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Water Balance in Leaf Tissue

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Abstract. Samples of the leaf tissue (14 cm^2) were placed in a plexiglass chamber which consisted of three parts. Water absorbed by the leaf tissue on one side of the sample was transported through the middle part of the sample to the opposite side and was transpirated there. The intensity of transpiration the intensity of water absorption and water saturation deficit (w.s.d.) were determined simultaneously in this tissue by gravimetry. Water balance was studied either in saturated samples of leaf tissue or in tissue where w.s.d. (10%, 20%, 30%, 40%) was established in advance. Although conditions for water absorption in leaf segments were optimal, w.s.d. originated in the saturated leaf tissue under all given external conditions (evaporation from 41·7 to 17·8 mg cm^{-2} h^{-1}). W.s.d. which was established in advance for the most part increased during the experiment and reached even high values (more than 60%). The equilibration was reached only under conditions of low evaporation and initial w.s.d. higher than 20% in young leaves and higher than 30% in adult leaves. A positive correlation between the ratio of the intensity of water absorption to the intensity of transpiration and w.s.d. was found only under conditions of lower evaporation (17·8 and 23·2 mg $cm^{-2}h^{-1}$). The maximal values of w.s.d. were limited in this way.

Water balance was studied: 1. in leaf tissue of upper, middle and lower leaves of fodder cabbage, 2. in leaf tissue of middle leaves of young and adult plants of fodder cabbage, 3. in leaf tissue of dicots (fodder cabbage) with different vessel orientation in respect to water transport, 4. in leaf tissue of monocots (banana-tree) with water transport upright to the vessel orientation.

Considerable change of water balance was observed when the water transport was prolonged by two incisions in the middle part of the sample.

Results of all these experiments revealed the possibility of water stress origin even in leaf tissue sufficiently supplied with water.

Little attention has been paid so far to the simultaneous investigation of the intensity of transpiration and water absorption even if the water balance (difference between water income and output) determines the water content of the whole plant, its organs, tissues and cells.

Water balance has been studied either in the whole plant (KRAMER 1937, TARLOWSKI 1955, 1956, TOTSUKA and MONSI 1959, 1960, KÜSTER 1960) or in cells (TAMIA and TAZAWA 1956).

The aim of this work was to develop a method for water balance studies in leaf tissue samples and to determine the laws regulating origin of water stress in the isolated leaf tissue.

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Fig. 1.

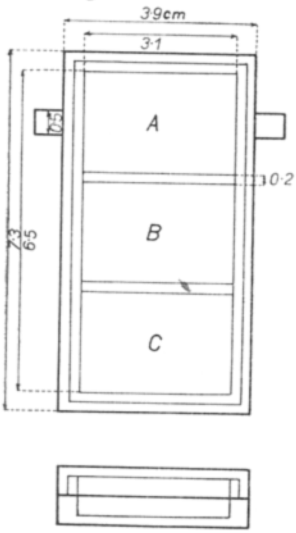

Fig. 2.

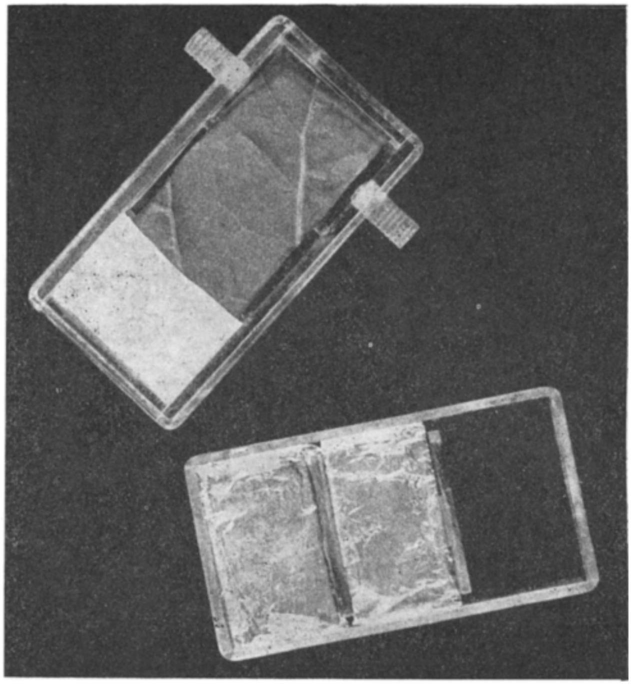

Fig. 1. Ground plan, front view and side view of a plexiglass chamber for studying water balance in leaf tissue.
Fig. 2. The position of leaf tissue sample in an open chamber.

Material and Methods

90 to 180 days old potted fodder cabbage (*Brassica oleracea* L., cv. *acephala*) plants grown in the greenhouse (temperature from 18 to 25°) were used throught the experiment. The plants had 8 to 9 resp. 11 to 13 leaves. Banana-tree (*Musa sapietum* L.) was also used.

The leaves were cut off in the early morning before every experiment and their petioles immersed in water and left in darkness in a saturated atmosphere for 24 to 48 hours for saturation. Oblong samples (14 or 24 cm^2) were cut in such a manner that no major vessels was running parallel with the longer axis of the sample. The larger samples were left to dry in a climatic chamber (25°, from 35 % to 40 % R.H.) to reach the desired w.s.d. (10 %, 20 %, 30 %, 40 %) and at that time the smaller samples of 14 cm^2 were cut from them to prevent undesirable drying of the edges during handling the samples.

Special plexiglass chambers were designed for studying the water balance in leaf tissues (inner dimension: $65 \times 31 \times 8$ mm). These chambers consisted of three parts of the same size (Fig. 1). Part C was lined with two plates of gel (polymer of ethylenglycolmonomethacrylate) which loses water very easily and in a reversible way. Parts B and C were covered with aluminium foil so that the samples were in darkness and were not overheated at the same time. Part A was provided with holes on the sides and the conditioned air (constant temperature, humidity and rate of flow) passed through them. These small chambers were placed during the experiment in a large climatic box with constant temperature. Each small chamber was illuminated using a light channel provided with 100 W krypton bulb, mirrors and water filter. The sample of leaf covered the whole area in part A and B and was slid with one shorter edge between two plates of gel saturated with water in part C (about 3 mm from the edge). A sufficient supply of water was ensured in this way (Fig. 2). The water absorbed by leaf tissue in part C passed through part B and was transpired to the surrounding air in part A.

The transpiration, water absorption and w.s.d. were established gravimetrically at given time intervals, usually every 30 minutes ($\pm$ 30 sec). The weight of the whole chamber and of leaf tissue samples was done on analytical balances with accuracy 10^{-4} g. Every experiment was repeated tenfold. The relationship between $s_{\bar{x}}$ and n was quite satisfactory under these conditions.

In some experiments the stomata opening was determined in the transpiring part by direct observation under the microscope.

The rate of transpiration was regulated by external conditions in such a way, that evaporation (measured as evaporation from green filter paper saturated with water) under conditions IV was less than a half of evaporation under conditions I (Table 1).

Table 1
Different types of external conditions

Type of conditions	Temperature (°C)	Relative humidity (%)	Density of illumination (λ=400 to 700 nm) (cal cm^{-2} min^{-1})	Linear rate of air flow (m s^{-1})	Evaporation (mg cm^{-2} h^{-1})
I	25	0	0·19	$3{\cdot}48 \times 10^{-2}$	41·7
II	25	31	0·19	$3{\cdot}48 \times 10^{-2}$	34·1
III	25	66	0·19	$3{\cdot}48 \times 10^{-2}$	23·2
IV	25	79·3	0·19	$1{\cdot}63 \times 10^{-2}$	17·8

The leaf temperature was checked during the experiment by thermistor thermometers. The temperature was never higher than 29° even under conditions of lowest evaporation.

For detailed description of the method see Pospíšilová 1967.

Results and Discussion

1. The influence of external conditions on water balance in leaf tissue

The water balance was observed in samples of tissues from the 3rd, 4th, 5th leaf under different types of external conditions (I, II, III, IV). W.s.d. was adjusted to 0 %, 10 %, 20 %, 30 % or 40 % before the experiment.

Only small differences in the intensity of transpiration appeared between samples with different initial w.s.d. under conditions of high evaporation (I, II). In particular the amount of water loss during five hours was practically the same. The intensity of transpiration decreased with increasing initial value of w.s.d. under conditions of lower evaporation (III, IV) (Fig. 3).

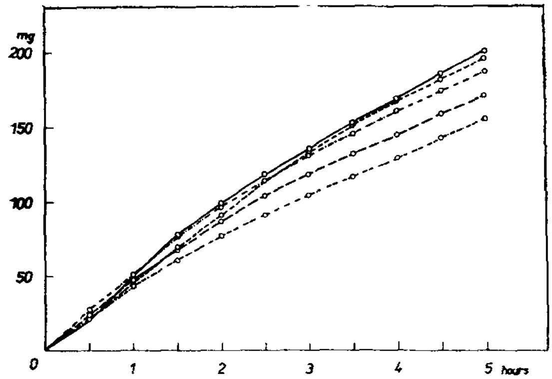

Fig. 3. Time-course of water loss by transpiring part of sample at different levels of initial w.s.d. (w.s.d. 0% ———, 10% - - - - -, 20% — · — · —, 30% — — —, 40% — — · — — ·) under conditions III. Abscissa: time in hours, ordinate: amount of transpirated water in mg.

This difference may be explained by a gradient in the water potential between leaf and air which was greatest under conditions I and lowest under conditions IV. Lowering of water content in leaves decreased the gradient of water potential between leaf tissue and surrounding air. This drop became more marked when the whole gradient was low (III, IV) and was less apparent with high gradient (I, II).

The intensity of transpiration decreased throughout every experiment. This was probably caused by gradual closing of stomata, by incipient drying of cuticula and cell walls surrounding intercellulars and by a decrease in sample area resulting from their drying.

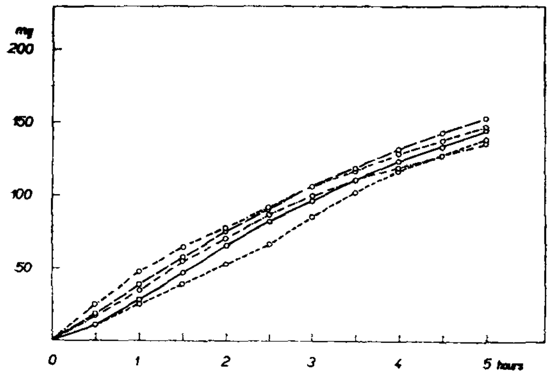

Fig. 4. Time-course of water absorption by sample on leaf tissue at different levels of initial w.s.d. (w.s.d. 0% ———, 10% - - - - -, 20% — · — · —, 30% — — —, 40% — — · — — ·) under conditions IV. Abscissa: time on hours. Ordinate: amount of absorbed water in mg.

The initial value of w.s.d. influenced the rate of water absorption at the beginning of the experiment, *e.g.* in treatment IV only the amount of water absorbed after 30 and 60 minutes was higher with higher initial w.s.d. (Fig. 4).

W.s.d. appeared under conditions I, II and III in water saturated samples of leaf tissue and increased under the same conditions in tissues with initial w.s.d. from 10 % to 40 %. W.s.d. increased about 20—25 % in every experiment

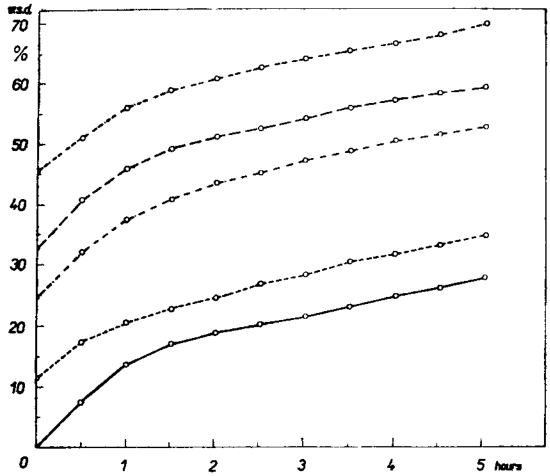

Fig. 5. Time-course of water saturation deficit development in leaf tissue at different levels of initial w.s.d. under external conditions II. Abscissa: time in hours, Ordinate: w.s.d. in %. (W. s. d. 0% —, 10% ---, 20% -.-.-, 30% ——— 40% --.--.)

under conditions of higher evaporation (I, II) often independently of the initial w.s.d. (Fig. 5). W.s.d. under conditions III increased similar to that at lower initial w.s.d. but the highest initial w.s.d. increased only about 7 % (Fig. 6.) The conditions of lowest evaporation (IV) resulted in a high increment of w.s.d. only in saturated samples and slight one in samples with initial w.s.d. of 10 % or 20 %. The deficit remained practically unchanged in samples with an initial w.s.d. of 30 % and 40 % and even slightly decreased at the beginning of the experiment (Fig. 7).

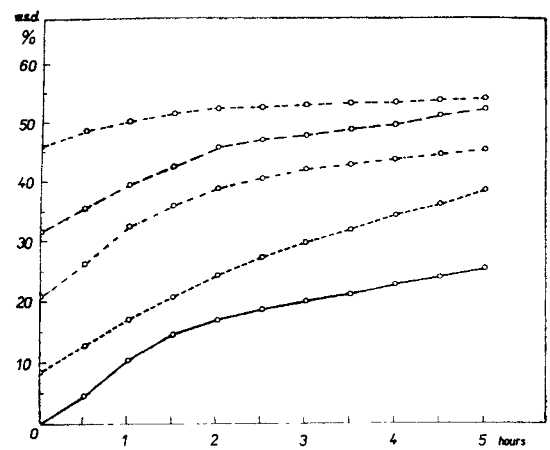

Fig. 6. Time-course of water saturation deficit development in leaf tissue at different levels of initial w.s.d. under external conditions III. Abscissa: time in hours. Ordinate: w.s.d. in %. (W. s. d. 0% –, 10% ---, 20% -.-.-, 30% — — — 40% --.--.)

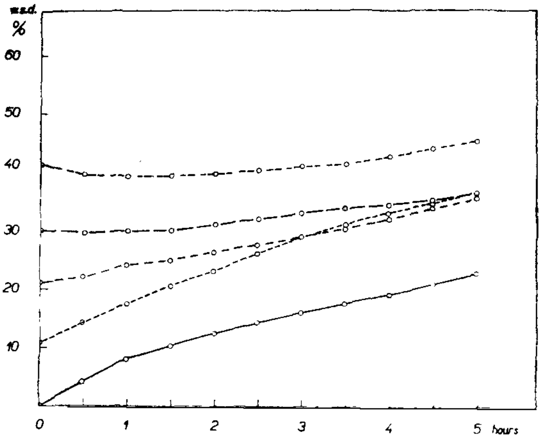

Fig. 7. Time-course of water saturation deficit development in leaf tissue at different levels of initial w.s.d. under external conditions IV. Abscissa: time in hours. Ordinate: w.s.d. in %. (w.s.d. 0 % ——— 10% - - - 20% —.—.— 30% — — —, 40% - - . - -)

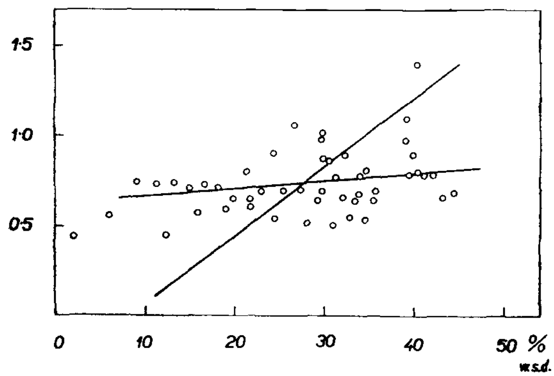

Fig. 8. Relationship of ratio of intensity of absorption to intensity of transpiration and of w.s.d. under conditions IV. Abscissa: w.s.d. in %. Ordinate: ratio of intensity of absorption to intensity of transpiration.

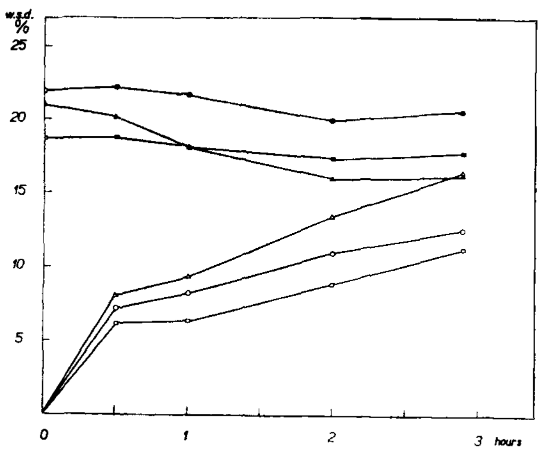

Fig. 9. Time-course of w.s.d. development in leaf tissue from upper(△), middle(○) and lower (□) leaves at initial w.s.d. 0% (○) and 20% (●) under conditions IV. Abscissa: time in hours. Ordinate: w.s.d. in %.

The ratio of the intensity of water absorption to transpiration increased with increasing value of w.s.d. (positive correlation) only under conditions of low evaporation (III and IV) and thus the maximal value of w.s.d. was limited (Fig. 8).

Results of all these experiments indicate that high w.s.d. may be found in leaf tissue sufficiently supplied with water from one side, under conditions where the evaporation and also the intensity of transpiration are not extremely high. An equilibrated water balance existed only under conditions of lowest evaporation and with an initial w.s.d. of 30 % and 40 %.

2. Water balance of leaf tissue in relation to leaf insertion and age

Leaf growth and development is accompanied by many changes in anatomical and physiological features. In order to confirm the general validity of our results we tried to compare experiments on tissues from upper, middle and lower leaves and on middle leaves from older and younger plants.

The upper (2nd leaf), middle (4th leaf) and lower (7th to 9th leaf) leaves differed in relative water content, which was lowest in the upper leaves and highest in the lower leaves.

The intensity of transpiration was higher in middle and upper leaves in comparison with lower ones at the initial w.s.d. of 0 %. The highest intensity of transpiration was observed in upper leaves at an initial w.s.d. of 20 %, the lowest was under the same conditions in middle leaves.

The lowest ratio of intensity of water absorption to intensity of transpiration took place in samples from upper leaves at an initial w.s.d. of 0 %. W.s.d. appeared very soon in these leaves. On the other hand only in samples from upper leaves the initial w.s.d. of 20 % decreased in such a way, that at the end of the experiment (after three hours) there were no more differences between samples of initial w.s.d. of 0 % and 20 %. W.s.d. in samples from middle and lower leaves at initial w.s.d. of 0 %, was lower at the end of the experiment than in upper leaves but remained higher at an initial w.s.d. of 20 % (Fig. 9). The relationship between the ratio of the intensity of absorption to the intensity of transpiration and w.s.d. was most clearcut in uppre leaves.

ČATSKÝ (1962) studying the development of w.s.d. in plants dried *in situ* found at the beginning of the experiment the highest w.s.d. in young leaves. These differences disappeared in the course of the experiment and from the average w.s.d. values he ascertained a distinct preference of young leaves for water supply.

The ratio of the intensity of water absorption and transpiration was practically equal in the samples from middle leaves of younger (from 90 to 120 days) and older (from 150 to 180 days) plants at an initial w.s.d. of 0 %. There were no differences between these samples during the experiment. This ratio was higher in samples from younger plants at an initial w.s.d. of 20 % and only in these samples w.s.d. decreased in the course of the experiment (Fig. 10).

Although some difference in the intensity of transpiration and water absorption and of w.s.d. development were established, the basic trend of water balance was the same in all the experiments. The general validity of our preceding results on water stress origin in samples supplied unilaterally with water was confirmed in this way.

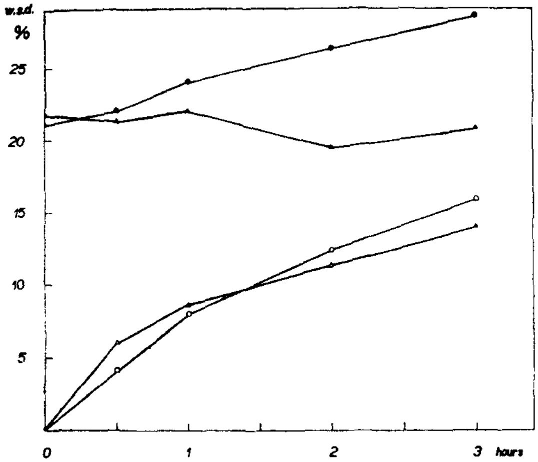

Fig. 10. Time-course of w.s.d. development in leaf tissue from middle leaves of younger (△) and older (○) plants at initial w.s.d. 0% (▲) and 20% (●) under conditions IV. Abscissa: time in hours. Ordinate: w.s.d. in %.

3. Water balance in leaf tissue with different vessel orientation to water transport direction

The water balance of different areas of the leaf blade and thus under conditions of different vessel orientation in respect to water transport direction was compared under lowest evaporation (IV) in following experiments. Four directions of water transport were chosen: from the base to the top, from the top to the base, from the middle to the margin and from the margin to the middle. There were no differences in water loss and water absorption between these variants. The highest w.s.d. was found in the transport from the margin to the middle and the lowest in the transport from the middle to the margin. W.s.d. of the lengthwise type of transport (used for all preceding measurements) was between these extreme values (Fig. 11).

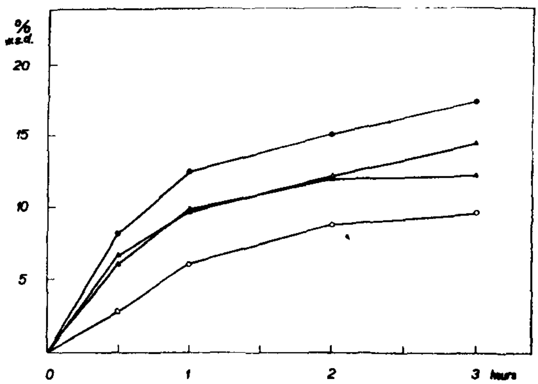

Fig. 11. Time-course of w.s.d. development at different positions of samples on leaf blade (Transport of water from middle to margin ○, from margin to middle ●, from base to top △, from top to base ▲) under conditions IV. Abscissa: time in hours. Ordinate: w.s.d. in %.

The intensity of transpiration and of water absorption and w.s.d. was also studied, using the same method, in samples from leaves with parallel vessels (*Musa sapientum* L.). The water was transported upright to the direction of

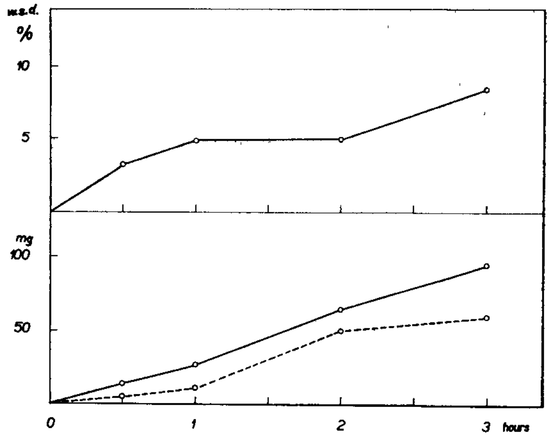

Fig. 12. Time-course of water balance in leaf tissue of banana under conditions IV. Abscissa: time in hours. Ordinate: above: w.s.d. in %, below: ———— quantity of transpired water and – – – – – quantity of absorbed water in mg.

the vessels (Fig. 12). Although the transpiration of banana leaves was in average lower then the transpiration of fodder cabbage leaves, w.s.d. also appeared during the experiment.

Both experiments brought us to the conclusion that water transport was practically independent of vessel orientation in leaf tissue samples. The continuity of water columns in the vessels was apparently disturbed during the cutting and thus the role of vessels in water transport was relatively small. Samples of leaf tissue may be hence used as a macromodel of leaf mesophyl.

4. The influence of water transport prolongation on water balance

Water balance was studied in further experiments in leaf tissue from fodder cabbage (under conditions IV) where the length of water transport was prolonged by two incisions in the middle of the sample. This prolongation

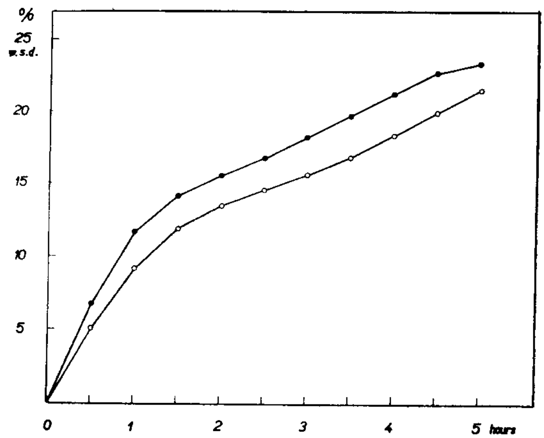

Fig. 13. Time-course of w.s.d. development in samples with prolonged water transport length (●) and in control samples (○) under conditions IV. Abscissa: time in hours. Ordinate: w.s.d. in %.

clearly influenced the water balance of leaf tissue in a negative way. Althought the transpiration in the incised samples was only slightly lower than in controls, the water absorption was lower in quite clear-cut way. W.s.d. developed sooner and reached higher values in samples with longer transport (Fig. 13).

These experiments show that water transport may play an important role in water balance of leaf tissue.

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Vzorky listového pletiva byly umístěny v trojdílných plexitových komůrkách tak, že voda absorbovaná listovým pletivem na jedné straně vzorku byla transportována střední části tohoto vzorku do jeho protilehlé části, kde byla vydávána transpirací. Gravimetrickou metodou byla současně měřena intensita transpirace, intensita absorpce vody a vodní sytostní deficit (v. s. d.) tohoto pletiva. Vodní bilance byla sledována jednak u nasycených vzorků listového pletiva, jednak u vzorků s určitým předem navozeným deficitem (10%, 20%, 30%, 40%). Ačkoliv podmínky pro absorpci vody byly optimální, vznikal při všech zvolených typech vnějších podmínek (výparnost 41,7 až 17,8 mgcm^{-2}h^{-1}) v nasyceném listovém pletivu deficit. Také předem navozený deficit během pokusu většinou stoupal a dosahoval i vysokých hodnot (přes 60%). K vyrovnané vodní bilanci docházelo pouze při podmínkách nejnižší výparnosti a počátečním deficitu větším než 20% u mladých listů a větším než 30% u starších listů. Také pouze při podmínkách nižší výparnosti (17,8 a 23,2 mgcm^{-2}h^{-1}) byla zjištěna kladná korelace mezi poměrem intensity absorpce vody a intensity transpirace a v. s. d., takže byly ohraničeny hodnoty maximálního deficitu.

Vodní bilance byla sledována: 1. u listového pletiva horních, středních a dolních listů kapusty, 2. u listového pletiva středních listů mladších a starších rostlin kapusty, 3. při různé orientaci žilek vzhledem ke směru transportu vody u listů dvouděložné rostliny (kapusta), 4. při transportu kolmo na směr žilek u listů jednoděložné rostliny (banánovník).

Značné zhoršení vodní bilance bylo zjištěno při prodloužení transportní dráhy dvěma zářezy ve střední části vzorků listového pletiva.

Výsledky všech těchto pokusů ukázaly reálnou možnost vzniku vodního deficitu v listovém pletivu i při jeho dostatečném zásobování vodou.

Я. Поспишилова, Институт экспериментальной ботаники ЧСАН, Прага: **Водный баланс листовой ткани.** — Biol. Plant. 11 : 119—129, 1969.

Образцы листовой ткани были помещены в плекситовой камере состоящей из трех одинаковых частей таким способом, что вода принятая тканью на одной стороне образца транспортировалась через среднюю часть образца и расходовалась при транспирации противоположной частью ткани. Одновременно была гравиметрически определена интенсивность транспирации, интенсивность абсорбции воды и водный дефицит ткани. Водный баланс исследовался не только у образцов с полным насыщением, но и у образцов с некоторым дефицитом (10, 20, 30 и 40%). Хотя условия абсорбции воды были оптимальными в насыщенной ткани листа возникал водный дефицит при всех избранных типах внешних условий (эвапорация от 11,7 до 17,8 мг см$^{-2}$ч$^{-1}$). Также дефициты 10, 20, 30 и 40% в течение опытов увеличивались и достигали даже более 60%. Равновесный водный баланс происходил только в условиях самой низкой эвапорации (17,8 мг см$^{-2}$ ч$^{-1}$) и первоначального дефицита около 20% у молодых листьев и около 30% у старших листьев. Также лишь в условиях самой низкой эвапорации (17,8 и 23,2 мг см$^{-2}$ч$^{-1}$) была установлена положительная корреляция между отношением абсорбции и транспирации и водным дефицитом листовой ткани и таким способом найден максимальный дефицит. Исследовался водный баланс 1. ткани верхних, средних и нижних листьев капусты, 2. ткани средних листьев младших и старших растений капусты, 3. при различной ориентировке нерватуры по отношению к направлению транспорта воды у листьев двухдольного растения (капуста) 4. при транспорте воды перпендикулярно к направлению нерватуры у однодольного растения (банан).

Значительное ухудшение водного баланса было найдено в случае удлинения пути транспорта воды двумя зарубинами в средней части образцов листовой ткани.

Результаты всех этих экспериментов показали реальную возможность возникновения водного дефицита в ткани листьев даже при его достаточном снабжении водой.