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Gasometric Method of Water Deficit Measurement in Leaves

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Abstract. — A gasometric method was developed for measuring water deficit in leaves. For a leaf at full turgor the amount of water penetrating into the tissue after removing the air from intercellular spaces by means of a vacuum pump, is equal in volume to the gas removed from the intercellular spaces. In a leaf with a water deficit the amount of the infiltrating water is greater than the removed gas volume by the amount egual to the water deficit. Determination of the volumes of the gas removed and penetrating water enables water deficit, if any, to be calculated. Comparative measurements carried out on five plant species confirmed the correctness of the method suggested. Reduction of the measuring time allowed to eliminate completely the sources of errors associated with the growth of tissue and loss of dry weight during respiration.

STOCKER (1929) developed a very simple method for determining the water saturation deficit (WSD) in terms of the percentage of water contained in leaves at full-turgor. The use of this procedure has been found to be limited because of the following difficulties and intrinsic errors:

1. The time of saturating leaves with water is rather long and can be as long as 3 days.
2. During saturation, infiltration of the tissue can occur.
3. During prolonged saturation, the tissue takes up water even due to extension growth and related factors respectively (ČATSKÝ 1965).

Other investigators (SHREWE 1924, HAMNER and CARLSON 1945, WEATHERLEY 1950, ČATSKÝ 1960, 1962, 1963, STREBEYKO 1966) attempted to develop more accurate methods for measuring water deficit. HEWLET and KRAMER (1963) criticized the existing methods of evaluating water deficit and came to the conclusion that data obtained by different investigators are in no way comparable.

Gasometric data on the intercellular space (CZERSKI 1964) have suggested a new possibility of measuring the water deficit in plant tissues. The water penetrating into the tissue of a fully turgid leaf under reduced pressure conditions is equal in volume to the air removed. When the leaf exhibits a

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water deficit, the amount of water absorbed is greater than the intercellular air space. This excess of water absorption associated with incomplete saturation of the tissue, may be a measure of water deficit in that tissue. The deficit evaluated in this way is the difference in the volumes of the water absorbed and air removed.

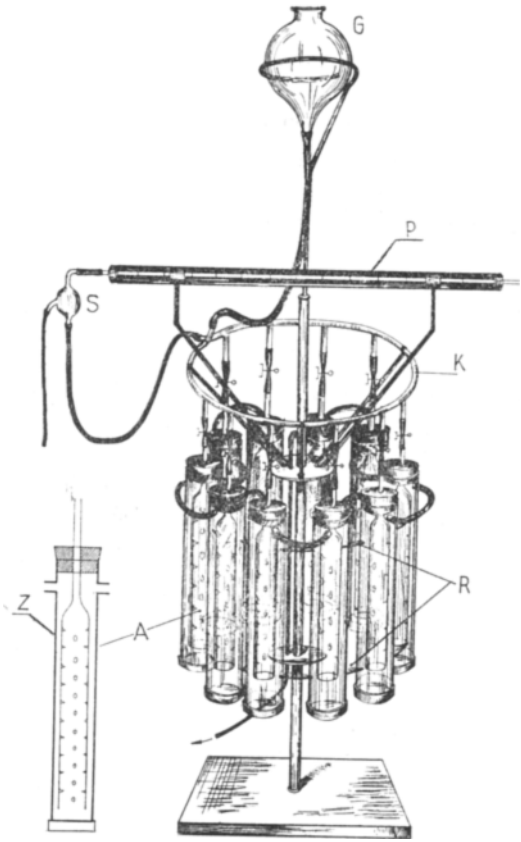

Fig. 1. Apparatus for determination of water deficit. For description see text.

Method

The apparatus described below (Fig. 1.), is a modification of the apparatus devised for measuring intercellular space volumes (CZERSKI 1964). It consists of 10 identical extractors A held peripherally by a plexiglas plate R. Each extractor contains a plexiglass vessel Z equipped with a rubber bung which carries a glass cylinder. The conical upper portion of the cylinder is connected

to a capillary K and then to a measuring burette P. The capillary K and burette P are connected by rubber tubing through a bubble collector S. The collector allows disjoined gas bubbles to coalesce before their being admitted to the measuring capillary. The extractors are connected by heavy-walled rubber tubing to a vacuum pump. The procedure for the preparation of the apparatus as well as the way of carrying out measurements were the same as described earlier (CZERSKI 1964).

Results

During measurements by different methods of the water deficit in the leaves of one plant, ČATSKÝ (1965) obtained different values of WSD in the same material using different methods and their modifications. This fact has made a comparison of the gasometric method with other methods of measuring water deficit rather difficult. The usefulness of the present method was therefore ascertained in terms of the following assumptions:

1. For a leaf in full turgor, i.e. exhibiting no suction force, the volume of the gas removed from intercellular spaces is equal to that of the penetrating (infiltrating) water.
2. Upon removal of the gas from intercellular spaces the leaf characterized by incomplete turgor will absorb water to fill up these spaces and to compensate the water balance of the tissue.
3. The difference between the volume (mass) of the penetrating water and the volume of the gas removed is equal to the water deficit of the tissue.
4. In a leaf whose weight in full turgor is already known, a decrease in water content due to transpiration may be artificially brought about. The subsequent measurement (by the gasometric method) ought to give a value consistent with the calculation based on the comparison of leaf weights. The difference, if any, between the calculated and observed deficits will be a measure of the inherent error of the method.

Since the temperature of the leaf-infiltrating water did not exceed 18° C, no correction was made for volume and 1 g. of water was assumed to have a volume of 1 cm³. Water deficit was calculated by Stocker's equation.

$$WSD = \frac{W_s - W_a}{W_s - DW} \cdot 100 \qquad (1)$$

where W_s = the water content at full saturation, W_a = the actual water content, and DW = the dry weight.

For the verification of the above assumptions, the following experiments were performed: A pot with *Begonia semperflorens* LINK et OTTO was kept in a lumistat chamber with relative humidity 98%. The fresh weight of a studied fully saturated leaf (FW_s) and the intercellular spaces volumes in that leaf were determined. After water infiltration and removal of water from the leaf surface, the leaf weight was re-determined (FW_1). The data are listed in Table 1. The intercellular space volumes were found to be equal or

nearly equal to the volume of the infiltrating water, which proves that the tissue examined was completely saturated with water.

A further experiment was carried out with turgor-deficient *Begonia* leaves. A fully saturated leaf was cut off, weighed (FW_s) allowed to transpire then re-weighed (FW_t) to determine the amount of the water transpired (Table 2.).

The experiments enable us to draw the following conclusions:

1. During infiltration, a turgor-deficient leaf absorbs water not only to fill its intercellular spaces but also to make up the water balance of cells.
2. The amount of water absorbed for compensation of the tissue water deficit is equal to the difference between the initial fresh weight (FW_s) of the fully water saturated leaf and its fresh weight (FW_t) after a partial loss of water due to transpiration.

Table 1

Measurements of water deficit in fully turgid leaves of *Begonia semperflorens*

Fresh weight FW_s mg	Fresh weight after infiltration FW_i mg	Intercellular space mm³	$FW_s - FW_i$ mg H_2O
660	750	90	90
1070	1280	216	210
970	1135	162	165
640	745	100	105
1180	1390	210	210
1200	1415	214	215
930	1090	175	160

For symbols and calculation see text.

Table 2

Measurements of water deficit in turgor-deficient leaves of *Begonia semperflorens*

Fresh weight at full saturation FW_s mg	Fresh weight after transpiration FW_t mg	$FW_s - FW_t$ (= WD_{abs} calculated) mg	Fresh weight after infiltration FW_i mg	V_g mm³	WD_{abs} measured mg H_2O	WSD %
1082	905	177	1220	138	177	16·3
1740	1615	125	2020	280	125	7·1
1490	1370	120	1735	248	117	8.0
1515	1360	155	1760	248	152	10·2
1052	940	112	1220	158	122	10·6
1540	1385	155	1840	298	157	10·0
990	875	115	1100	104	121	11·6
1980	1850	130	2345	354	141	6·5
580	500	80	660	78	82	13·7
1410	1220	190	1675	270	185	13·4

For symbols and calculation see text.

Table 3
Measurements of water deficit in *Reineckea carnea* KTH leaves

Fresh weight at full saturation FW_s mg	Fresh weight after transpiration FW_t mg	$FW_s - FW_t$ mg	Fresh weight after infiltration FW_i mg	Intercellular space volume V_g mm³	WD_{abs} mg	WSD %
300	240	60	420	112	60	20·0
698	580	118	935	234	121	22·0
405	350	55	560	146	64	17·7
568	410	158	800	222	168	35·5
582	440	142	830	250	140	31·3
660	520	140	890	216	154	27.8
640	500	140	920	265	155	28·2
570	440	130	795	228	127	29·5

For symbols and calculation see text.

Table 4
Measurements of water deficit in *Zebrina pendula* Schmitzlein leaves

Fresh weight at full saturation FW_s mg	Fresh weight after transpiration FW_t mg	$FW_s - FW_t$ mg	Fresh weight after infiltration FW_i mg	Intercellular space volume V_g mm³	WD_{abs} mg	WSD %
370	340	30	426	54	32	8·7
611	570	41	714	103	41	7 2
590	548	42	690	103	39	7·8
540	492	48	605	66	47	9.7
630	580	50	720	88	52	8·4
580	540	40	660	76	44	7·4
540	480	60	620	81	59	11·4
510	470	40	590	76	44	8·5
540	490	50	620	82	48	9·8
540	510	30	635	94	31	5·9
450	410	40	515	64	41	9·6

For symbols and calculation see text.

3. In studying a leaf with an unknown water content, the water deficit of the tissue may be calculated by Eq. (2). Thus, the fresh weight of the leaf in the full turgor (FW_s) equals the value of the absolute water deficit (WD_{abs}) *plus* the fresh wieght of the leaf after transpiration (FW_t). Then

$$WD_{abs} = (FW_i - FW_t) - Vg \qquad (2)$$

where WD_{abs} = the weight of water required for the full saturation of the leaf, FW_i = the fresh weight of the leaf after infiltration, FW_t = the

fresh weight of the leaf after transpiration, and V_g = the volume of the gas removed (eguals the volume of intercellular space).

Further experiments were carried out in a very similar manner on *Reineckea carnea* CTH and *Zebrina pendula* Schmitzlein leaves. Data are collecte ł in Tables 3 and 4.

The gasometric method was verified by measuring the water deficit also in incompletely turgid leaves. It was found that the deficits of leaf-halves had approximately the same values. The experiment was carried out as follows: *Brassica oleracea* L. leaves were cut in halves and water deficit was immediately determined in one half of a leaf. The other half was allowed to lose some water by transpiration and then its deficit was measured. With the initial deficit of the one half of the leaf already known, the deficit of the other half after some additional loss of water could be calculated and compared with the observed value. The calculation was made as follows: The defici determined in one half of a leaf immediately after being cut from the plant was 5·4%. Since the fresh weight of the second half of the leaf was 1256 mg,t its fresh weight with full saturation should be 1327 mg. As the weight of the second half-leaf after transpiration was 1124 mg, the amount of water required for its complete saturation could be calculated (1327—1124 = 203 mg). When the dry weight of the leaf was determined and the experimental data were put into the Stocker equation, the water deficit was calculated as 16·3%, whereas the experimentally stated value was 18%. The above described experiments (Table 5.) were performed on *Brassica oleracea* L. and *Beta vulgaris* L. var. *saccharifera* LNGE, (cv. AJ4) leaves.

Discussion

The purpose of the present investigations was to develop a method for determining water deficit applicable in field conditions, easy to perform, and connected with a minimum error. The necessity of using an oil vacum pump to produce a subatmospheric pressure in the extractors renders field measurements difficult. This appears, however, to present no insurmountable difficulties in measuring the water deficit of plants in natural conditions. This suggestion is based on the data collected in Tables 2—5. As can be seen from these Tables, the measurement of the initial weight of the tissue under investigation is very important. Further losses of water preceding the actual measurement have no effect on the correctness of determination. It is therefore sufficient to determine the weight of leaves in field conditions and the water deficit may be determined after their having been ransferred to a laboratory.

STOCKER's method (1929) appears to be the most suitable method for measuring water deficit, but as already indicated the measurement is of rather long duration; this may result in changes of the material under investigation and thus in a higher error of determination. The present method obviates the difficulties of the Stocker's method. This is particularly true as

Table 5

Water saturation deficits (in per cent) in leaf-halves of *Brassica olera ca* L (BO) and *Beta vulgaris* L. var. *saccharifera* LNGE cv. AJ4 (BV)

Immediately after being cut off		After transpiration			
		calculated		experimental	
BO	BV	BO	BV	BO	BV
5·4	15·8	16·3	33·8	18·0	33·6
12·1	11·7	23·6	27·3	22·8	32·1
8·4	21·6	17·4	35·7	17·5	33·2
7·2	18·5	16·2	34·0	16·1	33·5
0·0	21·6	9·7	36·5	12·0	33·0
7·0	16·2	19·3	30·1	16·8	31·5
2·9	17·3	12·4	36·1	13·9	37·2
1·0	21·4	9·2	37·3	10·9	36·3
	15·4		31·1		32·6
	24·9		37·8		33·8
mean 5·5	18·4	15·5	33·9	16·6	33·7

regards an infiltration of the material investigated during the saturation of leaves with water, which is the drawback of the Stocker method but is the principle underlying the gasometric method of measurements. The time of measuring water deficit by the gasometric method is about 30 min., but the actual saturation of tissue with water takes 1 to 15 min. A question arises whether the tissue could be completely saturated with water within this short period of time. After the gas has been removed, water penetrates rapidly into the intercellular spaces and this makes possible its contact with the surface of all cells adjacent to these spaces. In these conditions cells can rapidly replenish their water contents and the tissue will become completely saturated with water. A confirmation of the complete saturation of a tissue with water within 1 min. is the fact that when kept in water for a protracted period of time the tissue fails to absorb more water.

According to ROSENE (1943) and STREBEYKO (1956), the cytoplasm is very slightly permeable. Radish hairs, for example, have been found to absorb water at a rate of 0·2 to 3·1 μm^3 per μm^2 per minute. A prismatic cell model, $60 \times 60 \times 20$ μm in size, has a surface area of 5600 μm^2 and a capacity of 24,000 μm^3. If water penetrates through 1 μm^2 of the cytoplasm surface at a rate of 3 μm^3 per minute, the whole surface will allow 16,800 μm^3 of water to pass within this time. If the cell is assumed to contain 80% of water, its capacity will be equal to 19,200 μm^3. If the water deficit of the cell should be as high as 50%, it would be compensated within 0·5 min.

Individual cells of a leaf tissue contact with one another and the free surface accessible to water infiltration may be less that calculated for the model. However, the presence of large intercellular spaces in the leaf tissue results in a surface of cells high enough to allow the water deficit to be compensated very rapidly.

It was found that the volume of air removed from intercellular spaces is

equal to that of the water infiltrating the leaf only if that leaf is in full turgor. When not fully turgid, the leaf will absorb an amount of water greater than the volume of the gas removed by the amount required to achieve complete saturation. A question arises whether the determination will be correct also for tissues showing a greater water deficit, i.e. when the intercellular spaces may undergo contraction. With beet and cabbage leaves made to exhibit water defcits of 33.9% and 15.5% -Table 5.), respectively, the intercellular spaces were found to undergo no change in volume. However, at still higher deficits leaves of certain plants are likely to exhibit changes in the intercellular space volume high enough to affect the accuracy of the present method. In such cases it appears sufficient to determine the weight of a leaf under investigation and to keep it in a water vapour-saturated atmosphere until the turgor partially grows and then to carry out the actual water deficit measurement.

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J. Czerski, Oddělení fysiologie rostlin University ve Varšavě: **Gazometrická metoda ke stanovení vodního deficitu v listech.** — Biol Plant. **10** : 275—283, 1968.

V práci je popsána nová gazometrická metoda ke stanovení vodního deficitu v listech. Principem metody je stanovení objemu vzduchu vysátého vývěvou z mezibuněčných prostor sledovaných listů U plně turgescentního listu se množství vody vniklé do pletiva při podtlaku rovná objemu plynu vysátého z intercelulár U listu majícího vodní deficit je množství vniklé vody větší než objem plynu z intercelulár o množství odpovídající vodnímu deficitu Váhové stanovení vniklé vody a současné stanovení objemu plynu z mezibuněčných prostor tedy umožňuje výpočet vodního deficitu Srovnávací měření na pěti druzích rostlin potvrdila správnost navržené metody. Zkrácením doby potřebné k měření se zcela vyloučily zdroje chyb spojené s růstem pletiva a prodýcháním sušiny během měření

Е.Черски, Отделение физиологии растений, Варшавский университет **Газометрический метод измерения водного дефицита в листьях.** — Biol Plant **10** . 275—283, 1968.

Разработан метод измерения водного дефицита в листьях. Измерительное устройство работает на принципе извлечения газа из межклетников вакуум-насосом и измерения его объема При полном тургоре количество воды поступившей в ткань равно по объему газу извлеченному из межклетников. В условиях водного дефицита объем воды больший чем объем газа на величину равную водному дефициту По измерении объема воды и газа можно расчитать водный дефицит Сравнительные измерения на пяти видах растений дали хорошие результаты оправдывающие применение предлагаемого метода. Уменьшение длительности измерения исключило ошибки связанные с ростом ткани и потерей сухого веса при дыхании.