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# Stomatal Density in Leaves of Various Xerophytes - A Preliminary Study

DAVID W. STROBEL AND MARSHALL D. SUNDBERG\*

**ABSTRACT** — Recent general botany and plant anatomy textbooks state that stomatal density of xerophytic leaves is higher than that found in leaves of mesophytes. In contrast, previous textbooks indicate that stomatal density in xerophyte leaves is reduced. The purpose of this study is to examine the leaves of succulent and non-succulent xerophytes to determine if opposite trends in stomatal density correlate with the xeromorphic strategy employed. The species examined were the succulents *Crassula argentea*, *Kalanchoe blossfeldiana*, *K. diagrammontiana*, *K. tubiflorum*, and the non-succulents *Nerium oleander*, *Ammophila breviligulata*, and *Ficus elastica*. Stomatal densities of succulents were determined directly from epidermal peels. Stomatal densities of non-succulents, whose stomates occur in crypts, were determined from cross-sections using stereological methods. Results indicate that stomatal density is reduced in leaf succulents and increased in non-succulent types.

## INTRODUCTION

Stomates are intercellular openings in the surface of leaves and stems through which gases are exchanged between the intercellular spaces of tissue and the atmosphere. If water vapor is lost through stomates and water is at a premium in arid environments, it seems reasonable that plants with fewer stomates per unit surface area would be best adapted to the arid environment. This was the conclusion of Haberlandt (1) and the physiological plant anatomists of the late nineteenth century. As recently as 1947 (2) a reduction in number of stomates was cited as a typical plant adaptation to the xeric environment.

Contemporary plant anatomists (3,4,5) state that there is actually an increase in stomatal density in leaves of xeromorphic plants, an observation first reported by Volkens (6). The explanation for this apparent anomaly is that when stomates are closed during the hottest part of the day, little water is lost. But there is also little gas exchange, so photosynthesis is inhibited. When the stomates reopen there is great demand for gas exchange to facilitate rapid photosynthesis (7). Numerous stomates are therefore advantageous.

The species cited by Haberlandt (1) are succulents whereas those cited by Esau, Cutter, and Fahn (3,4,5) are non-succulents. Therefore, it was our hypothesis that different trends in stomatal density are correlated with the xeromorphic strategy employed. The purpose of this study was to compare the stomatal densities in leaves of various succulent and non-succulent xerophytes.

## MATERIALS AND METHODS

Seven species of xeromorphic plants were examined during the winter and spring of 1983. The succulent types included: *Crassula argentea*, *Kalanchoe blossfeldiana*, *K. diagrammontiana*, and *K. tubiflorum*. Non-succulent species were *Ammophila areviligulata*, *Ficus elastica*, and *Nerium oleander*.

Two methods were used to determine stomatal density. Epidermal peels were made of all succulent leaves and stomatal frequency was determined directly from the number of stomates per calibrated field of view. At least three fields of view were averaged for each leaf region examined. The stomates of most

non-succulent xerophytes are sunken or occur in crypts. **although in many cases (eg., *Ficus elastica*)** individual stomates are sunken beneath the epidermis, in others (eg., *Ammophila* and *Nerium*) there are multiple stomates per crypt and so the number of stomates cannot be determined from an epidermal peel.

A stereological technique was used to determine stomatal frequency of non-succulent leaves (8). Stereological estimates were calculated from counts made from commercially prepared leaf cross-sections. The image of the specimen was displayed on a video monitor along with a uniform grid test probe (8). Relative stomatal density was determined by counting intersections of stomates and epidermal cells with the test probe. An estimate of stomatal density was obtained from the formula  $D = 1/a(A)$  where  $D$  is the stomatal density,  $a$  is the average area of an epidermal cell, and  $A$  is the proportion of stomates to epidermal cells. The precision of this estimate is directly related to the number of counts used to obtain the relative density (9). For each specimen examined stereologically over 2000 counts were made, assuring a 95% confidence level.

It is known that stomatal density may vary with leaf position on the plant and sample position on the leaf (10). Several preliminary counts were made to determine the amount of inherent variation in an individual plant. The parameters examined were: leaf position along the axis, sample position along the leaf axis, sample position across the width of the blade, and conditions under which the sampled plants were grown. Leaves were numbered successively from the youngest expanded leaves at the tip of the plant toward the older leaves lower down the stem. A 95% confidence level was employed on all statistical tests.

## RESULTS AND DISCUSSION

Leaves of *Ficus elastica* (non-succulent) were used to determine inherent variation in a single plant. Stomatal density near the midrib did not differ significantly from densities near the margins on any of the leaves along the axis (Leaf 2,  $t = 2.00$ ; Leaf 11,  $t = 1.83$ ; Leaf 20,  $t = 0.27$ ;  $P > 0.05$ ; Figure 1). However, stomatal frequency on a young leaf, L2, was significantly lower than on the older Leaf 20 ( $t = 13.38$ ,  $P < 0.05$ ; Figures 1, 2). The argument

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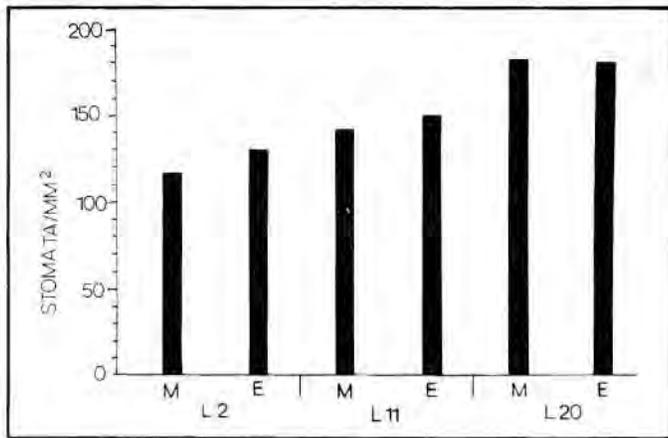


Figure 1. Effect of lateral sample position across the leaf blade on average stomatal density in *Ficus elastica*. M, near midrib; E, near margin; L2, L11, L20, samples on leaves 2, 11, and 20, respectively. Leaves are numbered from youngest to oldest expanded leaf.

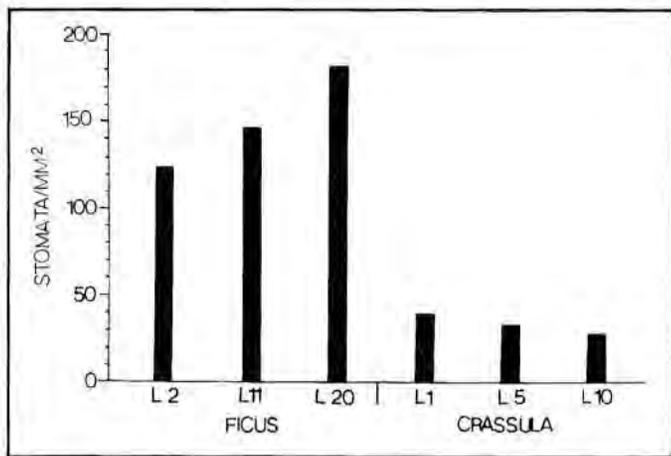


Figure 2. Effect of sample leaf position along the shoot axis on average stomatal density in *Ficus elastica* and *Crassula argentea*. L = leaf number, progressing basipetally from the most recently expanded leaf.

proposed by Maximov (11) to explain similar results in other non-succulent plants is that the lower leaves are closer to the water source, the roots; therefore, they are less likely to be water stressed and are able to support a higher stomatal density. This explanation is supported by the observation that stomates may continue to differentiate as the leaf expands (4). This trend of increasing stomatal density on successively older leaves was not universal. There was no significant difference between Leaf 1 and Leaf 10 of the succulent species *Crassula argentea*, ( $t = 2.00, P > 0.05$ ), although there appeared to be a trend of decreasing frequency in successively older leaves (Figure 2). There was also no clear pattern along the axis of a single leaf (Figure 3). In *Ficus* there was a decrease in stomatal density from the tip to the base of the leaf, but this trend was not significant, ( $t = 1.00, P > 0.05$ ). In *Kalanchoe diargremontiana* (succulent), however, there was a significant decrease from tip to base on the upper surface ( $t = 7.33, P < 0.05$ ) and a significant increase from tip to base on the lower surface, ( $t = 3.67, P < 0.05$ ). The significance of these opposite trends on different sides of the same leaf is unclear.

Significant differences in stomatal density of a single species are correlated with the growing conditions of the plant (Figure 4). *Kalanchoe diargremontiana* grown under humid conditions had significantly fewer stomates than a sibling grown under more

typical dry conditions ( $t = 3.94, P < 0.05$ ). This trend agrees with numerous reports in the literature, beginning with Volken (6), involving a wide array of species. Furthermore, seedling leaves exhibited a significant increase in stomatal density over that of mature plant leaves ( $t = 4.30, P < 0.05$ ).

The results of these preliminary studies indicate that for reliable comparisons of stomatal density in succulent and non-succulent xeromorphic leaves, it is essential that the plants be grown under comparable, preferably dry, conditions. Likewise, because stomatal density may vary significantly between young and mature leaves, it is important that only mature leaves are included in the sampling.

Although differences in stomatal density on different areas of the same leaf have frequently been cited (10, 11), these trends do not appear to be significant, especially in terms of the overall objective of this study.

The average stomatal densities on both the upper and lower leaf surfaces of four succulents were compared with the frequencies observed on the leaves of three non-succulents (Figure 5). Succulents had a dramatically lower number of stomates per mm<sup>2</sup> than non-succulents. Less than 50 stomates/mm<sup>2</sup> were observed in all succulent species, more or less evenly distributed over both the upper and lower surfaces. These numbers are comparable to those reported for the succulents *Sedum acre* and *Sempervivum tectorum* (1). They support the traditional interpretation of xeromorphic leaf adaptation; that is, reduced stomatal density decreases the surface area through which water is lost, thereby reducing the need for this limited resource. The fact that stomates occur on both sides of the leaf is not surprising, since the leaves are more rounded in cross section than the flattened leaves of non-succulents. Furthermore, in succulents, the leaves, especially younger leaves, tend to be more upright on the stem so that both the upper and lower sides are exposed to full sunlight during parts of the day.

Two to over 10 times as many stomates/mm<sup>2</sup> occurred on leaves of non-succulents, but these were restricted to the lower surface. By being restricted to the lower surface, therefore shaded from direct sunlight, water loss during the hottest part of the day is reduced. Yet the high stomatal density assures that when the stomates are open, rapid gas exchange may occur. The distributions observed on non-succulents in this study also

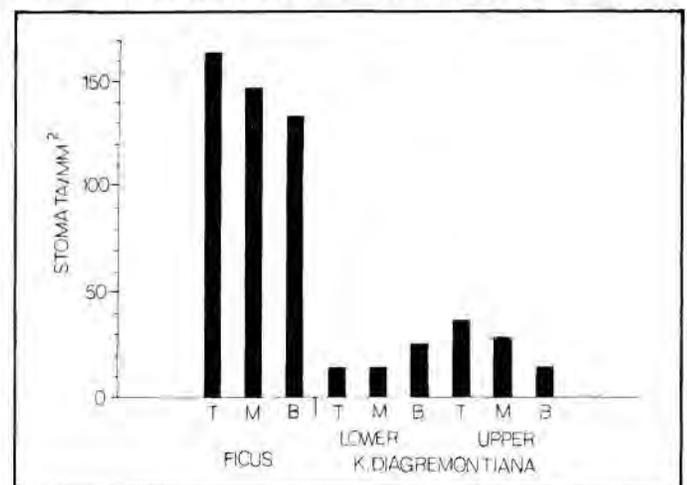


Figure 3. Effect of sample position along the leaf axis on average stomatal density in *Ficus elastica*, *Kalanchoe diargremontiana*. T, near tip; M, near middle; B, near base of leaf. Values of both lower and upper epidermis are presented for *Kalanchoe*.

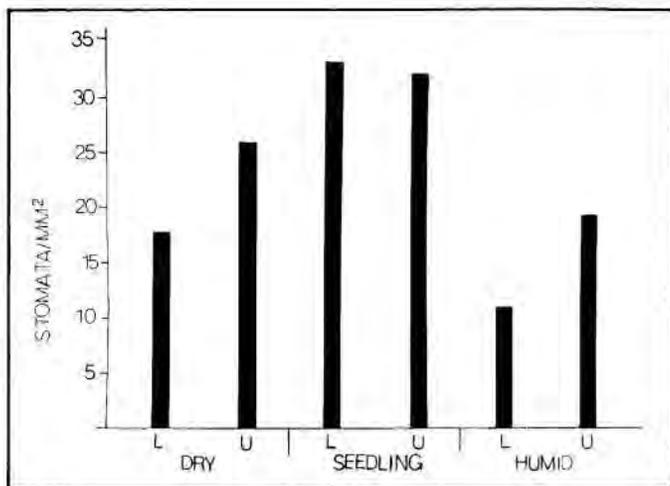


Figure 4. Effect of age of plant, seedling vs. mature, and growing conditions, typical dry vs. humid, on average stomatal density in *Kalanchoe diargemontiana*. L, lower epidermis; U, upper epidermis.

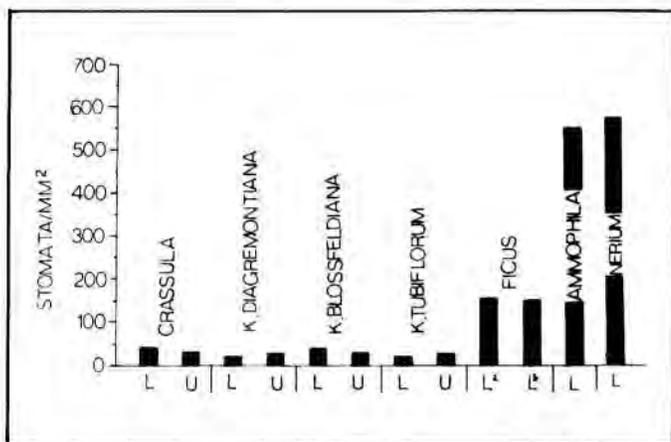


Figure 5. Average stomatal density on leaves of seven xerophytes. *Crassula* and *Kalanchoe* are succulents and both upper, U, and lower, L, surfaces are presented. *Ficus*, *Ammophila*, and *Nerium* are nonsucculents. Data obtained directly from epidermal peels, L<sup>a</sup>, and stereological technique, L<sup>b</sup>, are presented for *Ficus*.

Popham (12) reports 145 stomates/mm<sup>2</sup> in *Ficus elastica*, a number nearly identical to our results. There was virtually no difference in the density calculated for *Ficus* using either direct determination from leaf peels (150.5/mm<sup>2</sup>) or stereological technique (146/mm<sup>2</sup>), indicating that the two methods are of comparable precision. The data obtained for *Ammophila* and *Nerium* approach that cited for *Olea europaea* (12, 13).

## CONCLUSIONS

Two divergent stomatal strategies, characterized by either an increase or a decrease in density, appear to have evolved in xeromorphic plants. Succulents exhibit a relatively low stomatal density and tend to be water conservers, while non-succulents exhibit a relatively high stomatal density and tend to be water spenders. These trends parallel a number of other well-documented differences between succulents and non-succulents such as the type of root system (shallow vs. deep), metabolism (CAM vs non-CAM) in addition to tissue succulence.

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