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https://doi.org/10.5109/22550

出版情報：九州大学大学院農学研究院紀要. 2 (3), pp.93-116, 1928-06. Kyushu Imperial University
バージョン：
権利関係：

Journal of the Department of Agriculture, Kyushu Imperial University, Vol 2, No. 3.
June 1, 1928

VARIATION OF THE WATER CONTENT OF LEAVES AS RELATED TO THE WILTING OF PLANTS[1]

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I. INTRODUCTION

As it has been pointed out by several authors (14, 16, 17, 7, 19), the water content of the leaves of plants is not always constant, but shows a variation during the day, as is the case in regard to the foliar transpiring power (11). Among them, LIVINGSTON and BROWN (14) paid much attention to the diurnal change of foliar water content, as a factor regulating the transpiring power of leaves. When the rate of water supply does not cover the water loss from the tissue of leaves, the tissue should become less moist and dry out more or less. The first step of this internal drying of the tissue, which may be attained temporarily in a leaf at the time of maximum evaporation of a day, has been termed incipient drying by these authors. If such a drying process in a leaf tissue continues, the water content and correspondently the turgor pressure of the tissue should decrease. Finally all turgidity would be destroyed and a point, at which the wilting of a leaf is first externally to be seen, is now reached; and the more the water content of leaves decreases, the more the state of wilting proceeds. Therefore, any definite degree of wilting in a tissue would be correspond to a definte water deficit in the tissue, although experimental determination of such a definite

[1] Contributions from the Botanical Laboratory, Kyushu Imperial University No. 20.

relation between them might not be an easy task. In literature we can find few contributions dealing with this problem (5, 20). Among others CALDWELL (5) discovered, that a plant has a definite amount of water in the leaves when wilting begins and at the time of so-called permanent wilting. But our knowledge of this relation is at present quite insufficient. The writer worked, therefore, during 1919-1920, when he was studying on the water relation of wilting plants in the Plant Physiological Laboratory of the Johns Hopkins University Baltimore, on the problem in question, with some reasonable results. But unavoidable circumstances compelled him to leave these results unpublished up to the present. Recently the writer was able to continue the work on this problem somewhat further in the Botanical Laboratory of Fukuoka. The present paper is based on the results obtained in those two laboratories.

It is the writer's pleasant obligation to express his gratitude to Professor B. E. LIVINGSTON of the Johns Hopkins University for his helpful suggestions in this work and his great kindness in permitting the writer to use the facilities and equipment of his laboratory.

II. MATERIAL AND METHOD

The plant materials used in this work were *Coleus Blumei*, *Glycine Soja* and *Mimosa pudica*. They were planted in pots. When it was desired to wilt them, they were placed on a table without further watering. For determining the water content of leaves, the common method of weighing, drying and reweighing was employed. As the leaves were plucked, they were placed immediately in tarred glass tubes and tightly stoppered. After being weighed, the open tubes were placed in a drying oven of temperature 100-103°C, and the final dry weight obtained by reweighing the re-stoppered tubes without removing or handling the leaves, but after cooling in a desiccator.

For the cultivation of plants, several kinds of soil or their mixtures, with different water holding capacity were used, the water capacity having been determined by the method of HILGARD (6). Sand, loam, humus and mixtures were principally used for the work in Baltimore and were numbered according to the value of the water holding capacity, which was given in percentage on the basis of the oven-dry volume (Tab. 1).

Tab. 1. The water holding capacity of soils used in experiments at Baltimore.

Number of soils	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Constitution of soils	S	S3+L1	S1+L1	S1+L3	S3+H1	L	L3+H1	S1+H1	L1+H1	S1+H3	L1+H3	H
Water-holding capacity, in volumetric %	40.7	43.0	45.8	50.7	55.7	57.7	67.5	71.1	79.5	81.4	86.5	103.1

S=Sand, L=Loam, H=Humus.

For the experiments carried out in our laboratory at Fukuoka other kinds of soil were used. The water holding capacity of these soils was however not directry determined. These soils were numbered a, b, c and d, according to the value of the water residue in soils at the critical wilting point of *Mimosa pudica* cultured in each soil, instead of the water holding capacity (Tab. 2).

Tab. 2. The water content of soils at the critical point of wilting of *Mimosa pudica* rooted therein (Soils used at Fukuoka).

Number of soils	a	b	c	d
Kinds of soil	Sandy garden-soil	Ordinary garden-soil	Mixture of sand and humous soil	Humous soil
Critical water content given in % of oven-dry volume of soil ...	5.49	8.95	9.64	20.33

As the index of the water content of leaves, its percentage on the basis of fresh weight or dry weight was adopted in many cases in the literature. But the fresh or dry weight of leaves is of a variable nature, especially in our case, where the plant materials were studied in the progressive phases of wilting. The writer preferred, therefore, to use the amount of water contained in the unit area of leaves as the better index for our purpose, although some sampling error, which may be caused by the difference of the thickness of leaves, must be expected. The area of the leaf was measured by means of a planimeter. The percentage content of water based on the fresh and dry weight was also taken into consideration for comparing their applicability. In the parts of experiment, which were carried out in the laboratory at Fukuoka, the so-called powder method for determining the water content (8, 10, 13) was also employed.

III. EXPERIMENTS

1. *Coleus.*—A variegated yellow purple and a purple variety

Four equally grown plants of variegated variety, which were cultivated in pots in common garden soil, were well watered in the day of beginning the experiment. One of them was tested for the water content of its leaves on the day of watering, while the others were allowed to wilt. Among them one was studied for the same purpose, when it began to wilt slightly, another plant, when it reached the day of the so-called permanent wilting in the sense of BRIGGS and SHANTZ (2, 3, 4, 21), and the last one, when it became excessively wilted. Another series of experiment was made with the purple variety.

Determinations of the foliar water content of each plant were always tried at the hours 13:30 and 23:30. Five mature leaves on one side of the leaf pairs were collected for the day-time determination, and the five opposite leaves were removed for the night-test. The values found and the ratios between the day and night values are given in the table 3.

Taking the water content per unit area (1 cm^2) into account, we see, that the water content of leaves becomes smaller and smaller as the wilting progresses. The values of the water content given by the percentage on the basis of the dry or fresh weight showed neverthless some points of non-uniformity, though there was, roughly speaking, the same tendency in the relation in question. The reason, why such a lack of uniformity appears, must be due to the circumstance, that the variations of the dry and fresh weight of plant materials used are wholly neglected in these cases. In the case where the foliar water content of the variegated variety was given in the percentage on the basis of the dry weight, the values for the slightly wilted material came much larger than those for the healthy material. This must be namely an index error probably caused by the negligence of the diminution of dry matter in the wilted material.

As regards the relation between the day and night values of the water content, there was a different tendency as the plant material used was different. As for the variegated variety, the night values were

Tab. 3. Water content of the leaves of *Coleus*, cultivated in common garden soil at the four different stages of wilting healthy (I), slightly wilted (II), permanently wilted (III) and excessively wilted (IV).

Stages of wilting	Per 1 cm² area in mg.			% on dry weight			% on fresh weight		
	Day value (I)	Night value (II)	Day-night ratio (I/II)	Day value (I)	Night value (II)	Day-night ratio (I/II)	Day value (I)	Night value (II)	Day-night ratio (I/II)
Variegated variety									
I	19.18*	20.00	*0.96*	1320	1596	*0.83*	93.0	94.0	*0.99*
II	18.34	18.78	*0.98*	1547	1737	*0.89*	93.8	94.4	*0.99*
III	**17.41***	**17.48**	*1.00*	1409	1504	*0.94*	93.4	93.8	*1.00*
IV	16.58	17.10	*0.97*	1053	1246	*0.85*	91.3	92.5	*0.99*
Purple variety									
I	19.49*	19.33	*1.01*	1588	1335	*1.19*	94.2	93.5	*1.01*
II	18.07	18.82	*0.96*	1350	1220	*1.11*	93.1	92.5	*1.01*
III	**17.15***	**16.95**	*1.01*	1133	1051	*1.08*	91.9	91.3	*1.01*
IV	14.87	14.44	*1.03*	916	885	*1.04*	90.3	90.0	*1.00*

*The ratio of the water content at the point of permanent wilting to that at the state of full turgidity: 17. 41/19. 18=**0.91**, 17. 15/19. 49=**0.88**.

larger than that of the day in all stages of wilting, while in the case with purple variety the relation appeared conversely. The reason, why such a different relation occured in this case, might be attributed to the difference of properties on the plants themselves, or to the difference of the environmental circumstances. To tell the truth, the atmospheric condition of the days, during which the experiments with the variegated variety were carried out, was much dryer than that of the days with another variety. It was supposed that the water content of leaves might fall into the lower values at the arid period of the day or at the day-time, as it was suggested by LIVINGSTON and BROWN (14). This was at least one of the reason, why the day value for the variegated variety came to light somewhat lower than the night value. In connection with this relation it was worthy of remark, that the day-night ratio of the water content became nearer to unity as the wilting proceeded, until the plant fell into the state of permanent wilting. That is to say, the degree of diurnal fluctuation of foliar water content reaches its minimum at this point. On the contrary the purple variety, which was experimented on during rather wet days, showed no such tendency, namely, that the value of the foliar water content in the day time should become lower than that of the night value of the same 24 hour period. This plant seemed therefore simply to lose its leaf moisture decreasingly during the process of wilting without visible fluctuation.

By comparing the results obtained in both series of experiment, there is a remarkable fact brought to light, namely, that the water content of leaves at the time of full turgidity, of slight wilting and of the permanent wilting seems to be approximately alike, or probably constant in each stage, though the values shown in the percentage on the basis of the dry weight are found to correspond less accurately. This might be caused by the probable fact, that the content of the dry matter or its variation in those two materials does not agree to each other (Tab 3). It is suggested therefore by this experiment, that the leaves of *Coleus* contain a constant amount of water corresponding to any definite stage of wilting.

2. *Glycine*.—A variety named Wilson

In this series of experiments soy bean plants seven or eight weeks old, which were cultivated in five different soils, were used : sand, sand-loam mixture, loam, sand-humus mixture and humus. In each pot, there were six plants; and each plant bore one pair of simple leaves and two or three well grown compound leaves. The water content of the leaves was determined on the day of full turgidity, at the beginning of wilting and at the time of permanent wilting. The collection of leaves for each determination was made between the hours 11 and 16, when the leaves were in the required critical state of wilting; one middle leaflet and two side leaflets in the first compound leaves being picked at each time of determination from different plants in each pot.

The results of this experiment are shown in the table 4, where the observed values for each plant as well as the relative values compared with the average value are given. The values found by the area or dry weight method of indication showed, that the foliar water content of material used decreased remarkably as the plants wilted, although it was less remarkable in the case of the fresh weight method. The latter result is of course not so reliable to the material, because the method of indication used is not fitted.

According to the results found by the area method, which must be the most reasonable method for our purpose in this case, the foliar water content seems to become larger, as the water holding capacity of the soil used grows larger. This is noticeable in the leaves in any stage of wilting, but most clearly in those at the time of permanent wilting. This fact is not so clearly indicated by the values found by the fresh or dry weight method, which must involve in themselves methodical error in this case (see 8, 10, 13, 19). This difference of the foliar water content, caused by the difference of culture soils indicates presumably, that the water retaining power of the cells in the leaf of plants, which were cultivated in the soil of higher water holding capacity, may be larger than that of the plants cultivated in the soil of lower water holding power or in the soil with higher amount of available water[2].

On the other hand the ratio between the foliar water content at the time of the permanent wilting and at the time of full turgidity appeared to be the larger, the larger was the water holding capacity of the culture soils. This seems to indicate, that the resistance of the leaf tissues against the water desiccating power becomes higher in the plants cultivated in the soils with higher water holding capacity.

[2] It must be kept in mind, that the plants used in this experiment were all cultivated in soils watered every day. Thus the plants, which were cultivated even in the soils of lower water holding capacity, never suffered from the lack of available water in the soil.

Tab. 4. Water content of the leaves of *Glycine*, cultivated in five different soils, at the time of full turgidity (I), at the beginning of wilting (II) and at the time of permanent wilting (III).

Number of culture soils	Per 1 cm² area in mg. at the stage of				% on dry weight at the stage of				% on fresh weight at the stage of			
	I	II	III	III/I	I	II	III	III/I	I	II	III	III/I
Observed values												
1	10.03	9.11	5.65	*0.56*	451	309	262	*0.58*	81.8	75.5	72.4	*0.89*
3	11.70	10.22	6.00	*0.51*	588	404	300	*0.51*	85.5	80.2	75.0	*0.88*
6	11.14	10.25	6.28	*0.56*	432	379	285	*0.66*	81.2	79.1	74.0	*0.91*
8	13.49	12.95	9.40	*0.70*	499	406	419	*0.84*	83.3	81.2	80.7	*0.97*
12	14.16	13.05	10.54	*0.74*	487	466	451	*0.93*	83.1	82.3	81.8	*0.98*
Average	12.10	11.12	7.57	***0.61***	491	393	343	*0.70*	83.0	79.7	76.8	*0.93*
Relative values												
1	83	82	75	—	92	79	76	—	99	95	94	—
3	97	92	79	—	120	103	87	—	103	101	98	—
6	92	92	83	—	88	96	83	—	98	99	96	—
8	111	116	124	—	102	103	122	—	100	102	105	—
12	117	117	139	—	99	119	131	—	100	103	107	—
Average	100	100	100	—	100	100	100	—	100	100	100	—
Deviation $\left(\pm\frac{\Sigma\Delta}{n}\right)$	±11.2	±13.4	±25.2	—	±8.6	±10.0	±21.4	—	±1.2	±2.4	±4.8	—

3. *Coleus*.—A pale yellow variety

Twelve plants, which belong to one and the same variety, were cultivated in twelve different kinds of soil. In these materials the foliar water content was determined at the stage of permanent wilting only. The purpose of this experiment was to study the influence of the moisture condition in the culture soils upon the critical water content of leaves.

At the hour 16:30 on the day of permanent wilting, three middle aged leaves were collected for the day-time test, and the three opposite leaves at the hour 23:30 of the same day for the night-time study. The day-night ratio of the foliar water content found in each plant was in almost all cases very near to unity. The ratios between the average value of the day and the night, which were calculated for the water content per unit area and for the percentage on the basis of the fresh weight was 1.003 and 1.000 respectively, while the ratio obtained by the dry weight method was 0.943. The variation of the values found at these two test times was, that is to say, but a little for each case, as it was expected. Therefore the average results of each two tests for each plant was adopted as the index for the critical water content in question (Tab. 5).

The index based on the area method being taken into consideration, it was found, that the amount of water held in the leaves of each plant was very near to that in every other: the maximum and minimum value were namely 17.73 mg. and 14.05 mg. respectively for 1 cm^2 area, the average value being 15.99 mg. In other words, the mean deviation of the relative values for the average, which was calculated after the formula $\pm \frac{\Sigma \Delta}{n}$, was only 4.2 %. However, there was a tendency to be seen, even if very slightly, that the water content of the plants cultivated in the soils with higher water holding capacity is somewhat smaller than that found in other plants.

This relation could be found also in the results obtained by the fresh weight method. But according to the results based on the determination by the dry weight method, the difference of the foliar water content of plants cultivated in different kinds of soil came to light more remarkably. But the letter result is, presumably, only a mere show caused by the methodical error, because the foliar dry matter content of plants, which were cultivated under different soil conditions, would show not a little variation.

Tab. 5. Water content of the leaves of *Coleus*, cultivated in twelve different kinds of soil, on the day of permanent wilting.

Number of culture soils	Per 1 cm² area in mg.		% on dry weight		% on fresh weight	
	Observed values	Relative values	Observed values	Relative values	Observed values	Relative values
1	16.21	101	1740	145	94.3	102
2	**17.73**	111	1299	108	92.9	101
3	16.85	105	1280	107	92.8	101
4	16.40	103	1157	96	92.1	100
5	16.02	100	1123	94	91.4	99
6	15.86	99	1265	106	92.4	100
7	15.88	99	1049	87	92.5	100
8	16.68	104	1395	116	93.2	101
9	15.42	96	995	83	92.0	100
10	14.72	92	1088	91	91.7	99
11	16.06	100	1098	92	91.7	99
12	**14.05**	88	894	75	90.0	98
Average	**15.99**	100	1199	100	92.3	100
Deviation$\left(\pm \frac{\Sigma\Delta}{n}\right)$	—	±4.2	—	±13.7	—	±0.8

Observed values are given in the average of day- and night-value.

4. *Glycine*.—A variety named Wilson

In this series of experiments, the water content of the first ordinary simple leaves of the soy bean plants eight weeks old, which were cultivated in five different soils, as in the case of the experiment 2., was determined. The determinations were made at the time of full turgidity and of permanent wilting, four leaves being collected for each determination from different individuals in the same pot.

The results obtained by this experiment coincided with those of the experiment 2. (Tab. 6). The difference of the foliar water content of plants cultivated in different kinds of soil again appeared more remarkable in the stage of permanent wilting than in the stage of full turgidity. The ratio between the values of water content at these two stages

Tab. 6. Water content of the first ordinary simple leaves of *Glycine*, cultivated in five different kinds of soil, at the time of full turgidity and of permanent wilting.

Number of culture soils	Per 1 cm² area in mg. at the time of			% on dry weight at the stage of			% on fresh weight at the stage of		
	full turgidity	permanent wilting	Ratio	full turgidity	permanent wilting	Ratio	full turgidity	permanent wilting	Ratio
Observed values									
1	9.02	4.56	*0.51*	439	197	*0.45*	81.5	66.3	*0.81*
3	10.21	5.96	*0.58*	428	245	*0.57*	81.1	71.0	*0.88*
6	10.44	5.34	*0.51*	459	238	*0.52*	82.2	70.4	*0.86*
8	12.79	7.96	*0.62*	534	344	*0.64*	84.3	77.5	*0.92*
12	13.16	9.55	*0.73*	512	339	*0.66*	83.7	77.3	*0.92*
Average	11.12	6.67	***0.59***	474	273	*0.57*	82.6	72.5	*0.88*
Relative values									
1	81	68	—	93	72	—	99	91	—
3	92	89	—	90	90	—	98	98	—
6	94	80	—	97	87	—	100	97	—
8	115	119	—	113	126	—	102	107	—
12	118	143	—	108	124	—	101	107	—
Average	100	100	—	100	100	—	100	100	—
Deviation $\left(\pm\frac{\Sigma\Delta}{n}\right)$	±13.2	±25.0	—	±8.2	±20.2	—	±1.2	±5.6	—

studied was also found to be larger in the plants cultivated in the soils with higher water holding capacity. In this connection, there was a remarkable fact to be seen in the two series of experiment 2. and 4., namely, that the ratio in question, under the results obtained by the area method, was found to be similar in those plants cultivated in the same kind of soil, the average value for each series of experiment being 0.61 and 0.59 respectively (Tab. 7). It seems therefore, that the relation of the water content of a given plant at the stage of permanent wilting to the normal water content of the same plant is likely to be the same for a given condition of soils.

Tab. 7. Comparison of the ratios of the values for the water content of the leaves of *Glycine* at the point of permanent wilting to that at the state of full turgidity found by two series of experiment (Exp. 2. and Exp. 4.), determinations being made by the area method.

Number of culture soils	Experiment 2.		Experiment 4.		Average		Deviation of values in %
	Observed values	Relative values	Observed values	Relative values	Observed values	Relative values	
1	0.56	105	0.51	95	0.535	100	±5
3	0.51	94	0.58	106	0.545	100	±6
6	0.56	105	0.51	95	0.535	100	±5
8	0.70	106	0.62	94	0.660	100	±6
12	0.74	101	0.73	99	0.735	100	±1
Average	**0.61**	102	**0.59**	98	0.600	100	±2

5. *Coleus.*—Another variety with variegated leaves

In this case another variegated variety of *Coleus*, which was cultivated in four different soils (soil a, b, c and d), was used for experiment, two series of experiment being carried out. The water content of leaves was determined at the stage of permanent wilting in comparison with the content at the stage of full turgidity, and the results calculated for 1 cm^2 leaf area as well as for the percentage on the basis of the dry weight, were taken into consideration (Tab. 8). But the results obtained by the dry weight method seemed clearly to be unreasonable, because the water content at the stage of permanent wilting came to light sometimes as if it was larger than that at the stage of full turgidity.

Tab. 8. Water content of the leaves of *Coleus*, cultivated in four different soils, at the time of full turgidity and of permanent wilting.

Number of culture soils	Values at full turgidity			Values at permanent wilting			Ratio II/I
	First exp.	Second exp.	Average (I)	First exp.	Second exp.	Average (II)	
Per 1 cm² area in mg.							
a	23.1	21.7	22.4	21.2	20.8	21.0	*0.94*
b	22.9	22.7	22.8	20.8	20.0	20.4	*0.89*
c	21.6	22.1	21.9	19.6	19.2	19.4	*0.89*
d	23.8	20.2	22.0	21.5	17.8	19.7	*0.90*
Average	22.9	21.7	22.3	20.8	19.5	20.1	***0.91***
% on dry weight							
a	1355	1288	1322	1194	1294	1244	*0.94*
b	1196	1155	1176	1121	1021	1071	*0.91*
c	1303	1406	1355	1129	1612	1371	*1.01*
d	1318	966	1142	1162	1031	1097	*0.96*
Average	1293	1204	1249	1152	1240	1196	*0.96*

According to the results obtained by the area method, there were no unreasonable data to be found, and the results of the two series of experiment were alike in general. The difference of the water content of leaves as related to the difference of the culture soil was however scarcely recognizable, though there was some tendency toward the value of the water content in the soils with higher water holding capacity appearing somewhat smaller than that of other soils. This will be probably caused by the matter of fact, that there was small variation in the water relation of the soils used for this experiment.

The ratio between the value at the stage of permanent wilting and that of the stage of full turgidity, being calculated for each plant by the average value of the two series of experiment, was 0.89-0.94, the average value of all materials used being 0.91. This value agreed well with the value obtained by the experiment 1. In other words this ratio seems to be a definite value for the *Coleus* plants.

6. *Glysine*.—Another variety

The soy bean plants used in this experiment were well grown to the maturity of flowering. Three kinds of soil (Soil a, b and d) were used for cultivation. The determination of water content was carried out at the stage of full turgidity and the stage of permanent wilting, the results of determinations being given in the values per unit area of the leaf, the values in percentage on the basis of the dry weight and the values per 1 cm³ powder obtained from the leaf tissue (8, 10).

Tab. 9. Water content of the leaves of *Glycine*, cultivated in three different kinds of soil, at the time of full turgidity and of permanent wilting.

Number of culture soils	Values at full turgidity			Values at permanent wilting			Ratio II/I
	First exp.	Second exp.	Average (I)	First exp.	Second exp.	Average (II)	
Per 1 cm² area in mg.							
a	7.99	7.32	7.66	3.91	4.14	4.03	0.53
b	8.44	7.61	8.03	4.31	3.76	4.04	0.50
d	8.18	8.22	8.20	3.97	4.37	4.17	0.51
Average	8.20	7.72	7.96	4.06	4.09	4.08	**0.51**
% on dry weight							
a	337	322	330	196	187	192	0.58
b	380	344	362	192	174	183	0.51
d	369	355	362	186	189	188	0.52
Average	362	340	351	191	183	188	**0.54**
Per 1 cm³ tissue powder in mg.							
a	963	1030	997	532	593	563	0.56
b	1114	1160	1137	578	548	563	0.50
d	1074	1151	1113	533	598	566	0.51
Average	1050	1114	1082	548	580	564	**0.52**

The experiment were carried out twice and the results agreed in general to two cases. The three kinds of results obtained by the three different methods of indication also showed considerable agreement, the ratios between the values for the stage of permanent wilting and those for the stage of full turgidity being alik for each case. The averages of the values in question were namely 0.51, 0.54 and 0.52 respectively.

The values for the water content found in the materials cultivated with different kinds of soil showed little variation, but there was a tendency for the value for the plant cultivated in the soil with higher water holding capacity to be somewhat larger than the others. Therefore we may presume, that this tendency would be more marked, if the soils used for this experiment were more highly different from each other in their water holding capacity. In truth the water holding capacity of the soils were in this case varied less than in the case of experiments 2. and 4., where the tendency in question came to light more clearly.

7. *Mimosa*

Mimosa pudica plants, which were cultivated from seeds in pots filled with the soil b, were used for experiment. The determination of the foliar water content for the time of full turgidity was made two hours after the watering the pots. The second determination was done at the critical point of wilting, where all leaves of the plants used lost just their reactivity, even for the strong mechanical stimulations because of the insufficiency of the turgidity in the pulvinus (9). As the leaf of this plant is a compound one composed of many small leaflets, the determination of the water content in the leafblade itself could not be undertaken. The leaves used for experiment were picked from their insertion point at the stem.

The experiment were repeated five times with five different individuals, five leaves being gathered for each experiment. The values of the water content were given in the percentage on the basis of the dry weight. For this material the area method could not be applied. As

Tab. 10. Water content of the leaves of *Mimosa*, cultivated in soil b, at the time of full turgidity and of critical wilting.

	% on dry weight						per 1 cm³ tissue powder in mg.
Number of material	1	2	3	4	5	Average	
Values at full turgidity (I)	212.0	212.1	210.2	203.8	214.8	210.6	1141
Values at critical wilting (II)	109.1	110.7	108.6	117.2	111.2	111.4	608
Ratio (II/I)	*0.51*	*0.52*	*0.52*	*0.58*	*0.52*	**0.53**	**0.53**

the meterial gathered from each plant was too scanty for the powder method, all materials used for the five experiments were used together at once for this purpose, and the values of the water content per 1 cm^3 tissue powder were calculated from the total sum of values found from total materials (Tab. 10)

According to the results on the basis of the dry weight, the ratio of the values found at the two stages of plants were 0.51-0.58, the average being 0 53. The ratio found by the powder method was also 0 53

8 *Mimosa*

In this series of experiments *Mimosa pudica* plants, which were raised from seeds and cultivated in four pots with different kinds of soil a, b, c and d, were employed. The foliar water content was determined in the same way as in experiment 7. But in this case, after several leaves were picked from each material at the state of full turgidity for the first test, their branches were cut off for the purpose of hastening the wilting process. When the leaves on the branches cut off reached the critical point of wilting, several of them were picked for the second test.

According to the results obtained in this experiment (Tab. 11), no definite relation between the foliar water content of plants and the water holding capacity of soils used for cultivation was to be seen. But the

Tab. 11. Water content of the leaves of *Mimosa* at the time of full turgidity and at the point, at which the leaves on branches cut off are reached the critical state of wilting.

Number of culture soils	% on dry weight at the time of			per 1 cm^3 tissue powder at the time of		
	full turgidity (I)	critical wilting (II)	Ratio (II/I)	full turgidity (I)	critical wilting (II)	Ratio (II/I)
a	251	113	*0.45*	1172	530	*0.45*
b	235	97	*0.41*	1198	393	*0.33*
c	292	111	*0.38*	1391	466	*0.34*
d	247	114	*0.46*	1247	451	*0.36*
Average	256	109	***0.43***	1252	460	***0.37***

the degree of water lost at the critical point of wilting was fairly alike in each material. The ratios between the water content of two test periods were namely 0.38-0.45 with the average value of 0.43, when the values were found by the dry weight method; and the values found by the powder method were 0.33-0.45, the average being 0.37.

Thus the value of ratios in question was found somewhat smaller in this case than in the case of the previous experiment. This difference was caused partly by the too high values for the first test and partly too low values for the second test in this case. Of course there must be several causes relating to this difference, but the principal one would seem to be the difference of the testing method.

IV. DISCUSSION

The wilting of plants is, as is known, a general symptom of water deficiency in the tissues. It must be therefore a matter beyond question, that the water content of leaves decreases progressively during the process of wilting. But the phenomenon of wilting is a chain of several stages of water deficiency in tissues, which correspond to the physiological states of plants, as the incipient drying (14), the temporary wilting, the permanent wilting (3, 4) etc. If a given state of wilting is nothing more than a symptom of a definite state of the water deficit in tissues of a plant, there must be a definite water content of tissues corresponding to this state of wilting However, to determine this definite water content experimentally is very difficult, unless we may catch exactly a critical point in the progressive phase of wilting. The point of so-called permanent wilting in the sense of BRIGGS and SHANTZ (3, 4) or the critical point of wilting of *Mimosa pudica* in the sense of KÔKETSU (9) is the only critical stage of wilting, that has received attention in the literature (22). Another critical point of wilting, which we may point out, if not so exactly, is the point of the beginning of visible wilting phenomenon in the leaf. A plant must contain definite amount of water in the leaf tissue at these two critical points of wilting. CALDWELL's work (5) was based on this idea and he has proved it experimentally. Our results of experiment on *Coleus* (Exp. 1.) testified further to his conclusion.

The conclusion, that a plant has a definite water content of leaves at a definite stage of wilting, does not mean, however, that this definite water content is specific for a given plant. The results of the experiments 2. and 4. on *Glycine Soja* cultivated in different soils showed, that a given plant contained different amount of water in the tissue of leaves at a given critical stage of wilting, when it is cultivated under different soil conditions. This relation is seen most clearly at the point of permanent wilting. The same fact is, though somewhat less markedly, derived from the experiments on *Coleus* cultivated in soils with different water holding capacity (Exp. 3). The occurence of the different values of water content in question seems therefore to be relate at least partly to the difference of the water relation in soils (see 15). Though this relation was not directly brought out in our experiment, it was shown indirectly by the values of water holding capacity or the water retaining power of the soils used. If therefore we use for experiment soils with less variation in water relation, the fact above mentioned will come less marked. This must be the reason, why we could not find the remarkable difference in experiments 5, 6 and 8.

On the other hand, there is an interesting fact to be found. In *Glycine* plants (Exp. 2, 4 and 6), we see, that the water content at the stage of permanent wilting is the larger, as the water holding capacity of the soil used is higher. On the contrary, the results found in *Coleus* (Exp. 3 and 5) are *vice versa*. The reason of this phenomenon is not easily analysable. But we assume, that this phenomenon has some relation to the difference of the xerophytism of plants. If we assume, that a mesophytic plant such as *Coleus* has small power to derive water from soils, while a semi xerophytic plant such as *Glycine* has larger power, then a plant such as *Coleus* will fall into the critical state of wilting and will contain less moisture in the leaves, when it is cultivated in a soil with higher water holding capacity but with higher resistance against the water depriving power, the circumstances for a plant such as *Glycine* being reversed. Another point of view in this connection is, that a xerophitic plant has a higher power of adaptation for a soil with higher resistance against the water depriving power and is adapted to reserve water in tissues. However the state of things is complicated, if we consider the water content of leaves at the critical point of wilting of different plants. As it is shown in the tables, the water content per unit area of leaves is much higher in the leaves of *Coleus* than in the leaves of soy bean plants. Thus the water content at the critical point of wilting may be higher in the mesophytic plants than in the semi xerophytic plants. However this relation may not apply the succulent plants.

If the water content of leaves decreases lineally during the process of wilting, so the day value of the water content of leaves at a given stage of wilting will be larger than the value for the following night, thus the day-night ratio coming larger than the unity. This relation might occur, when the desiccating power of air conditions will be relatively high. However, if a given plant loses its tissue moisture relatively slowly, the foliar water content in the day time may be smaller than that of the following night, as is the case in the healthy leaves (14). As is known (1, 11), the transpiring power of healthy or wilted leaves shows generally a larger value in the day time than at night. In other words, the water loss from a leaf is smaller at night. If the water supply to a leaf from the stem may overcome the water loss at night, so the water content of a leaf at night will become larger than that in the previous day time. In this case, the day-night ratio of the water content must be smaller than unity. But the value of this ratio will theoretically approach unity, the more the wilting process of a plant proceeds, until the water supply can no longer cover the water loss.

Thus we may consider two different instances as to the variation in the day-night ratio of water content of leaves. The results obtained from the experiment 1. on two varieties of *Coleus* might represent this two probable instances, though there may be other reasons for this state of things. In the case of the variegated variety, we see a regularized march of the day-night fluctuation of the foliar water content. The fluctuation becomes however smaller and smaller, until the plant falls into the state of permanent wilting, the fluctuation at the next stage of wilting becoming once more somewhat larger. This relation is shown clearly by the variation of the day-night ratio for different stages of wilting. This fact is remarkable, if we reflect upon a similar fact found in the march of daily variation of transpiring power of a wilting leaf. As it was pointed out by BAKKE (1) and KÔKETSU (11), the daily variation of the transpiring power of a wilting leaf, which was determined by means of hygrometric paper, becomes smaller and smaller, as the plant becomes more wilted, until the plant falls into the state of permanent wilting.

On the other hand there is found another point of interest. The ratio between the water content at the critical point of wilting and that at the point of full turgidity of leaves seems to have a deep meaning in itsself. Though the values of this ratio is not constant for a plant, but may be affected by the culture conditions, it seems however characteristic for a plant. Thus the values found in the two different series of experiment on *Glycine*, which were based on the area method, (Exp. 2. and 4.), were 0.61 and 0.59, the values being given in the average from the values found in the plants cultivated in different soils. This ratio found for the two different varieties of *Coleus* in the experiment 1. were 0.91 and 0.88 respectively, while the value obtained in another variety of this plant (Exp. 5.) was also 0.91.

The values of this ratio seems therefore to be definite for a plant, if a plant is cultivated under similar conditions Under different culture conditions the values may, however, very not a little. The values found in the five different individuals of *Glycine* fluctuated between 0.51-0.74 with the average 0.61 in experiment 2. and between 0.51-0.73 with the average 0.59 in experiment 4, while the values found in *Coleus* cultivated in four four different soils gave a fluctuation between 0.89-0.94 with the average 0.91. In the case of *Glycine* the fluctuation was therefore very marked. This fact is without question correlated to the larger difference of the nature of the soil used for this plant. Roughly speaking, the higher the water holding capacity of the soils used, the larger are the values in question found in this case. There is, therefore, a parallelism between the values of the ratio and the values of the water content of leaves at the critical point of wilting.

The values in question for *Glycine*, which were used for experiment 6, were found to fluctuate between 0.50-0.53, the average value being 0.51. The reason why the values found in this case are relatively low, is probably caused principally by the fact, that the nature of material was different and the plants were cultivated in the soils with relatively low values of water holding capacity.

For the consideration of the water content of leaves the values obtained by the area method were adopted principally, because this method of indication was considered as theoretically the most reasonable method for our case. But in such a plant as *Mimosa* it is nearly impossible to employ this method for the determination of foliar water content. To consider the relation in question by the values obtained by the dry weight method and the powder method, it was found in the experiment 7. of this plant, that the ratio between the values for the water contents at the criticial point of wilting and of full turgidity is 0.53, the values obtained by the two different methods being the same. The values for *Glycine* which were found in the experiment 6. by these two methods, were 0.54 and 0.52 respectively, while the value obtained by the area method was 0.51 as already mentioned. That is to say, the values obtained by the dry weight or the powder method brought similar results, as in the case of area method.

In comparing the values of ratio obtained in *Mimosa* and *Glycine*, we see at a glance, that the values are very similar to each other. But we must bear in mind in this case, that the methods of determination of the critical point of wilting were different, BRIGGS and SHANTZ' method for the latter and KOKETSU's method for the former being used. Moreover the manner of gathering materials for experiment was different. However it may be assumed, at least, that the values in question for these two plants will be near to each other. In experiment 8., where the critical point of wilting was determined in the leaves on the detached branches of *Mimosa*, the values in question came to light somewhat lower than in the case of experiment 7., where the rooted plants were employed for experiment. The reason of this difference may be caused, as is already discussed, principally by the difference of testing methods.

Thus the critical foliar water content of a plant may come to light in the different values, according to the difference not only of the culture conditions but also of the testing method. Therefore, if we hope to compare the water relations of different plants, we must test with the same method and with materials cultured under the same conditions. On the other hand we must employ the reasonable methods of determination of water content for a given case In our case it is beyond question, that the fresh weight method of determination is least applicable. As regards the dry weight method, it will theoretically bring reasonable results in some limited cases, where the content of the dry matter in leaves is but slightly variable. But in such a case, where the dry substance varies not a little during the experiment, this method also is not applicable. Thus in many cases in our experiment the results obtained by this method appeared as in point of fact less reasonable, than in the case of area method. Therefore our consideration on the water relation in question was based principally on the results obtained by the area method. When this method was not applicable, we were obliged to depend upon other methods. KÔKETSU's powder method (8, 10) was introduced here as a theoretically reasonable method and proved an applicable method in this case.

Looking back upon the water relation of plants as related to the wilting once more, we may derive a point of interest from the data obtained in our experiments on different plants The water residue in the leaf at the critical point of wilting is very different in the different plants. This critical water residue given by the ratio to the water content at the full turgidity is very high in *Coleus* in comparing with that of *Glycine* plants, while this value for *Mimosa* is rather similar to that of *Glycine*. In other words *Coleus* plants lose the relatively small amount of their foliar moisture, before they fall into the critical state of wilting. The degree of the water loss seems in this connection to be an index showing the degree of the resistance of a plant to wilting. The higher is the ratio in question, the less xerophytic might a plant be This conclusion agrees closely with the MAXIMOW's words: " Xerophytes can endure without injury a greater loss of water than mesophytes " (20).

V. SUMMARY

1. The variation of the foliar water content as related to the wilting of plants was studied by determining the water content at the critical stages of wilting of leaves, *Coleus Blumei*, *Glycine Soja* and *Mimosa pudica* being used as the materials for experiments.

2. As the index to show the water content of leaves, not only its percentage values on the basis of the fresh and dry weight also the amount contained in the unit area of the leaf were determined. The content per unit volume of the tissue powder was also attributively determined. However, it was proved, that the area method was preferable to the percentage methods. The consideration of results was made therefore principally on the values obtained by this method. But in the case of *Mimosa*, where this method was impracticable, the values obtained by the dry weight method was applied. The powder method was also proved to be a good applicable one.

3. The water content of leaves of a plant seemed to be very alike at a given critical stage of wilting, for instances at the beginning of wilting or at the point of so-called permanent wilting of a plant. But this value was affected not a little by the culture conditions. The higher the water holding capacity of culture soils, the larger was the critical water content of leaves of *Glycine*, while the results found in *Coleus* were the opposite. This relation was assumed by the writer to have some bearing on the xerophytic nature of plants.

4 The water content of leaves at the critical wilting point of different plants was proved very different to each other. The ratio of

this critical water content to the content at the stage of full turgidity was also very different one from the other. The value of this ratio was found much higher in *Coleus* than in the other two materials, while the values for these latter two were near to each other.

5. The ratio in question seemed moreover to be specific to a given plant, and to show the degree of the resisting power of a plant to wilting. The value of this ratio might be, therefore, an index to compare the degree of the xerophytism of plants, the more xerophytic being a plant, the lower is the value.

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