A RE-EXAMINATION OF THE RELATIVE TURGIDITY TECHNIQUE FOR ESTIMATING WATER DEFICITS IN LEAVES

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Summary

The relative turgidity technique consists in comparing the initial and turgid water contents, on a percentage basis, of disks punched from leaves, the turgid water content being obtained by floating the disks on water.

Three main sources of error associated with flotation are recognized: (1) changes in dry weight of the disks; (2) continued increase in water content after the attainment of full turgidity; (3) injection of the intercellular spaces at the cut edges of the disks.

An examination of these sources of error led to the following conclusions concerning them:

(1) By regulating the light intensity approximately to the compensation point, dry weight changes can be reduced to unimportant proportions. This obviates the necessity of taking duplicate samples since the final dry weight can be used for calculating both the initial and turgid water content of the disks.

(2) Water uptake by floating leaf disks can be divided into two phases, phase I in response to the initial water deficit, and phase II the continued uptake, due to growth. The aim of the technique is to measure phase I alone. Metabolic inhibitors eliminated phase II but their use in the technique is unpractical (anaerobiosis) or objectionable (potassium cyanide). Low temperature (3°C) eliminated phase II but reduced phase I which itself appears to be divisible into more than one phase.

It was found that for *Ricinus communis* L. full turgidity was attained in 4 hr and in this period phase II increase did not occur. A similar period appears to be suitable for a wide range of material. This short flotation time also reduced any danger of dry weight changes.

(3) Injection errors were measured and found to be negligible for *Ricinus*. For *Sambucus nigra* L. they were considerable. Evidently the magnitude of this error varies with the species concerned.

Plastic flow or irreversible contraction of the cell walls of turgid or wilted disks respectively was not found to be of importance.

I. Introduction

The concept of "relative turgidity" (Weatherley 1950) as a measure of water deficits in leaves, has been found useful by a number of workers (Weatherley 1951; Oppenheimer 1954; Werner 1954; Farbrother 1955, 1956; Slatyer 1955, 1960; Catsky 1959; Wormer and Ochs 1959). However, during the ten years since its

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introduction, the original technique has been modified (Catsky 1959; Wormer and Ochs 1959; Slatyer 1960) and also its soundness questioned (Milthorpe and Spencer 1957). It seemed appropriate, therefore, to examine the technique anew: if possible to establish it in a firmer theoretical basis and to define more clearly the practical methods for measuring it.

The technique consists essentially in comparing the water content of leaf tissue when freshly sampled with the fully turgid water content and expressing the result on a percentage basis, thus:

$$RT = \{(FW-DW)/(TW-DW)\} \times 100, \tag{1}$$

where FW is initial fresh weight, TW is turgid fresh weight, DW is dry weight, and RT is the relative turgidity.

In principle, to obtain the initial and turgid water contents it is necessary merely to weigh freshly sampled leaf tissue (disks punched with a cork-borer are most convenient), to reweigh the same sample after it has been floated on water to full turgidity and finally to subtract from each of these values the weight of the sample after oven drying.

In practice, however, the use of final dry weight to estimate initial water content was found by Weatherley (1950) to be inaccurate, since a significant decrease in dry weight occurred during the 24-hr period of floating which had been adopted to permit the tissue to become fully turgid, and this led to the initial water content being overestimated. Weatherley therefore considered it necessary to collect simultaneously a duplicate sample of tissue which was oven dried immediately to give the initial dry weight needed for the accurate determination of the initial water content.

The use of a second sample in this way was complicated by the errors which inevitably arose from chance differences between the duplicate samples. These were mitigated to some extent by using the demonstrable correlation between fresh weight and water content of the samples, but this involved the determination of the appropriate regression coefficient and was rather cumbersome. In general, other workers using the method have not found it necessary to do this although they have used duplicate samples. The practice has been to compute the original dry weight of the sample, $DW_{a'}$, simply from the relation

$$DW_{a'} = (DW_b \times FW_a)/FW_b, \tag{2}$$

where subscript a refers to the sample which is subsequently floated, and subscript b refers to a duplicate sample which is oven-dried immediately after determining its initial fresh weight. This computation is based on the reasonable assumption that the DW/FW ratio will vary little between duplicate samples. Thus

$$RT = \{(FW_a-DW_{a'})/(TW_a-DW_a)\} \times 100. \tag{3}$$

A further difficulty arises from the form of the water uptake curve of floating leaf disks. It appears to be divisible into two distinct phases: an initial rapid uptake during the first few hours followed by a slow steady uptake lasting as long as the disks remain floating on water and healthy. It was suggested (Weatherley

1950) that the first phase represented uptake in response to the water deficit in the disks, whilst the slow persistent uptake was due to growth of the disks. Catsky (1959) is in general agreement with this interpretation.

The main objectives of the present investigation were to examine the soundness of relative turgidity as a measure of water deficits in plants and to modify the technique so that changes in dry weight could be eliminated and water uptake due to other factors than the initial deficit of the tissue minimized. This would render the collection of duplicate samples unnecessary and permit the direct use of equation (1) with elimination of experimental error due to variation between duplicate samples. It would also achieve a considerable saving in time, labour, and experimental material.

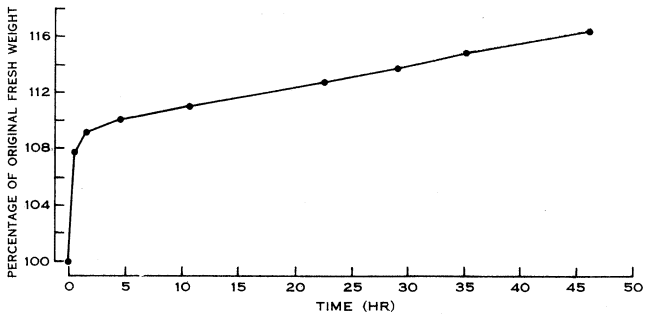

Fig. 1.—Change of fresh weight of floating leaf disks with time.

II. Materials and Methods

Except where otherwise stated, the material used was a commercial variety of the castor-oil plant *Ricinus communis*. Samples of 25 disks were taken from the leaves with a $\frac{7}{16}$-in. diameter leather punch closed with a piston at one end which facilitated rapid ejection of the disks from the punch after their collection. A rubber bung was used to support the leaves during punching.

Except where the effects of temperature and solutes were the subject of investigation, disks were subsequently floated on water in closed petri dishes at 20°C in a constant-temperature room. In addition, the disks were illuminated but details are given later as this factor was varied.

III. Results

(*a*) *Shape of the Water Uptake Curve*

Since it was hoped that the present work would be useful in a further evaluation of the relative turgidity technique it was important to establish that the experimental material (castor-oil leaves) was similar to the cotton leaves used by Weatherley (1950) in its water uptake pattern.

Accordingly a set of leaf disks was weighed, floated on water, and reweighed at intervals. The results are shown in Figure 1.

Weatherley (1950) described the water uptake by floating cotton leaf disks as comprising two distinct phases: an initial rapid uptake during the first few hours followed by a slow steady uptake lasting as long as the disks remained floating on water and healthy. It will be convenient to refer to these uptakes as phase I and phase II respectively. From Figure 1 it is apparent that the same uptake pattern occurred for disks from castor-oil leaves as for disks from cotton leaves. It was found that phase II could persist at a constant rate in castor-oil leaves for 96 hr, though by this time the disks showed visible signs of becoming moribund.

(*b*) *Changes in Dry Weight during Flotation*

In order to ascertain whether the changes in dry weight during flotation that Weatherley (1950) had noted were due to an imbalance between photosynthetic gains and respiratory losses, sets of disks were floated at various light intensities

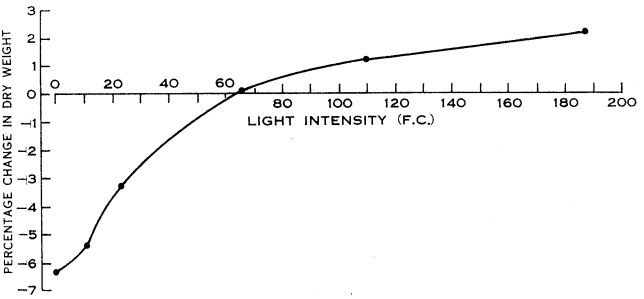

Fig. 2.—Effect of light intensity on dry weight of leaf disks floated on water for 24 hr.

and changes in dry weight noted. It was not necessary to consider the possibility that leakage of solutes from the disks during floating could lead to a decline in dry weight since Weatherley (1954) had already shown these losses to be negligible.

Six samples were floated on water at 20°C for 24 hr. One was contained in a dark box and the remaining five placed at different distances from a set of Sieray mercury vapour–tungsten filament lamps. Radiant heating of the illuminated petri dishes was minimized by a water stream at 20°C and even at the highest light intensity the temperature of the water was not raised more than 0·3°C. The different light intensities to which each sample was exposed were measured with a photoelectric cell calibrated in foot candles. Six duplicate samples taken at the same time were dried in an oven at 90°C for 24 hr immediately after their initial fresh weight had been determined. The final dry weights of the floated samples were determined similarly by oven drying.

The results are presented in Figure 2 where changes in dry weight are expressed on a percentage basis. It is apparent that change in dry weight was closely related to the light intensity to which the disks were exposed, and that at approximately 65 f.c. the dry weight remained constant. This presumably was the compensation point of the disks.

However, further work showed that the compensation point could vary between 40–80 f.c. from sample to sample. Hence it was not possible to eliminate dry weight changes entirely by floating disks for 24 hr at any predetermined light intensity.

Since this experiment established that changes in dry weight during floating were due to metabolic activity it seemed reasonable to expect such changes would be minimized by application of a metabolic inhibitor. Low temperature was tried as it was thought that this would be an effective but safe inhibitor. Fifteen sets of disks were floated at 3°C in the dark in a refrigerator. The average loss in dry weight was only 0·79% which is satisfactorily small. But at the same time it was noted that the turgid water content of disks floated at 3°C was significantly lower than that of disks floated at 20°C.

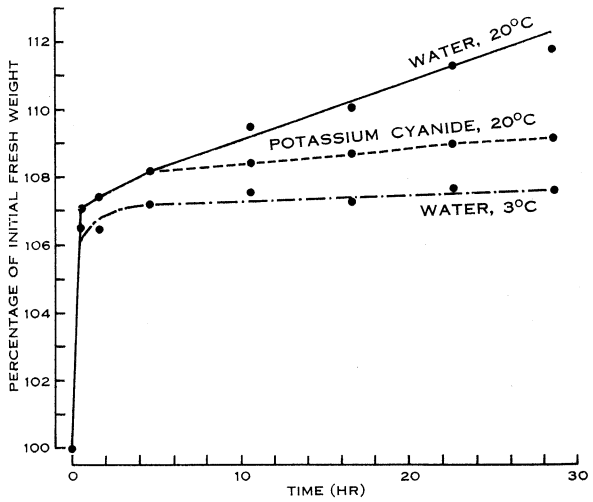

Fig. 3.—Effect of low temperature and 10^{-3}M potassium cyanide on change of fresh weight of floating leaf disks.

(*c*) *Effect of Metabolic Inhibitors on Changes in Fresh Weight during Floating*

In the previous section the somewhat surprising reduction in both phase I and phase II when disks were floated at 3°C was noted. It was decided to investigate further the action of metabolic inhibitors on changes in water content. Accordingly a comparison was made of the effects of low temperature and of potassium cyanide on water uptake. The cyanide was applied by floating disks on a 10^{-3}M potassium cyanide solution in the light at approximately the compensation point. A control set of disks was floated on water at 20°C.

Figure 3 shows both cyanide and low temperature virtually eliminated phase II, although the plateau attained by the low-temperature treatment was distinctly lower than that for the cyanide. This is confirmed by the results shown in Figure 4. The effect of this supression of part of phase I on estimates of relative turgidity could be appreciable. Thus, relative turgidities were measured for four sets of leaf disks previously equilibrated to the same water deficit by the technique of Weatherley

and Slatyer (1957), two of these sets were then floated at 20°C and gave values of of 60·8 and 61·5% compared with 69·8 and 70·5% for the two other sets floated at 3°C.

Finally the effect of anaerobic conditions was investigated. For this the apparatus consisted of shallow glass dishes fitted with conical tubulated lids. The dishes contained water and the air in the vessels was displaced by a stream of nitrogen. The leaf disks were held on a wire platform above the surface of the water until all the air had been displaced ($\frac{1}{2}$ hr), when the platform was lowered so that the disks floated on the water. In this way the disks could be deprived of oxygen from the first moment of floating. A duplicate apparatus through which only air

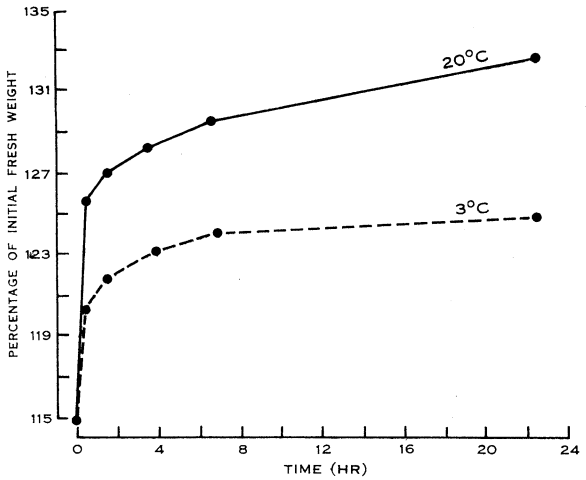

Fig. 4.—Effect of floating on water at 20 and 3°C on changes of fresh weight of leaf disks.

was passed served as a control. The rates of flow of the gases in the two chambers were kept very similar by the use of flow-meters. In order to minimize any drying effects the gas currents may have had on the disks, both the air and the nitrogen were saturated with water vapour by bubbling through long tubes filled with air-free water, before being admitted to the experimental chambers. Further details of this equipment are given elsewhere (Barrs 1959).

The effect of oxygen deprivation on phase I was assessed by comparing the change in fresh weight during the first hour of water uptake under aerobic and anaerobic conditions. The results of 10 such comparisons are collected in Table 1.

The average changes in fresh weight of 5·82 and 5·80% suggested the identity of the two uptakes within the limits of experimental error. This was confirmed by Student's t-test for paired samples, which showed there was no significant difference between the two treatments at the 0·05 level of probability ($t = 1{\cdot}218, t_{0.05} = 2{\cdot}262$). Hence nitrogen was without effect on phase I.

The effect of nitrogen on phase II was studied by floating the disks under air until phase I had been completed and phase II had been in progress for several

hours. Nitrogen was then passed for half an hour with the disks floating on the water, after which they were left under these anaerobic conditions for 12 hr, before being returned to aerobic conditions.

Figure 5 clearly shows how phase II was virtually reduced to zero under the anaerobic conditions. Return to aerobic conditions led to a marked increase in phase II above the original aerobic rate followed by a reduction to approximately the original rate. An interesting point is that this result could only be obtained in the absence of light. Illuminated disks showed no inhibition of phase II by nitrogen. Further work is planned in this connexion.

TABLE 1

CHANGES IN FRESH WEIGHT OF DISKS OF CASTOR-OIL LEAVES AFTER FLOATING ON WATER FOR 1 HR IN AN ATMOSPHERE OF AIR OR OF NITROGEN

Sample No.	Change in Fresh Weight in Air (%)	Change in Fresh Weight in Nitrogen (%)	Sample No.	Change in Fresh Weight in Air (%)	Change in Fresh Weight in Nitrogen (%)
1	6·22	6·22	6	5·55	5·15
2	7·12	6·63	7	4·97	5·34
3	6·87	7·14	8	6·20	6·18
4	6·92	6·86	9	4·71	5·01
5	4·87	4·71	10	4·72	4·80
Mean				5·82	5·80

It might be argued that observations on the effect of nitrogen on uptake in the first phase would have been better taken when the phase was nearer completion. However, Figure 2 shows that even after only 1 hr of floating over 80% of the phase I uptake has occurred, hence a large part of any metabolic-dependent uptake component of this phase would have been detected. Also, the relative turgidity of the disks in this experiment averaged 93% initially, and it seems unlikely that this water content would be low enough to cause material reduction in metabolic uptake had this in fact been present in phase I. Hence it seems reasonable to conclude that under anaerobic conditions phase II was eliminated whilst phase I remained unaffected.

IV. DISCUSSION

The relative turgidity technique consists in comparing the initial and the turgid water content of tissue on a percentage basis. In principle this is simple, in practice two possible classes of error must be taken into consideration, since dry weight may change during the period of floating and final water content may not be equal to fully turgid water content. Possible causes of the latter inequality include growth of the disks, injection of damaged tissue at the cut edges, plastic flow of cell walls in fully turgid tissue, and partially irreversible contraction of cell

walls in wilted tissue. Discussion will centre round the origin, importance, and, where necessary, ways of reducing these sources of error to acceptable levels.

Weatherley (1950) suggested that when leaf disks were floated on water the initial rapid uptake (phase I) was in response to the initial water deficit in the tissue, and that the prolonged slow uptake (phase II) was due to growth of the disks. If this is so then phase I might be expected to be relatively insensitive to metabolic inhibition compared with phase II. The experiments with inhibitors support this hypothesis.

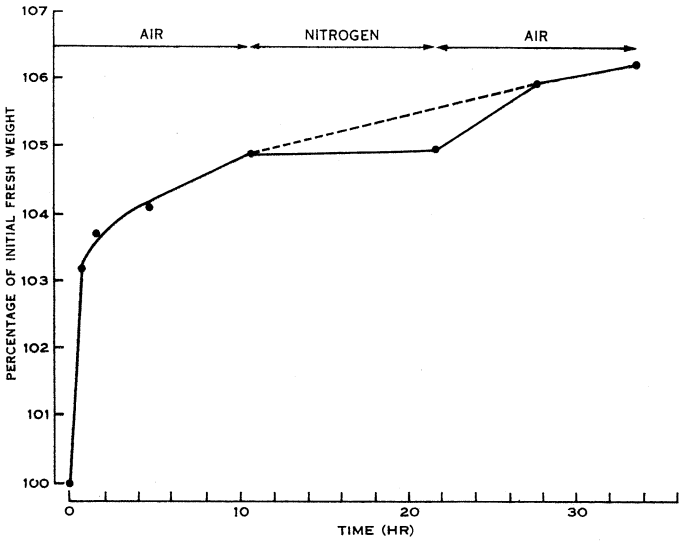

Fig. 5.—Effect of an atmosphere of nitrogen on changes of fresh weight of floating leaf disks.

All three inhibitors (low temperature, cyanide, and nitrogen) reduced phase II virtually to zero (Figs. 3 and 5) whereas phase I was unaffected by cyanide and nitrogen (Fig. 3 and Table 1). The reduction in phase I by low temperature was apparently anomalous; however, there is evidence (Weatherley 1962) that phase I may itself be divisible into two phases each of which probably represents separate regions of the cell. One of these is insensitive to temperature whilst entry of water into the other is completely stopped by a temperature a little above 0°C. Thus flotation of disks at 3°C leads to a saturation of one zone only, and this is represented by the low plateau obtained with this treatment (Fig. 4).

If it is accepted that phase II is due to growth but that phase I occurs simply in response to the initial water deficit of the tissue, than it is apparent that the key to relative turgidity measurement lies in an accurate assessment of the phase I uptake, unobscured by any phase II component. This leads to a consideration of whether phase II follows phase I or occurs simultaneously.

The experiments with nitrogen and cyanide indicate that the former is the case, phase II only commencing when phase I is virtually complete. Thus Table 1

shows that during the first hour of flotation uptake was no less under anaerobic than under aerobic conditions. Since demonstrably phase II is inhibited under anaerobic conditions it may be concluded that there was no phase II uptake during the first hour of flotation during which time the bulk of phase I uptake occurred. It will be recalled that the disks were held above the water for half an hour whilst nitrogen was passed through the vessels, so that it is very likely that any potential phase II uptake would have been inhibited from the very beginning of flotation. It might be argued that it would be difficult to detect an increment due to phase II after only 1 hr of floating; however, Figure 3 clearly shows that even after 4 hr the uptake by disks floated on 10^{-3}M potassium cyanide is equal to that of disks floated on water. Hence it is only after this time that phase II uptake is apparent.

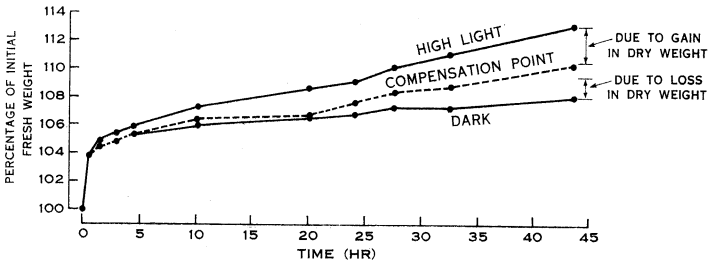

Fig. 6.—Effect of light intensity on the change of fresh weight of floating leaf disks with time.

The fact that phase II follows phase I renders the method of Yemm and Willis (1954) inadmissable. They eliminate uptake due to phase II by obtaining the slope of uptake during this phase and extrapolating back to zero time. This makes the tacit assumption that phase II starts from the beginning of flotation, thus their method will underestimate the turgid water content, Admittedly the error would usually be small, but the extra number of determinations their method requires in order to obtain the phase II slope is evidently gratuitous.

Dry weight changes during floating were shown in Figure 2 to be due to an imbalance between photosynthesis and respiration. Depending on the light intensity, disks appeared either to gain or to lose in dry weight, and at about 65 f.c. there was no change, i.e. this light intensity was approximately the compensation point. That the elimination of dry weight change in itself is not sufficient to ensure an accurate estimate of fully turgid water content is brought out in Figure 6.

Figure 6 shows the changes in fresh weight and in final dry weight of disks floated at a relatively high light intensity (120 f.c.), at the compensation point (65 f.c.), and in the dark. Original dry weights were established by oven drying parallel samples immediately after determining their initial fresh weights. For disks floated at the compensation point, fresh weight continued to increase with time. Clearly this was due to phase II uptake. Even in the case of disks floated in the dark there was a net increase in fresh weight due to phase II uptake exceeding dry weight losses. In the case where dry weight increased with time (high light intensity) there

did not seem to be any interaction between the increase in dry weight and phase II uptake, subtraction of the dry weight increase resulted in the percentage gain in fresh weight becoming very close to that of disks floated at the compensation point.

Since it has already been established that for accurate estimation of fully turgid water content phase II must be eliminated, and that this phase is only apparent after 4 hr of floating, the problem of dry weight change during the floating period is reduced to preventing significant dry weight change during 4 hr. In Sections III it was mentioned that sufficient variation in the compensation point was found to cause significant changes in the dry weight of some samples when a 24-hr floating period was used. However, when the floating period was reduced to 4 hr this variation became very small. This is shown in Table 2.

TABLE 2

COMPARISON OF RELATIVE TURGIDITIES BASED ON INITIAL AND FINAL DRY WEIGHT OF DISKS OF CASTOR-OIL LEAVES

Floating period 4 hr

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Initial dry weight (g)	0·0734	0·0756	0·0674	0·0661	0·0685	0·0804
Final dry weight (g)	0·0726	0·0748	0·0673	0·0655	0·0679	0·0798
Difference (g)	0·0008	0·0008	0·0001	0·0006	0·0006	0·0006
Relative turgidity using initial and final dry weight (%)	90·8	91·1	93·1	93·3	93·7	94·0
Relative turgidity using final dry weight only (%)	90·9	91·2	93·1	93·3	93·7	94·0
Difference (%)	0·1	0·1	0	0	0	0

In Table 2 also a comparison is made between relative turgidities obtained using both the initial and final dry weights and using the final dry weights only. It is apparent that the differences between these are negligible. Clearly, when disks are floated for only 4 hr at the approximate compensation point changes in dry weight are no longer large enough to be significant in the calculation of relative turgidity.

This reduction of the floating period from 24 to 4 hr at the approximate compensation point (65 f.c.) seems to be an improvement on Weatherley's (1950) original method since dry weight changes are eliminated and with them the necessity for the collection of a second sample. Also the inclusion of uptake due to phase II is virtually avoided, making the final water content a more accurate estimate of the fully turgid value.

The results of the experiments with potassium cyanide or nitrogen indicate that application of these inhibitors achieves the same ends. However, the extra labour and equipment required to expose the disks to nitrogen make the adoption

of this technique as a standard laboratory procedure impracticable. It is less troublesome to expose disks to cyanide during floating, but occasionally anomalous results again make this practice objectionable. Under these conditions fresh weights occasionally rose abnormally high or declined to levels below the initial value.

The applicability of a reduced floating period has not been investigated further in this paper because there already exists in the literature a considerable body of evidence that satisfactory results are obtained for a wide range of material under

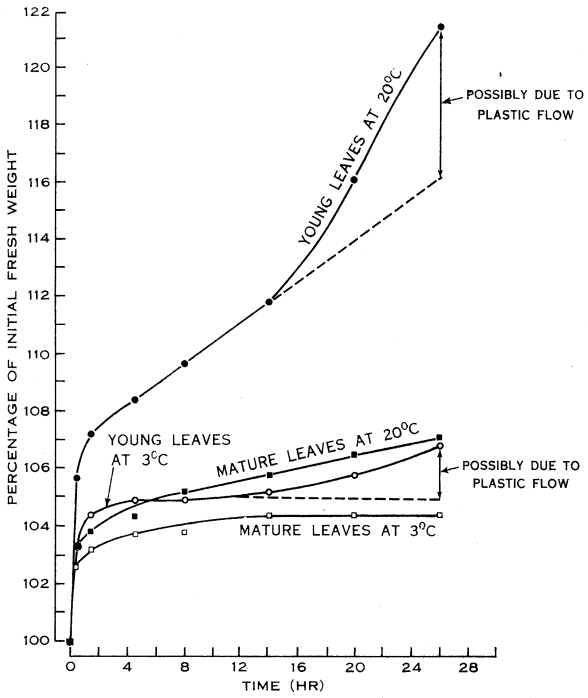

Fig. 7.—Effect of floating leaf disks on water at 20 and 3°C on changes in fresh weight of young and mature tissue.

these conditions. Thus, Bliss, Kramer, and Wolf (1957) found that tobacco leaf disks reached full turgor after floating for 4 hr. Catsky (1959, 1960) again using tobacco leaf found a 3-hr period satisfactory. Namken and Lemon (1960) report that disks from corn leaves reached full turgor after floating for 4 hr. Wormer and Ochs (1959) found that peanut leaf disks attained full turgor in 1 hr. Carr and Gaff (1959) considered disks from the juvenile leaves of *Eucalyptus globulus* (Labill.) to have reached full turgor after floating on water for 2 hr. Finally, reference may be made to the uptake curve for cotton leaf disks given by Weatherley (1950) in the paper in which the relative turgidity technique is first suggested. The curve shows that phase I is completed after 4 hr of floating.

Milthorpe and Spencer (1957) suggested that the relative turgidity technique could be in error as a result of changes in cell volume due to irreversible changes

in the cell wall. Their work led them to conclude that in fully turgid cells the cell wall stretched due to a process they termed "plastic flow" and that during wilting a partially irreversible contraction of the cell wall occurred.

If plastic flow is the cause of phase II uptake it is of little technical importance since the error it introduces is avoided as outlined above. However, the term plastic flow suggests a passive process which should be little affected by a change to anaerobic conditions or metabolic inhibition. Phase II therefore would not appear to be due to plastic flow in the cell walls, and moreover the fact that a flat plateau in the water content curve is obtained under anaerobic conditions and in the presence of cyanide indicates that plastic flow does not occur. An exception was found in the behaviour of disks punched from young leaves which were not fully expanded. Figure 7 shows curves of fresh weight change for disks from young leaves floated at 20 and 3°C compared with disks from mature expanded leaves at 20 and 3°C.

After some 10 hr of floating the young disks at 20°C showed a marked positive departure from phase II. A similar though smaller effect occurred for young disks at 3°C. Since this effect persisted at 3°C it could well be termed a plastic flow. This was the nearest approach to a plastic flow that could be detected. Such an effect is easily avoided by restricting sampling to mature fully expanded leaves, and in any case it is not manifest until considerably after the 4-hr period to which it is proposed to limit floating. It is apparent that plastic flow is not a significant source of error in the relative turgidity technique.

The criticism, that partial irreversible contraction of cell walls on wilting might lead to an underestimate of the turgid water content and thus constitute an error in relative turgidity estimation, is surely unwarranted. If during wilting changes in the cell wall occur which make them less extensible so that the water content at full turgor is less than it was before wilting, this change *should* be reflected by a rise in relative turgidity. For although the water content might remain constant during the period of wilting, the supposed changes in the cell walls will constitute a form of "recovery" in that the relative turgidity/diffusion pressure deficit relationship will have altered and the water deficit lessened.

The possible existence of such a change in the walls was investigated directly by comparing the water uptake of tissue before and after inducing a considerable deficit. It was assumed that, should an appreciable contraction occur, the subsequent uptake would be demonstrably below the original amount. Disks from well-watered plants were floated at 20 and 3°C for 22 hr in order to establish their uptake curves. At the end of this time they were removed from the water and exposed to the atmosphere of the laboratory for 20 min; after inducing a considerable deficit in this way they were returned to water and the subsequent uptake followed.

Figure 8 shows that complete recovery occurred. At 3°C the water content returned to the same level as before dehydration, whilst with 20°C flotation the post-dehydration curve was paralled to, but slightly below, the pre-dehydration line extrapolated. This is according to expectation if phase II ceased during the period of dehydration and the first hour or two of re-uptake. It will be seen that after 4 hr of re-uptake the fresh weight was almost equal to the value just before

dehydration. This, incidentally, is added evidence that phase II does not occur during phase I. The experimental procedure used is perhaps objectionable in that the drying of the tissue was far more rapid than would occur naturally and the conditions under which drying took place were not well controlled. However, slow dehydration of the disks to a known reproducible level under the closely controlled conditions of the vapour equilibration technique of Weatherley and Slatyer (1957) followed by floating on water gave closely similar results. Similar turgid water contents were attained in all cases, even when disks had been dried initially to relative water contents as low as 50% which is far below the wilting point. It is concluded that for *Ricinus* irreversible contraction of the cell walls does not constitute an important source of error in the technique.

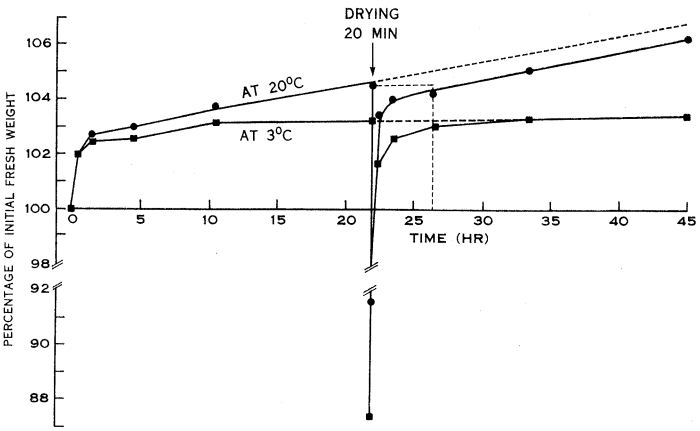

Fig. 8.—Effect of a short period of drying on water uptake by leaf disks floated at 20 and 3°C.

Whilst errors due to the injection of the cut edges of floating leaf disks were not important for cotton leaves (Weatherley 1950), it was thought desirable to assess the importance of this source of error in castor-oil leaves. In the present work the technique was somewhat different from that used on cotton leaves and involved a comparison of the percentage uptake of disks of $\frac{3}{4}$ and $\frac{7}{16}$ in. diameter. Since the former have a lower circumference : area ratio than the latter and injection is limited to the circumfrence, the uptake of the smaller disks should be relatively greater if injection at the cut edge did contribute materially to the uptake. Paired samples of smaller and larger disks were taken from the same material and floated for 24 hr, at the end of this time their changes in fresh weight were compared.

Table 3 gives the results obtained with *Ricinus* and *Sambucus*. For *Ricinus* the mean values indicate that the smaller disks did in fact take up relatively more water, but the result is only barely significant (Student's t-test: $t = 2{\cdot}16$, $t_{0.05} = 2{\cdot}11$) and the amount involved would not constitute a serious error. However, marginal injection can lead to large errors with some species. Thus, the results obtained with *S. nigra* show a much larger and highly significant effect (Student's t-test:

$t = 3{\cdot}04$, $t_{0.02} = 2{\cdot}82$). Some care should therefore be exercised in the choice of material for use with the relative turgidity technique, to ensure that marginal injection will not be an important component of water uptake.

This source of error has been investigated by other workers for a range of leaf tissue. Thus Rutter and Sands (1958) report the injection error in *Pinus sylvestris* (L.) to be negligible. Carr and Gaff (1959) found a small and apparently

TABLE 3

PERCENTAGE CHANGES IN FRESH WEIGHT BY SMALL AND LARGE DISKS OF RICINUS COMMUNIS AND SAMBUCUS NIGRA FLOATING ON WATER

Ricinus communis			*Sambucus nigra**		
Sample No.	Disk Diameter		Sample No.	Disk Diameter	
	$\frac{7}{16}$ in.	$\frac{3}{4}$ in.		$\frac{7}{16}$ in.	$\frac{3}{4}$ in.
1	3·78	3·10	1	7·05	6·37
2	2·52	2·64	2	5·19	3·84
3	3·41	3·84	3	6·64	4·78
4	3·73	3·61	4	5·21	4·55
5	6·25	5·09	5	7·53	6·99
6	4·98	3·84	6	8·84	7·21
7	4·55	4·42	7	7·35	5·61
8	4·15	4·45	8	6·76	6·28
9	3·15	4·00	9	7·57	5·10
10	6·81	6·67	10	5·68	6·38
11	6·68	6·98			
12	9·90	9·14			
13	6·00	5·00			
14	5·38	4·67			
15	4·86	5·35			
16	7·06	6·56			
17	7·84	6·84			
18	11·54	9·96			
Mean	5·70	5·34		6·78	5·71

* Fewer samples were taken for *Sambucus* because a smaller number were required to obtain a significant result.

constant injection error in *Eucalyptus globulus* which they considered could be taken into account by reducing the uptake per 8-mm disk by 0·4 mg/disk. Catsky (1960) found that the injection error in tobacco leaves was reduced by exposing the disk in such a way that only their cut edges were in contact with a free water surface. This was achieved by exposing disks in cylindrical holes in wet spongy polyurethane.

In general, an error due to injection is readily detectable as a much darker area of the leaf disk. For the materials used in the papers already cited the relative turgidity technique appears to be satisfactory in this respect. The present paper

adds one more species to this list, and one to the list of unsatisfactory material. A simple method of estimating the importance of this error (i.e. comparison of uptakes by large and small disks) is given and it is thought that this is of greater utility to any potential user of the technique than a list of species which have a low injection error. In view of the very wide range of plant material which has been used it would be difficult to draw up a list even approaching the degree of comprehensiveness that might be useful.

V. Conclusion

A revised form of the technique may be restated. Disks should be floated at constant temperature for 4 hr at the approximate compensation point (65 f.c.). It is then not necessary to estimate initial dry weight from a parallel disk sample. A subsequent period of oven drying of 1 hr at 90°C is sufficiently long to dry the disks to constant weight. This method permits the evaluation of relative turgidity in 5 hr as against 48 hr as required in the original procedure. In some circumstances, e.g. where relative turgidity estimates are being used to assess irrigation requirements, the earlier availability of the estimate may well be useful.

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