

Some Problems of the Determination of Stomatal Aperture by the Microrelief Method

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Received January 31, 1966

Abstract. The applicability of the microrelief (or reprint) method for stomatal aperture determinations was studied in middle-aged sugar beet leaves.

It was found that the picture of the central aperture (CA) of stomata on the reprints could be observed only if their eisoal aperture (EA) was sufficiently wide. If the width of EA was small (about 1—3 μ), it was impossible to determine the width of CA by means of the reprint method, in view of the proportional dimensions of the upper closing flaps. A lower concentration of the chlorophorm solution of methylmethacrylate used for taking reprints had a favourable influence on the amount of stomata whose width of central aperture was reproduced on the reprints.

Simultaneous measurements of the EA-width and of the diffusibility of leaves (determined porometrically) in the course of a day showed, on the whole, fairly corresponding changes. During the wilting of detached leaves their diffusibility fell to zero, where it remained. The width of EA also decreased at first, but at higher water deficits it partly again increased.

Quantitative determination of stomatal aperture is a rather difficult matter. A great number of various methods exist for this purpose, but when using any of them one must take into account a certain error. The cause of these errors are specific with each method. In most cases we are able to measure the stomatal apertures only in a small area of a leaf blade. The possibility of changes in the stomatal response in the course of a methodical procedure is also a very important question.

The microrelief (or reprint) method (BUSCALIONI and POLLACCI 1901) is often used for the determination of stomatal aperture. Among its advantages are its simplicity and speed, and it is very easily applicable under field conditions. The success of this method depends on the quality of the reprints obtained, and on their correct microscopical evaluation. If we want to know the changes of stomatal apertures in terms of absolute values, the changes in the width of the central aperture have to be observed, because that is the closest position in the stomatal pore in the majority of stomata types. Therefore it is important to obtain the picture of the central aperture on the reprint and to measure its width. One could estimate the state of stomata in relative

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values by measuring eisodial aperture width or widening of the stomatal apparatus, but this is possible only if we know the relations of these proportions to the width of the central aperture.

This work is a methodical contribution to the problem of the applicability of the reprint method to determinations of stomatal aperture. Its purpose is to find out whether the central aperture is always visible on the reprints, and to describe the kind of relation between the width of the eisodial aperture and the diffusibility of leaves.

Material and Methods

In the experiments sugar beet plants (*Beta vulgaris* ssp. *esculenta* SALISB. GÜRKE var. *altissima* ROESSIG, variety Dobrovická C) cultivated under field conditions were used. For the experiments middle-aged leaves were chosen. The measurements were taken in the second half of August 1963, when the plants were 90 to 100 days old.

The reprints were made using a 5 per cent chloroform solution of methylmethacrylate. They were stripped off from the leaves with the aid of transparent adhesive tape ("Sellotape"). The minimum number of stomata measured microscopically on each reprint was two hundred.

The diffusibility of the leaves was determined by means of a porometer (a very simple modification, Fig. 1.). The porometric chamber was made of glass (inner diameter 10 mm) and was sealed to the leaf surface with a Leick seal (LEICK 1928). A mercury column (40 mm high) falling in a capillary tube (inner diameter 2.52 mm) produced a constant small underpressure in the connected porometric chamber. The fall rate of this column was measured

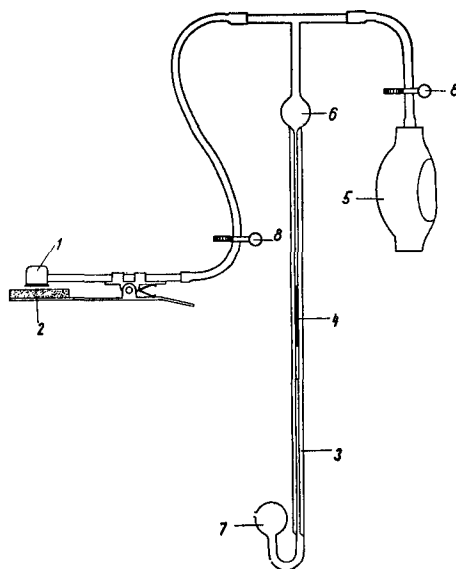


Fig. 1. Diagram of porometer. 1 — porometric chamber, 2 — polyurethane foam, 3 — capillary tube, 4 — mercury column, 5 — compressed rubber ball, 6, 7 — safety tanks, 8 — spring clamp.

and from it the diffusibility of leaves (in $\mu\text{l. sec}^{-1} \cdot \text{cm}^{-2}$) was calculated. The porometric chamber was sealed to the leaves only during the time of measurement, i.e. not longer than half a minute.

The determinations using both the reprint and porometric methods were carried out on the upper surfaces of leaves.

Experiments with detached leaves were arranged in the following way: The water-saturation deficit was determined by the leaf-disk method (ČATSKÝ 1960) and the initial measurements (reprints, porometer) were taken in selected leaves. Immediately afterwards the leaves were cut off, weighed and put in a little glasshouse under nearly constant conditions (air temperature $24 \pm 1^\circ \text{C}$, relative air humidity $65 \pm 5\%$, illumination 25 klx.). At 10-minute intervals the leaves were weighed, measured porometrically and reprints were taken. These measurements were carried out in the middle zone of leaf blades.

Results

The anatomy and external morphology of the stomatal apparatus in sugar beet leaves are shown in Fig. 2. According to the classical classification (SCHWEN-DENER 1881) the type of the stomatal apparatus is a modification of the *Amaryllis*-type. It is remarkable that this modification appears in many other dicotyledonous plants. The size and shape of the upper closing flaps (or upper borders) are of great importance for the applicability of the reprints method. They are highly cutinized and can be observed in a transverse section only after the staining of cuticula (e.g. by Sudan III-ethanol solution).

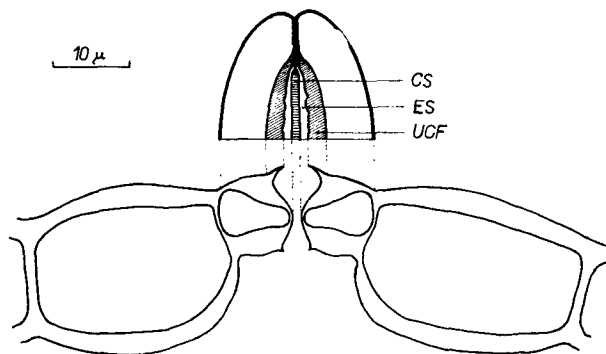


Fig. 2. Diagram of stomatal apparatus in leaves of sugar beet: transverse section and external morphology of guard cells. CA — central aperture, EA — eisodial aperture, UCF — upper closing flaps.

Stomatal reprints with the eisodial aperture (EA) of different width were evaluated, in order to find out whether and to what extent the central aperture (CA) can be observed. In this experiment the reprints were made with methyl-methacrylate solutions of different concentrations (Table 1). If the width of the EA was small (about $1-3 \mu$), reprints without any pictures of CA were obtained in most cases. Lower concentrations of the solution had a favourable influence on the occurrence of CA- pictures in the reprints.

In further experiments the relationship between the width of EA and the

Table 1.

The occurrence of the pictures of central apertures on the reprints (as percentage to the number of all stomata in each width-class), in dependence on the width of eisodial aperture and on the concentration of the methylmethacrylate solution used for taking reprints

Concentration of solution (%)	The width of eisodial aperture (μ)					
	1	2	3	4	5	6
5	0	2	10	53	86	91
10	0	0	0	36	65	85
15	0	0	0	2	12	26

diffusibility of leaves was measured. Fig. 3 shows results of determinations carried out in the course of a day in plants *in situ*.

The same measurements were taken in the leaves after detaching and during

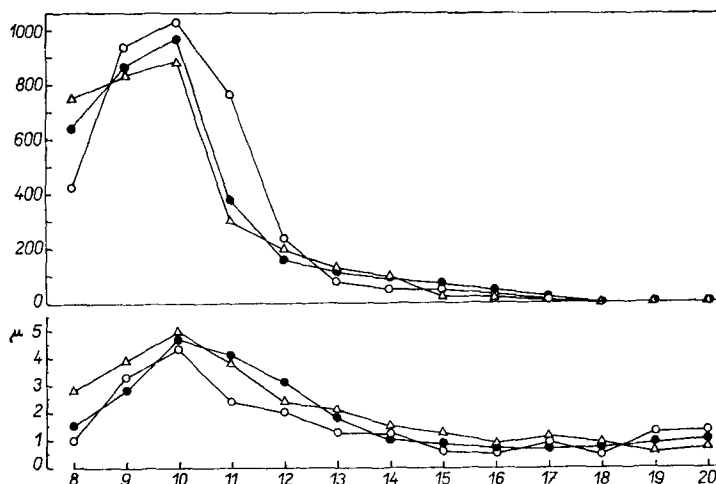


Fig. 3. The relation between diffusibility of leaves and the width of eisodial aperture in the course of a day (simultaneous measurements in three leaves). Abscissa: time (hours). Ordinate: diffusibility of leaves in $\mu\text{l. sec}^{-1} \cdot \text{cm}^{-2}$ (upper curves) and width of eisodial aperture in μ (lower curves).

their wilting (Fig. 4). Diffusibility falls to zero if water saturation deficit of leaves reaches the value of 22 to 28 per cent. The width of EA also decreases at first, but it increases partly again at higher water saturation deficits.

Discussion

The reprint method is very useful for obtaining a perfect picture of the leaf surface studied. The reprints can be made quickly and simply, and remain as permanent records. In studies of leaf epidermis we can use this method for determining the number and area of epidermal cells. The use of the reprint method in measurements of stomatal aperture is rather limited. In many cases the upper closing flaps of the guard cells are a great obstacle in obtaining the

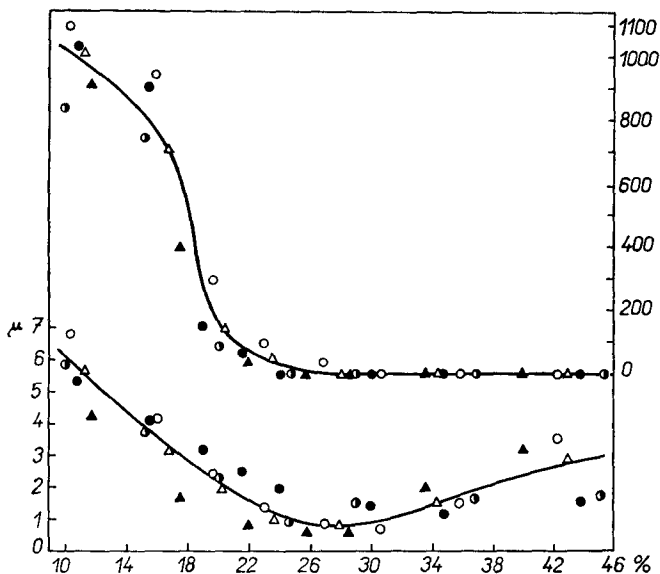


Fig. 4. The relation between diffusibility of leaves and the width of stomatal aperture during the wilting of detached leaves (simultaneous measurements in five leaves).
 Abscissa: water saturation deficit in per cent.
 Ordinate: diffusibility of leaves in $\mu\text{l. sec}^{-1} \cdot \text{cm}^2$ (on the right), and width of stomatal aperture in μ (on the left).

reprints of CA. Especially whenever the width of EA is small, it is practically impossible to get the picture of CA on the reprint.

WENZL (1939) made a rather detailed reexamination of the applicability of the reprint method to stomatal aperture study. He found that the application of this method is possible only with plants whose stomatal closing is achieved exclusively through the upper closing flaps, or if the movement of the flaps corresponds with the course of CA-changes. In such cases, however, it would be possible to determine the state of the stomata from the width of EA under one condition, i.e. that changes of this width run parallelly with changes in CA-width. This seems to be incorrect in some cases. The re-opening of EA of stomata with closed CA observed not only in reprints but also in directly microscopied leaves could cause mistakes. This was a phenomenon noticed also by SOLAROVÁ (1965) in fodder cabbage leaves.

The porometer used made it possible to determine reliably and speedily the diffusibility with a sufficiently high sensitivity even under field conditions. For measurements the upper leaf surface was chosen, offering a higher resistance than the lower surface in sugar beet. The resistance of the mesophyll is very small in this case, for it contains a lot of intercellular spaces and a typical palisade parenchyma is not differentiated. It may thus be assumed that the changes in diffusibility measured in the way described above were mostly determined by the changes of CA-width of stomata in the upper surface of the leaf.

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J. GLOSER, Botanický ústav ČSAV, Brno: Některé problémy při měření otevřenosti průduchů otiskovou metodou. — Biol. Plant. 9 : 28—33, 1967.

Na středně starých listech cukrovky byla studována použitelnost otiskové metody pro sledování otevřenosti průduchů.

Bylo zjištěno, že šířka střední skuliny je na otiscích zobrazena pouze při značně širokém otevření eisodiálního otvoru (EO). Je-li jeho šířka malá (1—3 μ), není možno vzhledem ke značně vyvinutým vnějším výběžkům zjistit otiskovou metodou šířku střední skuliny. Větší zředení roztoku metylmetakrylátu, použitého ke zhotovování otisků, mělo příznivý vliv na zvýšení procenta průchodů se zřetelně reprodukovanou šířkou střední skuliny.

Paralelní sledování šířky EO a difusibility listů (měřené porometricky) během dne ukazuje veelku souhlasné změny. Při vysychání odříznutých listů poklesá jejich difusibilita až k nulové hodnotě, kde trvale zůstává. EO se nejprve přivírá, avšak při vyšším vodním systostním deficitu listů se opět částečně rozevírá.

Я. ГЛОСЕР, Ботанический институт ЧСАН, Брно: Некоторые проблемы при применении метода отпечатков для исследования открытости устьичной щели. — Biol. Plant. 9 : 28—33, 1967.

У листьев сахарной свеклы была исследована возможность применения метода отпечатков для исследования открытости устьичной щели.

Было установлено, что ширина центральной щели изобразилась на отпечатках только в случае широко открытой эизодиальной щели (ЭЩ). В случае небольшой ширины (1—3 μ) является установление ширины центральной щели при помощи метода отпечатков невозможным. Главной причиной этой неудачи оказалась структура внешних краев устьиц. Более разбавленные растворы метилметакрилата, применяемого для изготовления отпечатков, имели благоприятное влияние на количество отпечатков устьиц с четко репродуцированной шириной центральной щели.

Одновременное измерение ширины ЭЩ и диффузной способности листьев (измеренной порометрически) в течение дня показывает приблизительно одинаковые изменения. После отрезания листьев понижается их диффузная способность до нуля, где она постоянно остается. ЭЩ сначала тоже начинает закрываться, но при большом водном дефиците листьев она снова открывается.