

**STOMATAL SIZE, DENSITY AND CONDUCTANCE IN LEAVES OF  
SOME XEROPHYTES FROM A THORN SCRUB IN VENEZUELA  
DIFFERING IN CARBON FIXATION PATHWAY**

**TAMAÑO, DENSIDAD Y CONDUCTANCIA DE ESTOMAS EN HOJAS DE  
ALGUNAS XEROFITAS DE UN MATORRAL ESPINOSO DE VENEZUELA  
QUE DIFIEREN EN RUTA DE FIJACION DE CARBONO**

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**ABSTRACT**

The objective of this study was to test the hypothesis that stomatal density, maximum leaf conductance and carbon fixation pathway are interrelated. Stomatal size and number of stomata per unit leaf area were measured in species from a thorn scrub in the semi-arid zones of northwestern Venezuela. Measurements of leaf diffusive conductance were made during different seasons. Some of the species were also grown in a greenhouse where the same measurements were made on well-watered plants to assess the maximal values of conductance that plants could display. Stomatal density, but not size, was related to carbon fixation pathway, with CAM plants showing the lowest and C3 plants the highest values. The same relation held for the proportion of leaf area occupied by stomata. Maximal leaf conductance in the greenhouse was not related to either stomatal density or percent leaf area occupied by stomata. In the field, a significant but small positive correlation ( $r = 0.48$ ) was found between percent stomatal leaf area and maximum conductance, C3 plants showing the highest and CAM plants the lowest values for both parameters. These preliminary results suggest that conductance is governed by carbon fixation pathway, and perhaps stomatal density, only when plants are under some degree of water deficit.

**KEY WORDS:** stomata, xerophytes, carbon fixation pathways, leaf conductance, thorn scrub, Venezuela

**RESUMEN**

El objeto del presente estudio fue el de comprobar la hipótesis de que la densidad estomática, la conductancia foliar máxima y la ruta de fijación de carbono están interrelacionadas. Se midieron el tamaño y el número de estomas por unidad de área foliar en especies de un matorral espinoso de las zonas semiáridas del noroeste de Venezuela. Durante las diferentes estaciones del año también se hicieron mediciones de la conductancia difusiva foliar. Algunas de las especies fueron cultivadas en un invernadero y las mismas mediciones fueron hechas en plantas irrigadas para determinar los valores máximos de conductancia que pudieran alcanzar las plantas. La densidad estomática, pero no el tamaño, mostró una relación con la ruta de fijación de carbono; las plantas CAM mostraron los valores menores, mientras que las C3 tuvieron los mayores. Esta relación se mantuvo para el porcentaje de área foliar ocupada por estomas. Para los valores de campo se encontró una correlación positiva significativa pero

pequeña ( $r = 0,48$ ) entre el porcentaje de área foliar ocupada por estomas y la conductancia máxima, con valores máximos para las plantas C3 y mínimos para las plantas CAM, para ambos parámetros. Estos resultados preliminares sugieren que la conductancia es gobernada por la ruta de fijación de carbono, y posiblemente por la densidad estomática, sólo cuando las plantas se encuentran bajo un cierto grado de deficiencia hídrica.

**PALABRAS CLAVE:** estomas, xerófitas, rutas de fijación de carbono, conductancia foliar, matorral espinoso, Venezuela

## INTRODUCTION

Plants from arid and semi-arid regions must regulate their water loss very strictly and to do this they resort to a variety of morpho-anatomical and physiological mechanisms. These mechanisms may operate dynamically in the short or long term, as shown by stomatal closure and paraheliotropic movements; they may also operate permanently by responses such as increases in pubescence, reduction in leaf size, and leaf abscission (Turner 1983). It is generally considered that selection pressures in xeric environments favour the C4 and Crassulacean acid metabolism (CAM) pathways of carbon fixation (see Kluge and Ting 1978, Pearcy and Ehleringer 1984), both of which show higher water use efficiency than C3 plants (see Kluge 1976). In fact, although C4 species do not necessarily dominate on xeric habitats and both CAM and C3 species do occur, the frequency and cover of C4 species are higher in xeric zones than in more mesic regions (Osmond et al. 1982).

One aspect of the morpho-anatomical features in which a difference between carbon fixation pathways may be detected is the configuration and operation of the stomata, i.e. maximal conductance, and stomatal size, number, and presence in one or both epidermes. Plants with the C3 and C4 pathways have a higher frequency

(density) of stomata per unit photosynthetic tissue area, by one order of magnitude, than CAM plants (Osmond et al. 1982). Also, it is generally assumed that conductance to water vapour is, under similar environmental conditions, higher in C3 than in C4 plants (see Gifford 1974) and that conductance in CAM plants is zero for most of the day (see Kluge and Ting 1978).

The proportion of leaf area occupied by stomata may be related to water vapour conductance. Körner et al. (1979) found that stomata could cover from 12% to 100% of the leaf area in several species, and determined that a comparison of maximal conductance between species was most suitably done using the total leaf area. We were interested in finding out the possible correlation between maximal leaf diffusive conductance to water vapour and stomatal area, rather than calculating the potential water loss per leaf, in xerophytes with different carbon fixation pathways growing under natural conditions. Hence, we restricted our basis of analysis to a unit leaf area, not total leaf area.

The same study was undertaken in well-watered plants under greenhouse conditions, to ascertain that the maximal conductances recorded in the field were representative of the highest values the species could attain.

The present study is part of an investigation on carbon and water balance

in plants from a thorn scrub in the semi-arid zones of northwestern Venezuela (Subregion C.1., Lara-Falcón Hills System, according to Huber and Alarcón 1988). The area studied possesses no altitudinal gradient. This scrub is composed of a sparse evergreen and deciduous tree stratum, with *Prosopis juliflora*, *Cercidium praecox* and *Capparis* as dominant, an arbustive stratum dominated by *Ipomoea carnea*, with *Jatropha gossypifolia* as a frequent constituent, and a herb stratum dominated by *Alternanthera crucis*. CAM is represented by the tree cactus, *Pereskia guamacho*, by columnar cacti of the tribe Cereae, by the climbing vine, *Cissus trifoliata*, and by herbs such as *Opuntia caribaea*, *O. wentiana*, *Melocactus caesius* and, occasionally, *Talinum triangulare*.

## MATERIALS AND METHODS

Plant material was collected in a thorn scrub near the city of Coro, Estado Falcón (11° 25' N, 69° 36' W, 20 m).

Rainfall data were taken from the Coro Airport Meteorological Station, at c. 9 km from the field site. Air temperature was measured with an air thermistor connected to a YSI telethermometer. Relative humidity was measured with an Abbeon hair hygrometer. Photosynthetically active radiation (PAR) was measured with a LI-COR LI-170 meter.

Only the leafy species, *Alternanthera crucis*, *Calotropis procera*, *Cissus trifoliata*, *Ipomoea carnea*, *Jatropha gossypifolia*, *Malpighia glabra*, *Pereskia guamacho* and *Talinum triangulare*, were studied. All these species are sun plants. *A. crucis* also grows under the shade of *Prosopis* trees, but for this study only plants under full exposure were sampled.

Samples for stomatal size and density were taken only at the height of the rainy season in 1977, which was when foliage was most abundant and vigorous in these deciduous species. Measurements of stomatal size (SS), stomatal density (SD) and diffusive resistance were made on leaves from plants growing in the field as well as in the greenhouse in Caracas, c. 1100 m. The greenhouse plants were maintained under daily irrigation.

*Amaranthus dubius*, a C4 species not common in the field site, was grown in the greenhouse to compare it to *Alternanthera crucis*, since the latter was the only C4 species detected in the field.

Plant material was processed within 48 h from collection in the field, or immediately in the case of greenhouse plants. Two methods were used to obtain samples for counting stomata. For the majority of field plants, epidermal peels were prepared following the procedure described by Ram and Nayyar (1974). Some field samples and all of the greenhouse samples were made by coating the epidermis with clear nail varnish and recovering the imprint by sticking transparent tape to the varnish coat (E. León, pers. comm.). Both methods were tested against each other for stomatal size and density and gave statistically similar results.

At least four fully expanded, non-senescent leaves per species were taken for preparing stomatal peels or imprints. Ten fields per species per epidermis were counted using a *camera lucida*. Twenty stomata per epidermis per species were measured under the microscope using an eyepiece micrometer calibrated against a stage micrometer. Stomatal size (not pore size) was determined as the area of an

ellipse:  $A = \pi ab$ ,  $a$  and  $b$  being the half-lengths of the long and short axes of the guard cells. The proportion of leaf area occupied by stomata (SA) was calculated as  $SA = SS \times SD \times 10^{-4}$  (stomatal cross-sectional area occupying 100 mm<sup>2</sup> leaf area).

Carbon fixation pathways were assigned using one or more of the following criteria: presence of Kranz (C4) or dorsiventral (C3) leaf anatomy (Fonseca 1983), occurrence of dark CO<sub>2</sub> fixation (Olivares et al. 1984, Delgado 1987, A. Herrera, unpublished results), and/or nocturnal acid accumulation (Olivares et al. 1984, Delgado 1987, Díaz and Medina 1983, A. Herrera, unpublished results), CO<sub>2</sub> compensation point (G. Montes, unpublished results), and patterns of short-term CO<sub>2</sub> incorporation into photosynthetic products (Fonseca 1983).

Leaf diffusive resistance for each surface was measured in triplicate by means of a LI-COR porometer (mod. LI-60) with dynamic response. Data were transformed into values of conductance to water vapour (g) using the equation given by Hall (1982). Data shown are the maximum values recorded during the daytime in the field from January 1977 until July 1979. Measurements in the greenhouse were made between 0700 h and 0900 h since the highest values in the field were always obtained during that period, probably because of a minimum daytime evaporative demand. PAR at the moment of measurement both in the field and in the greenhouse was higher than 400  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . Data reported are means. The statistical analysis was done using the SPSSX package and consisted of analysis of variance with log-transformed data, followed by Duncan's Multiple Range Test at  $p < 0.01$ .

## RESULTS

The rainfall regime for the period of sampling is shown in Fig. 1.

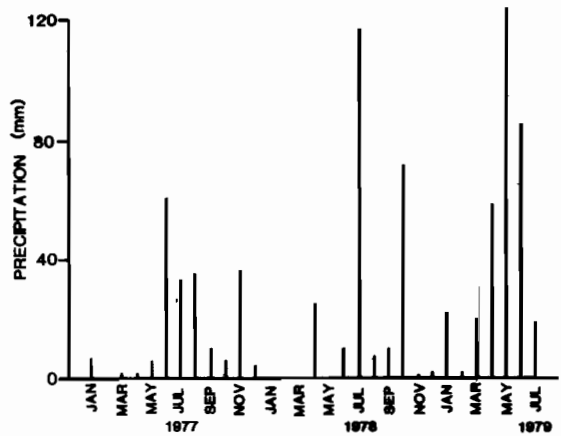


FIGURE 1. Monthly rainfall in the field site for the period of study.

Microclimatic conditions in the greenhouse resembled those in the field for an average daily course during the rainy season (Fig. 2) although in the greenhouse values of PAR and air temperature were slightly lower, and relative humidity higher, than in the field.

In leaves collected in the field, the number of stomata per unit leaf area followed a descending order relative to carbon fixation route: C3 > C4 > CAM (Table 1). Stomatal size showed no relation to carbon fixation pathway. Percent leaf area occupied by stomata roughly followed the order C3, C4 > CAM. Distribution of stomata over the leaf surface was apparently uniform for all the species examined.

The paucity in values of conductance for the upper epidermis in most species in the field (Table 1) is due to the fact

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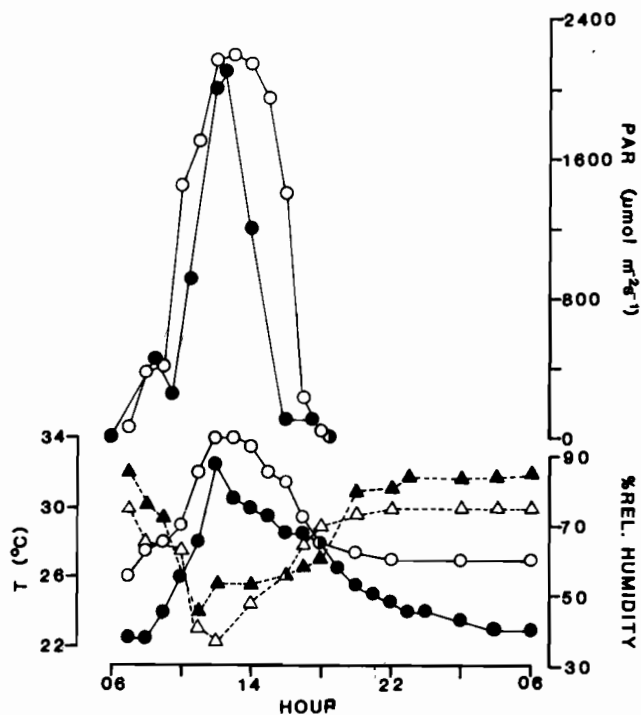


FIGURE 2. Microclimatic conditions for plants in the field (open symbols) and in the greenhouse (closed symbols). (A) PAR at full exposure. (B) air temperature (circles) and air relative humidity (triangles).

Table 1. Stomatal density (SD), stomatal size (SS), percent leaf area occupied by stomata (SA), and maximal leaf conductance to water vapour (g) for the upper (U) and lower (L) epidermis (EPI), in plants growing in the field. Values followed by the same letter are not significantly different at  $p < 0.01$ , nd = conductance below  $10 \text{ mmol m}^{-2} \text{ s}^{-1}$ .

SPECIES	EPI	SD ( $\text{s}/\text{mm}^2$ )	SS ( $\mu\text{m}^2$ )	SA (%)	g ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )
<b>C3</b>					
<i>I. carnea</i>	U	259 fg	549 efh	14.1 j	nd
	L	191 c	619 fg	11.9 j	70.2 ef
<i>J. gossypifolia</i>	U	209 ef	162 a	3.4 e	nd
	L	326	220 bcd	7.2 fg	16.0 a
<i>M. glabra</i>	L	354	169 ab	5.9 fg	27.1 abc
<i>C. procera</i>	U	234 eg	255 d	6.0 fg	68.0 de
	L	402	124	5.1 f	69.5 ef
<b>C4</b>					
<i>A. crucis</i>	U	127 d	180 c	2.3 abcd	nd
	L	114 d	487 ef	5.5 g	52.9 d
<b>CAM</b>					
<i>P. guamacho</i>	U	31 b	537 efg	1.7 ab	nd
	L	34 abc	481 e	1.6 a	18.8 ab
<i>C. trifoliata</i>	U	28 a	643 ghij	1.8 ac	nd
	L	35 c	657 ghij	2.7 bde	21.6 abc

that during most of the sampling period leaf resistance was too high to be measured with the porometer ( $g < 10 \text{ mmol m}^{-2} \text{ s}^{-1}$ ).

Although the species grown in the greenhouse were not exactly the same as

in the field, a similar pattern for stomatal density was observed for them (Table 2), *Amaranthus dubius* showing values for both epidermes that resembled more those of C3 species than of the other C4 species,

**Table 2.** Stomatal density (SD), stomatal size (SS), percent leaf area occupied by stomata (SA), and maximal leaf conductance to water vapour (g) for the upper (U) and lower (L) epidermis (EPI), in plants growing in the greenhouse. Values followed by the same letter are not significantly different at  $p < 0.01$ . \*Significantly different at  $p < 0.01$ .

SPECIES	EPI	SD (s/mm <sup>2</sup> )	SS ( $\mu\text{m}^2$ )	SA (%)	g (mmol m <sup>-2</sup> s <sup>-1</sup> )
<b>C3</b>					
<i>I. carnea</i>	U	173 de	434 ghij	7.5 k	1116.5 *
	L	224 gh	198 abcd	4.4 eghj	69.6
<i>J. gossypifolia</i>	U	210 efh	162 a	3.4 cde	238.7
	L	442	192 abcd	8.5 k	122.4
<b>C4</b>					
<i>A. crucis</i>	U	81 a	301 ef	2.6 b	81.9
	L	125 c	337 ei	4.2 dfhi	32.8
<i>A. dubius</i>	U	309	182 abc	5.7 ijk	64.1
	L	213 dfg	320 efg	6.9 ijk	173.1
<b>CAM</b>					
<i>C. trifoliata</i>	U	33	282 c	0.9	53.2
	L	54	369 efg hij	2.0 ab	23.4
<i>T. triangulare</i>	U	111 bc	322 c	3.6 cfg	57.7
	L	95 ab	170	1.6 a	28.5

*Alternanthera crucis*. The latter had a similar stomatal density to the CAM plant, *T. triangulare*. Stomatal size, again, bore no relation to carbon fixation pathway. For plants in the greenhouse, too, the percent leaf area occupied by stomata was generally highest for species with the C3 pathway

and lowest for species with the CAM pathway.

When both leaf surfaces were considered, conductances in plants growing in the field were always lower than in plants growing in the greenhouse. This was probably due to higher values of the

leaf-air vapour-pressure gradient in the field. Values ranged from 0.4 to 2.0 kPa in the greenhouse and from 3.0 to 7.0 kPa in the field, even during the rainy season. PAR level did not seem to be the reason for the differences in conductance, since measurements were taken at levels higher than  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ , at which many of the species studied have their photosynthetic rate saturated (Delgado 1987, Coronel et al. 1984, A. Herrera et al., unpublished, G. Montes and R. Urich, unpublished, Quijada 1987).

No correlation was found between conductance and stomatal size ( $r = 0.02$ ), conductance and stomatal density

( $r = 0.05$ ), or conductance and percent leaf area occupied by stomata ( $r = 0.14$ ) when data from the field and greenhouse plants were analyzed either separately or in combination, with the exception of a small significant ( $p < 0.01$ ) coefficient of 0.48 between conductance and percent leaf area occupied by stomata in plants growing in the field.

Table 3 shows that no trend was found for the ratio of stomatal density from the upper/lower epidermes in relation to carbon fixation pathway, when values were analyzed either by location or for both locations together.

**Table 3.** Ratio of stomatal density (upper epidermis/lower epidermis) in plants growing in the greenhouse and in the field. Values followed by the same letter are not significantly different at  $p < 0.01$ .

SPECIES	RATIO U/L	Significance	
		by location	both locations
<b>Field</b>			
<i>Ipomoea carnea</i>	0.8	a	abcdef
<i>Jatropha gossypifolia</i>	1.6	cde	efghij
<i>Calotropis procera</i>	1.9	de	fghijk
<i>Alternanthera crucis</i>	0.9	ab	abcefghij
<i>Pereskia guamacho</i>	1.2	abc	abcde
<i>Cissus trifoliata</i>	1.5	cd	efghi
<b>Greenhouse</b>			
<i>Ipomoea carnea</i>	1.4	a	
<i>Jatropha gossypifolia</i>	2.1	d	
<i>Alternanthera crucis</i>	1.7	cd	
<i>Amaranthus dubius</i>	0.7	a	a
<i>Cissus trifoliata</i>	1.6	c	
<i>Talinum triangulare</i>	0.9	a	abc

## DISCUSSION

Our results showing that the number of stomata per unit leaf area follows a descending order relative to the carbon fixation route, i.e. C3, C4 > CAM, confirm previous reports (see Osmond et al. 1982). Since only one C4 species was studied in the field, a generalization with regard to C4 species is not possible. Nevertheless, our values for *A. crucis* as well as for *A. dubius* are within the range reported for C4 plants (Das and Santakumari 1977). Table 4 summarizes data from the literature for plants with the three carbon fixation pathways. In general, there is little overlap in ranges of stomatal density between non-CAM and CAM species; however, no clear difference emerges between C3 and C4 plants.

The ratio of upper to lower epidermis stomatal density in this study showed significant differences between species but these differences could not be associated with any particular carbon fixation pathway.

Contrary to the data of Das and Santakumari (1977), values were not higher for C3 than for C4 plants. The amphistomatous CAM species, *Talinum triangulare*, showed more stomata on its adaxial face, and their size was not statistically different from that of stomata on the abaxial face. These observations are in disagreement with generalizations made by Gibson (1982) regarding CAM plants with succulent leaves.

Stomatal size was not a characteristic feature of any particular photosynthetic pathway. When stomatal size as well as density were taken into account, two general groups of species could be outlined, C3 plants showing on the whole higher values of percent leaf area occupied by stomata than C4 and CAM plants. However, *T. triangulare* (upper epidermis) and *C. trifoliata* (lower epidermis) had values which resembled more those for C3 species. This may be related to the fact that inducible CAM operates in *C. trifoliata* (Olivares et al. 1984) and *T. triangulare*

Table 4. Values of stomatal density (SD) for plants from the three carbon fixation pathway, extracted from the literature. U and L, upper and lower epidermis, respectively.

Carbon Fixation Pathway	SD (s/mm <sup>2</sup> )		range (U+L)	Reference
	U	L		
C3	105 ± 15	324 ± 47	50-480	Das and Santakumari (1977)
C4	124 ± 22	138 ± 22	49-236	Das and Santakumari (1977)
CAM			5-125	Gibson (1982)
leafy species	25 ± 1	25 ± 3		Kluge and Ting (1978)
stem succulents	34 ± 11			Kluge and Ting (1978)



(Delgado 1987); they show dark CO<sub>2</sub> uptake only under water deficit.

A high stomatal density in leaves of *Pereskia* sp. has been related to their reputed intermediate C3-CAM metabolism (Gibson 1982). In this study, though, values of percent leaf area occupied by stomata in *P. guamacho* were amongst the lowest recorded by us. Data reported by Díaz and Medina (1984), and unpublished observations (A. Herrera, R. Urich and G. Montes) indicate that *P. guamacho* is an inducible CAM species.

There was no clear segregation of conductance values according to carbon fixation pathways in the greenhouse. Values in the field were lower than in the greenhouse and could be ranked, according to pathway, in the order C3, C4 > CAM, with the exception of the C3 species, *Jatropha gossypifolia*, which showed conductances closer to those for the CAM species. A trend towards lower conductance values for C4 than for C3 plants has been highlighted by Gifford (1974) and Kriedemann and Downton (1981). Our data for *A. crucis* were not significantly different from those for the C3 species, *C. procera*.

Stomatal conductance did not appear to be governed by either stomatal density, size or percent leaf area occupied by stomata in leaves of field and greenhouse plants. A significant, but very small, correlation between stomatal conductance and percent leaf area occupied by stomata in the field suggests that the eventual differences in conductance due to stomatal density and to carbon fixation pathway would be noticed only under conditions of partial water deficit, and those differences would be offset by frequent watering in the greenhouse. Apart from

soil water content, another important difference between the two growth regimes may have been leaf-air water vapour gradient, which was higher for plants in the field, even during the rainy season.

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