as the "folding type" (STÄLFELT 1956; GESSNER 1956).

Differences in succulence in *C. macradenia* correspond to variations in epidermal thickness. In addition, an increase in succulence is associated with an almost proportional increase in the K content per unit of leaf area. Similar responses have been reported in Commelinaceae species (LÖTSCH 1971; DELGADO & MEDINA 1978), while in *Laguncularia racemosa* leaf succulence changes have been associated with Na concentration (BIEBL & KINZEL 1965). The results presented here show that there is a preferential accumulation of K in the epidermis resulting in higher concentrations of this ion per unit tissue water, while the reverse is true for Ca and Mg.

Accumulation of K in the leaves of *C. macradenia* is probably the result of unbalanced nutrient availability, with N deficiency probably playing a fundamental role. KLEINFELDT (1978) showed that the epiphytic *C. crassifolia* has a positive interaction with carton nest building ants of the genus *Crematogaster*. These ants build carton nests around the stem nectaries. It seems that the nest wall building material may constitute a significant nutrient source for the plant. In these nests stem nodes develop roots more abundantly than nodes on bare bark; moreover, plants which are not on ant nests showed significantly lower growth rates. Similar dependence on ants for nutrient supply were shown in *Tillandsia circinnata* populations by BENZING & RENFROW (1971).

If ant nests around *C. macradenia* were built with plant residues from the surrounding vegetation, one would expect a high K/N ratio in the nest material, since the low Amazon Caatinga vegetation is characterized by a relatively high K, and a particular low N content (CUEVAS & MEDINA 1986). LÖTSCH (1971) suggested that there is an antagonism between

K and soluble N, whereby high K levels in cells promote protein synthesis (increasing efficiency of N use), while high levels of non-protein N are characteristic of K deficient leaves. In the case of *C. macradenia* probably there is a low N supply together with high K availability as a result of the nutritional composition of the ant nests. These relationships could be clarified through the nutritional analysis of the ant nests. Cultivation of Commelinaceae species with low N supply results in a considerable increase in thickness of the upper simple epidermis (MOTHES 1932; GESSNER & SCHUMANN-PETERSEN 1948; LÖTSCH 1971; DELGADO & MEDINA 1978). A similar effect is induced by drought in *Callisia repens*, grown in vermiculite with normal nutrient supply, probably as a result of restricted nutrient flow to the surface of the root (DELGADO & MEDINA 1978).

Although the mechanism of N-K interaction is not yet understood, it seems clear that the nutritional unbalance is associated with the development of succulence. Our results suggest that this succulence is developed predominantly in the multiseriate epidermis because of the differential accumulation of K in this tissue. If K incorporated is preferentially transported into the epidermis cells, it would cause a flow of water into them, brought about by the decrease in water potential of the epidermis compared to the underlying parenchyma. The multiseriate epidermis seems to function as a water reservoir, a character of considerable ecological significance to epiphytic plants which depend on the frequency and not on the amount of rainfall for survival. The upper epidermis of *C. macradenia* does not have stomata, and cuticle seems to be highly impermeable to water. During dry spells water losses from the photosynthetic parenchyma may be at least partially substituted with water drawn from the multiseriate epidermis, thereby attenuating the impact of drought. Direct observation of drought stressed leaves under the microscope provide support to this interpretation, because they present a clearly shrank epidermis while the size of the photosynthetic parenchyma cells remains nearly constant.

It seems clear that the asymmetric distribution of cations within leaves tissues causes the epidermal succulence in species of Commelinaceae (DELGADO & MEDINA 1978) and Gesneriaceae. We hypothesize that this differential cation distribution is the cause for development of epidermal or hypodermal water reservoirs in other families such as Piperaceae (KAUL 1977), Guttiferae (POPP et al. 1988), Combretaceae (BIEBL & KINZEL 1965), and Bromeliaceae (MEDINA 1974). The accurate analysis of cation distribution in leaf tissues with techniques such as X-ray fluorescence, combined with scanning electron microscopy, should answer this question for leaves in which the water tissues cannot as easily isolated as in *C. macradenia*.

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