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COMPARISON OF THE LEAF EPIDERMIS OF SALIX ALBA L. IN DIFFERENT REGIONS OF THE LEAFY CROWN

SZERÉN PATAKY

Department of Botany, Attila József University, Szeged

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Introduction

In the course of investigating the leaf epidermis of amentiferous trees (Pataky, 1967), the question arose what an effect the conditions inside the foliage have upon some tissue elements of leaf epidermis.

According to Wagner (1957): "the more closed the foliage is the greater differences exist concerning the values and daily course of climatic elements, opposite to an open field". As demonstrated by Geiger and Amann (1961), the greatest fluctuations in temperature take place in the leafy crown; the greatest rise in temperature is found there. Kausch and Haas (1965) attribute the considerable difference, observed in the total amount of some chemical substances of leaves developed in the sunshine and in the shade inside the foliage of a free standing *Fagus silvatica* L., to microclimatic factors.

In the foliage, the different microclimatic conditions have the most direct influence on leaves supposedly through the epidermis. The question is whether or not there is a significant difference between the regions of leafy crown concerning some wellmeasurable constituents of leaf epidermis, i.e., whether the situation inside the foliage can be left out of consideration as diagnostized on the basis of epidermis.

With our investigations we wanted to get an answer to the following problems:

a) Whether or not the dissimilar ecological (or microclimatic) conditions of the leafy crown are reflected in some tissue elements — measurable exactly — of epidermis. (What is the number of stomata, length, width of guard cells, etc.).

b) As regards the single regions of foliage, which properties are changing?

c) In which properties is the degree of alterations significant and which are the comparatively stable properties of epidermis that can be used for diagnostic purposes, as well?

d) Is the reflection of the changing ecological effects of identical character and degree both in the upper and in the lower epidermis of leaf?

Materials and Methods

At selecting units and species, we have had regard first of all for the following points of view.

1. The ecological effects inside the leafy crown shouldn't be influenced by any conditions that doubtless occur in case of specimens from the same substance; therefore the material had been collected from trees standing alone.
2. The histological effect of the same ecological factors should be suitable for a simultaneous investigation in the lower, resp. upper epidermis of leaves.
3. We have compared fully developed, exactly determined leaves, resp. leaf parts with one another.

For investigating the conditions inside the leaf crown, we have selected a free standing specimen of *Salix alba* L. (Botanical Gardens, A. József University, Szeged).

Leaves (5—10 pieces) were collected from four different places of the leafy crown, for making preparations:

1. from the edge (outer part) of foliage
2. from the middle part „ „
3. from the inner part „ „
4. from the lower part „ „ out of

fully developed leaves between apex and base of a lateral branch. From the collected material there were made 2—4 epidermis preparations by maceration of every leaf, both from the lower and the upper surface of leaf (Fig. 1).

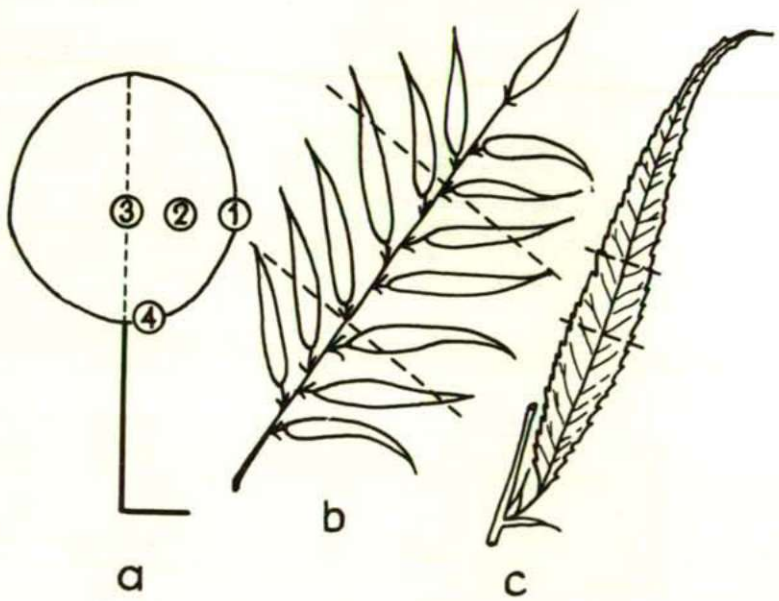

Fig. 1. *Salix alba* L. a) 1. edge of leafy crown, 2. middle of leafy crown, 3. inner part of leafy crown, 4. lower part of leafy crown;
b) lateral branch;
c) leaf pattern (the leaves) resp. leaf parts between the dotted lines are elaborated.

For staining the cleaned preparations, we have applied a triple staining with vesuvin, Ehrlich-f sour haematoxylin and Sudan III (Kisser, 1926).

We have measured the following tissue elements for comparison:

1. Number of stomata, piece/sq.mm (S).
2. Length of guard cells in μ (L). — (The greatest length of the two guard cells were measured in the direction of the longitudinal axis).
3. Width of guard cells in μ (W). — (The joint width of the two guard cells

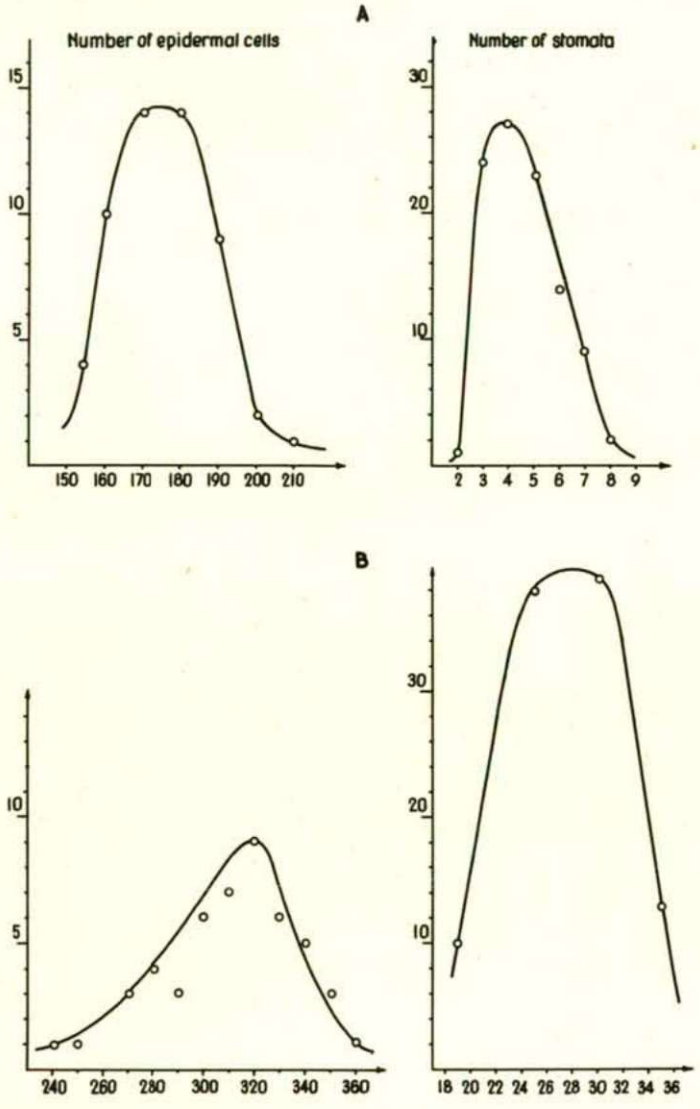

Fig. 2. Distribution curves of cell and stoma numbers of the epidermis, in:
A = upper surface epidermis
B = lower surface epidermis

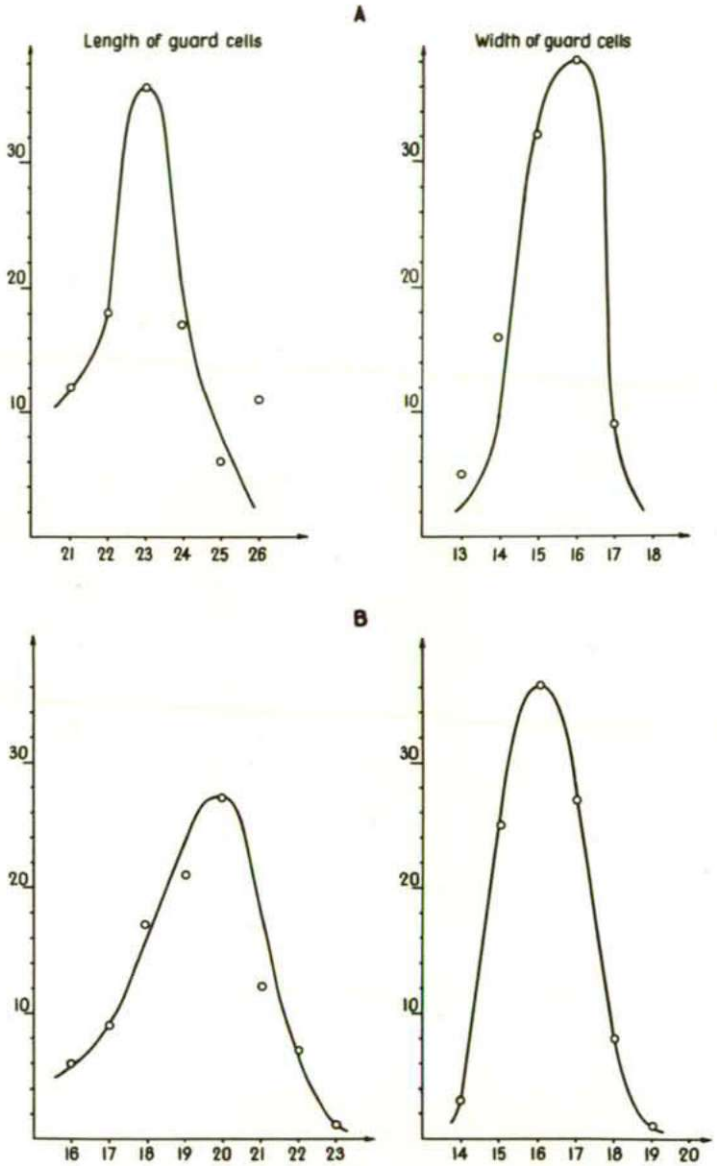

Fig. 3. Distribution curves of length and width data of guard cells, in:
A = upper surface epidermis
B = lower surface epidermis

were measured with the air slot between them).

4. Ratio length /Width of guard cells $\frac{L}{W}$ (A quotient of L and W of guard cells).

5. Number of epidermis cells, piece/sq.mm (E).

6. Stoma index ($I=\frac{S}{E+S} \cdot 100$).

The comparison of the properties enumerated above took place on the basis of the average values of data from 50 visual fields. (Measurements carried out with a Zeiss NF microscope, oc.x12,5, obj.x40).

The properties measured are showing a curve of normal distribution (Figs 2 and 3), the data are, therefore, estimated with variance analysis (Yule-Kendall, 1964). Calculations were performed by co-workers of the Laboratory of Cybernatics, Attila József University, with an electronic computer of type M:3.

Results

Structure and size of the epidermis tissue elements of *Salix alba* L. differ from one another on the upper and lower surface of the leaf. In the upper epidermis the cells are in every case larger than in the lower one (cf. Table 1).

The investigated properties of leaf epidermis are not homogeneous in the leafy crown, either. We have observed major differences between the single regions as to stoma number and epidermis cell number, and minor ones as to stoma index.

Size and quality of differences between the single regions of foliage are not the same in the upper and in the lower epidermis. The properties measured in the upper surface epidermis are differring generally significantly from one another, as regards nearly every region of the leafy crown. In the lower surface epidermis, the distribution of the same properties is more homogeneous, and in certain cases we can even notice some transition between the lateral and inner leaves of the foliage, e.g., as to stoma number (cf. Table 1). In the upper epidermis, there is a difference exceeding even a 0,1 p.c. SD-value between edge and interior of the leafy crown, resp. the lower and inner region of it. In the lower epidermis, only the difference between the edge and lower region of the leafy crown touches the SD level of 0,1 percent. Going from the edge of foliage towards the regions illuminated less strongly, the stoma number decreases gradually.

As to the cell number of epidermis, there are similarly essential differences between the single regions in the upper surface epidermis. There, the difference between the edge and middle of the leafy crown exceeds strongly even the SD-value of 0,1 percent.

Comparing the other regions of the leafy crown with one another, we find differences of 1 p.c. (edgeinterior of foliage) or 5 p.c. (interior-middle of foliage), except the middle and interior of foliage where the cell numbers are identical. In the lower surface epidermis, even between the most extreme values (lower part — interior of foliage), there is any difference only on the SD-level of 1 p.c. The differences between the

edge, middle and interior of foliage don't touch half the SD-value of 5 p.c., either. In these regions, therefore, the epidermis cell number is practically identical (cf. Table 1).

Table 1.

		Edge	Middle	Interior	Lower	SD		
		part of the leafy crown				0,1	1	5
						percent		
Stoma number	a	71	38	50	74	18,2	12,2	7,6
piece/sq. mm	b	231	199	171	152	67,6	44,8	30,4
Lenght of guard	a	29,7	29,7	26,5	25,9	2,2	1,4	1,0
cells in μ	b	25,9	24,4	23,3	25,0	2,4	1,5	1,1
Width of guard	a	21,1	19,7	17,7	20,7	2,0	1,3	0,9
cells in μ	b	17,5	16,8	16,6	18,1	1,4	0,9	0,6
Ratio L/W of	a	1,3	1,4	1,4	1,3	0,3	0,2	0,1
guard cells	b	1,4	1,4	1,3	1,3	0,3	0,2	0,1
Epidermis cell number	a	1823	1318	1545	1392	348,8	232,5	159,6
piece/sq. mm	b	2370	2340	2465	1783	683,2	455,2	312,4
Stoma index	a	3,7	2,8	3,2	5,1	1,4	0,9	0,6
	b	8,9	7,8	6,6	7,8	3,0	2,0	1,4

Alteration of tissue elements of the upper and lower surface epidermis of Salix alba L. inside the leafy crown. (a = upper surface epidermis; b = lower surface epidermis).

The stoma index is indicating the connection between stoma number and epidermis cell number, in a unit of field. In the upper surface epidermis, it is the smallest in the middle of foliage has the greatest value in the lower part of foliage the difference between the two extreme values being nearly double of the significance value of 0,1 p.c. In the lower surface epidermis, it is the smallest is the interior of folige; this value differs only from the stoma index of the edge of foliage on the level of 1 p.c.; it agrees on the other hand, with the stoma index of other parts of the foliage, in contradistinction to the upper surface epidermis.

The other properties investigated do not differ from one another in the most cases ,taking into consideration the situation inside the leafy crown. The results of our investigations are summed up in the following Table.

Evaluation of results

The stoma number in the single regions of the leafy crown shows significant alterations in the upper and lower surface epidermis. In the upper surface epidermis supposedly not the degree of illumination but

rather the water supply of leaves, the cc. of carbon dioxyde, the vapour content of air have effect on the stoma number. That is verified by the fact that at the edge and in the lower part of foliage, i.e. in places illuminated in a very different degree, there is no difference in stoma numbers; on the other hand, in the inner region of the leafy crown the stoma number decreases (cf. Table 1). In the lower surface epidermis, there is rather the decrease of the strength of illumination that has an influence on the stoma number, the most stomata being at the edge of foliage, that is to say, in the most illuminated parts; and the least of them are in the lower part of the leafy crown. [The effect of water supply and that of the single microclimatic conditions (light, CO_2 cc., vapour content) are interpreted, of course, in correlation with the morphogenesis of epidermis and of the leaves in different positions till the formation of the developed leaves.]

Summarized, therefore, on the basis of stoma number it is not possible to draw a conclusion of diagnostic value if the state of pattern inside the leafy crown is not known.

The length of guard cells changes hardly inside the foliage, only it is shorter in the interior of leafy crown. For diagnostizing, it is useful if the length of guard cells of the taxonomic categories under discussion is differring considerably from one another.

The width of guard cells between the regions of the leafy crown is showing a continuous transition: decreasing from the edge towards the interior of it (cf. Table 1). (The degree of the stomata being opened was left out of consideration. It is hardly suitable, according to our investigations, for characterizing the species.)

Ratio L/W of the guard cells — i.e., their shape, degree of their being globular — is not influenced by the conditions inside the leafy crown, either in the upper surface or in the lower surface epidermis. It can be used well for diagnostical aims if there are measurable differences between the single species.

The epidermis cell number falling to the unit of area is a function of the size of cells. According to Zalensky's law, the size of cells is decreasing parallel with an increase of the strength of illumination. As to the leafy crown, we have experienced during our investigations that the cells are bigger inside the foliage, i.e. in the regions less illuminated. The differences are of a considerable size between the parts of foliage illuminated differently, first of all in the epidermis of upper surface. The epidermis cell number can, therefore, not be used for diagnostic purposes.

The stoma index — the interior of foliage being left out of consideration — does not show any difference in the single regions, even on a 5 p.c. level. It can therefore be used for diagnostic aims as one of the complementary data. Our results concerning the formation of stoma number are, however, contrary to Zalensky's law, in view of the light conditions inside the leafy crown. Our statements about the stoma number are of course, reflected in changes of stoma index inside the foliage, as well.

Summary

We have investigated the leaf epidermis of a free standing tree (*Salix alba* L.) in function of the situation inside the leafy crown.

On the basis of our investigations it can be ascertained that:

1. Some tissue elements of epidermis change depending upon the conditions inside the foliage. A cause of alteration may have been the water supply of leaves being of different degrees and also the lasting influence of the different microclimatic conditions (light conditions, CO_2 cc., vapour content) prevailing in the morphogenesis (development) of leaves (Wagner, 1957; Geiger, 1927; Zalensky, 1964).

2. The degree (significance) of alterations is the greatest in stoma number and epidermis cell number.

3. The least extensive changes were observed in respect of the length-width ratio (L/W) of guard cells and of the length and width size of guard cells, as well in the stoma index. For diagnostic aims, therefore, the L/W ratio of guard cells, the stoma index, possibly as a complementary parameter the stoma number can be used.

4. Comparing the upper and lower surface epidermis of leaves, we have observed that on the upper surface the size of cells is generally bigger (cf. Table 1), the degree of differences found in the single tissue elements is, in the majority of cases, significant between every region. In case of using the epidermis for diagnostic aims, the lower epidermis of the leaf affords more reliable results.

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Address of the author:

Dr. Szerén Pataky
Department of Botany,
A. J. University, Szeged, Hungary