THE EFFECT OF WATER STRESS ON THE MOVEMENT OF [^{14}C]SUCROSE AND OF TRITIATED WATER WITHIN THE SUPPLY LEAF OF YOUNG BEAN PLANTS

By Z. PLAUT* and LEONORA REINHOLD†

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Summary

When [^{14}C]sucrose was applied to one of the primary leaves of bean plants and the small application site subsequently removed, far higher amounts of alcohol-soluble ^{14}C were detected in the leaves of plants under water stress than in controls. The effect could not be accounted for by translocation out of the leaf, nor by synthesis of sucrose into non-alcohol-soluble compounds, nor by respiration. Increased movement of ^{14}C out of the supply area in stressed leaves appeared to be responsible, and the mechanism of [^{14}C]sucrose transport within the leaf was therefore investigated.

Subdivision of leaves into sections at brief intervals after ^{14}C application showed that movement of sugar from the treated area was not random, but was directed towards the midvein. This pattern was independent of moisture tension. Radioautographs supported this conclusion, indicating that in both stressed and control leaves labelled sugar was not dispersed through the mesophyll but passed from the application site down a sidevein into the midvein.

The effect of stress on the movement of THO in the leaf was the opposite of that on [^{14}C]sucrose, i.e. far less THO was recovered from stressed leaves after removal of the supply area. Accelerated translocation of THO out of the leaf did not account for this result. Nor did exchange with the atmosphere, since the transpiration rate of the control was at least seven times that of the stressed plants. The gradient in specific activity of THO across the leaf was considerably steeper for control than for stressed leaves.

A steam girdle round the stem drastically reduced ^{14}C movement in the leaf in both stressed and control plants. THO movement did not appear to be affected by the girdle.

On the basis of the above results it was concluded that movement of applied sugar in the leaf, even over short distances from the application site, was not by capillary flow but involved phloem transport; and that one or more phases of this transport were stimulated by water stress.

I. INTRODUCTION

During a recent study of the influence of water stress on the transport of [^{14}C]sucrose in bean plants (Plaut and Reinhold 1965) we noted a striking effect on the behaviour of the ^{14}C-treated leaf. After removal of the small ringed area where the drop of labelled sucrose solution had been applied, leaves of stressed plants were found to contain far higher amounts of alcohol-soluble ^{14}C than was the case with

* Botany Department, Hebrew University of Jerusalem, and Volcani Institute of Agricultural Research, Rehovot, Israel; present address: Department of Biology, Brandeis University, Waltham, Mass.

† Botany Department, Hebrew University of Jerusalem, Israel.

controls. Different rates of translocation of [^{14}C]sucrose out of the leaf did not account for this effect in the large majority of experiments. Nor was the effect due to different rates of synthesis of sucrose into starch or other non-alcohol-soluble substances, since it was found that during the first 3 hr after ^{14}C supply very little synthesis occurred, and no significant difference could be detected in this respect between stressed leaves and controls. It therefore seemed likely that in plants under stress much more sugar had moved out of the treated area into the rest of the leaf blade. The present paper reports a more detailed investigation into the nature of this effect.

II. Methods

(*a*) *Application and Analysis of Labelled Sucrose*

For details regarding the conditions of growth of the bean plants used in this investigation (*Phaseolus vulgaris* cv. Brittle Wax) and the irrigation treatments see Plaut and Reinhold (1965). Uniformly labelled [^{14}C]sucrose solution (specific activity 10–13 mc/m-mole sucrose), containing 500 p.p.m. of sodium lauryl sulphate and 25 p.p.m. boric acid, was applied as a 0·01 ml drop to the lower surface of a primary leaf within an area defined by a tygon ring, about 10 mm to one side of the midvein. The ring was covered with transparent tape to reduce evaporation. After the desired translocation period (light intensity 1000 f.c., temperature 25°C) the leaf area within the ring was removed with a punch and discarded. The rest of the plant was divided into sections which were treated and analysed as described by Plaut and Reinhold (1965). Since the stressed leaf effect under investigation was prominent both when the translocation period was relatively short (15–30 min) and when it was of considerably longer duration (45 min–15 hr—see Plaut and Reinhold 1965), short translocation periods were preferentially used in most of the experiments. All experiments were carried out in quadruplicate, each replicate being analysed individually.

(*b*) *Radioautography*

After removal of the ^{14}C-treated area within the ring, in certain experiments leaves were frozen between two blocks of solid CO_2 for 15 sec, then placed between two perforated aluminium plates in a freeze-drying apparatus at −5°C. Radioautographs were subsequently prepared from the freeze-dried leaves using standard X-ray film (Kodak) and an exposure time of 8 days.

(*c*) *THO Application and Analysis*

In certain experiments the [^{14}C]sucrose was dissolved in tritiated water of specific activity 150 μc/ml. The solution was applied to the leaves as described in Section II(*a*). The plants were divided into sections as usual [Raney and Vaadia (1965) showed that THO content was unaffected by conditions of cutting] and the segments were stored in closed tubes at −10°C. Their water content was subsequently distilled in a high-vacuum apparatus (vapour pressure 2×10^{-3} mmHg) for 3 hr. The volume of the distillate was measured and its radioactivity determined in a Packard liquid scintillation counter using a dioxan–naphthalene solvent system

containing 0·75% PPO and 0·03% POPOP. Methanol (2%) was also added to prevent freezing. Quenching was always below 5%. In the tables radioactivity is expressed as specific activity.

(*d*) *Relative Turgidity*

Relative turgidity was measured by the method of Barrs and Weatherley (1962).

(*e*) *Steam Girdling*

Steam was applied to a 1 cm band of stem immediately below the primary leaf node for 1 min, 60 min before application of [^{14}C]sucrose to the leaf.

(*f*) *Respiration*

Plants were placed in glass vessels through which CO_2-free air streamed continuously at the rate of 1 litre/min. The relative humidity in the vessels was 40–60%. After 10 min, [^{14}C]sucrose was applied to a leaf. Respiratory CO_2 was absorbed in a series of 0·05N KOH traps, then precipitated with $BaCl_2$ and counted in a gas-flow counter. Total CO_2 evolved was determined by back-titration of the KOH.

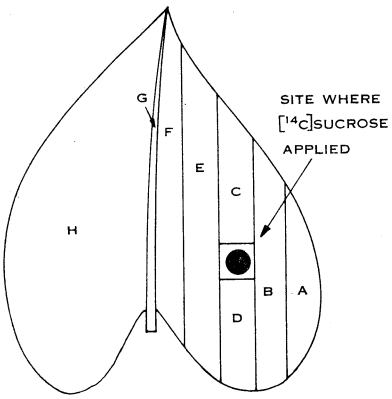


Fig. 1.—Diagram showing sections into which the bean leaf was divided in the experiment summarized in Table 1.

III. Results

(*a*) *Distribution of* ^{14}*C within the* [^{14}*C*]*Sucrose-treated Leaf*

One possible explanation for the "stressed leaf effect" (i.e. for the fact that far more ^{14}C seemed to move out of the ringed area on the supply leaf in stressed plants than in controls) was that the dry leaf tissues in the former case might act as wicks, bringing about a mass flow of labelled sugar solution from the treated area. A flow of this kind would presumably bring about a random distribution of ^{14}C in all directions from the treated spot. The following experiment was carried out to check whether this was in fact obtained.

At various intervals after [^{14}C]sucrose application to the primary leaves of two groups of plants, one stressed and one control, the treated leaves were cut into seven pieces as indicated in Figure 1 and analysed. Table 1 shows that the amount of ^{14}C which had moved in the direction of the midvein, or which had already reached the latter, was much greater than the amount which moved

in the opposite direction. At least 50% of the total ^{14}C recovered from the leaf was in fractions E, F, and G, whereas only about 10% was in A and B. Water stress did not affect this distribution significantly, nor did duration of ^{14}C supply.

TABLE 1

DISTRIBUTION OF ALCOHOL-SOLUBLE ^{14}C WITHIN PRIMARY LEAF OF BEAN PLANTS AT VARIOUS TIMES AFTER APPLICATION OF [^{14}C]SUCROSE

For location of leaf sections A–G, see Figure 1. The experiment was performed in quadruplicate

Translocation Period (min)	Treatment	^{14}C as % of Total ^{14}C in Leaf Sections							Total ^{14}C in Sections A–G (counts/min)
		A	B	C	D	E	F	G	
15	Stressed	0·5	12·2	28·5	7·5	13·8	13·0	24·6	8,130
	Control	0	5·8	22·9	7·0	25·2	12·7	26·5	2,360
30	Stressed	1·2	10·2	18·8	14·1	14·8	15·9	25·1	8,070
	Control	0·7	6·1	20·9	22·3	11·8	15·5	22·8	1,451
Standard error		0·5	4·9	12·7	7·1	4·5	3·8	7·0	1,590

An analysis of variance of the values for absolute number of counts, made according to a split-plot design (main treatments water regime and translocation period, subtreatment direction of movement), showed no significant interaction between

TABLE 2

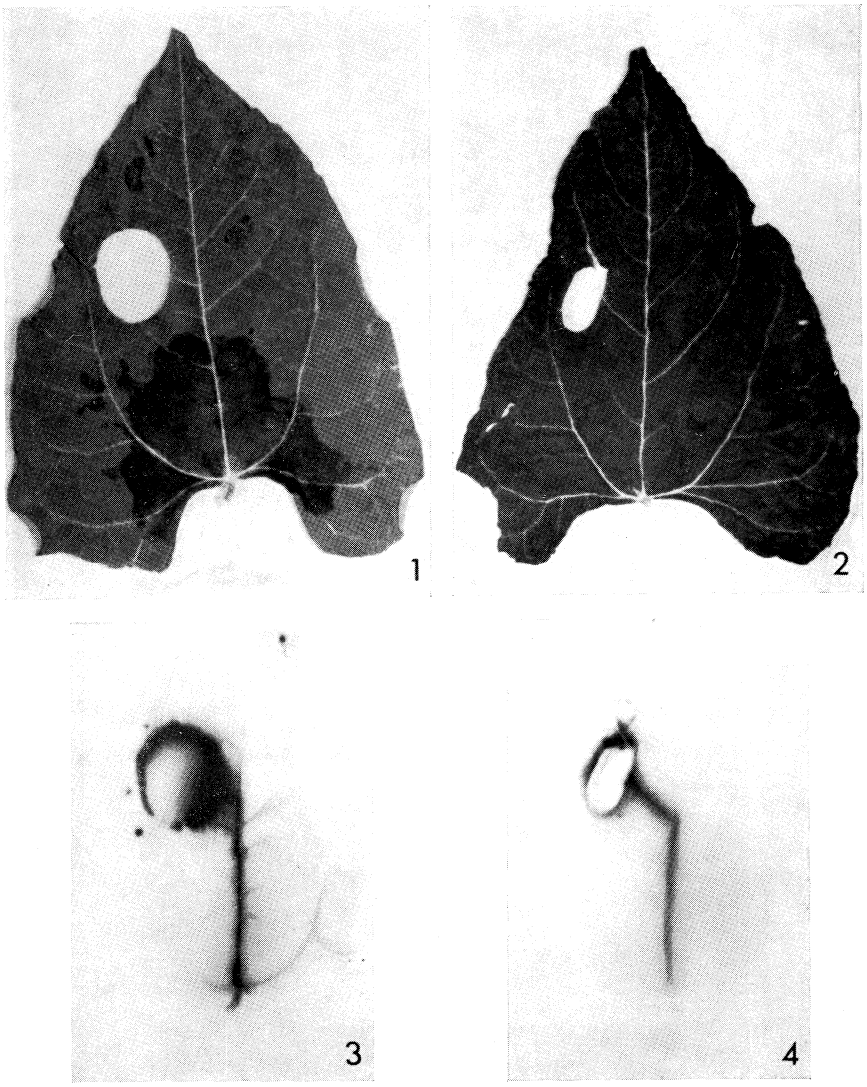
ALCOHOL-SOLUBLE ^{14}C PRESENT IN RING OF TISSUE 3 MM WIDE SURROUNDING APPLICATION SITE AND IN OTHER PLANT PARTS 30 MIN AFTER SUPPLY OF [^{14}C]SUCROSE TO PRIMARY LEAVES OF STRESSED AND CONTROL BEAN PLANTS

The experiment was performed in quadruplicate

1	2	3	4	5
Treatment	^{14}C in Ring as % of Total ^{14}C in Blade	^{14}C in Leaf Blade including Ring (counts/min)	^{14}C in Midvein (counts/min)	^{14}C in Rest of Plant (counts/min)
Stressed	41·5	36,080	662	1,846
Control	34·8	1,030	87	158
Standard error	17·5	11,900	247	648

stress and direction of movement, or between translocation period and direction of movement. On the other hand, the differences between the various leaf parts were highly significant for both translocation periods. It may thus be concluded that the movement of ^{14}C from the treated area was not random in all directions.

MOVEMENT OF LABELLED COMPOUNDS IN BEAN PLANT



Figs. 1 and 2.—Photographs of the primary leaf of a stressed (Fig. 1) and a control (Fig. 2) bean plant. The site of application of [^{14}C]sucrose has been removed.

Figs. 3 and 4.—Radioautographs of the primary leaf of the stressed and control plant, respectively, shown in Figures 1 and 2, 30 min after the application of [^{14}C]sucrose. Exposure time 8 days.

The distribution of ^{14}C within the treated leaf was also inspected by means of radioautography. The leaves of eight stressed and eight control plants were supplied with [^{14}C]sucrose for 30 min and then prepared for radioautography as described in Section II(*b*). A typical radioautograph from each group is shown in Plate 1, Figures 3 and 4. It will be noted that in neither case is the radioactivity dispersed throughout the blade—^{14}C appears to flow down a side vein directly into the midvein. In the stressed leaf radioactivity is also detectable within veins on the opposite side of the midvein, though in very low amounts. The radioautographs

TABLE 3

EFFECT OF A PREVIOUSLY APPLIED STEAM GIRDLE ON THE DISTRIBUTION OF ALCOHOL-SOLUBLE ^{14}C THROUGH STRESSED AND NORMAL BEAN PLANTS AT VARIOUS INTERVALS AFTER APPLICATION OF [^{14}C]SUCROSE TO PRIMARY LEAF

The experiment was performed in quadruplicate

1	2	3	4	5	6	7	8
		^{14}C in Girdled Plant as % of Value for Non-girdled Plant					
Translocation Period (min)	Treatment	Treated Leaf*	Midvein	Petiole	Second Internode and Growing Point	First Internode plus Hypocotyl	Root
15	Stressed	0·3	5·7	4·4	0·9	3·2	26·7
	Control	3·5	11·2	15·5	50·0	78·4	48·1
30	Stressed	2·6	6·6	9·1	11·9	34·8	27·9
	Control	37·7	0·9	15·5	21·8	60·8	48·2
180	Stressed	20·9	26·3	9·1	†	1·7	†
	Control	65·7	71·7	50·9	23·6	4·6	0·5

* Without punched area. † Count not significantly different from background.

of the stressed leaves were all blacker than those of the controls, suggesting a higher concentration of ^{14}C. In particular, the immediate vicinity of the site of ^{14}C application was much darker in the case of the stressed leaves, especially on the midrib side (see Plate 1, Fig. 1). This result raised the possibility that the high amounts of ^{14}C characteristically recovered from stressed leaf blades might to a large extent be located within 2–3 mm of the site of application.

A further experiment was therefore performed in which, after 30 min of ^{14}C supply, a ring of tissue 3 mm wide surrounding the punched area was removed from the leaf and analysed. Results for this sample as well as for other plant parts are given in Table 2. Column 3 shows that the stressed leaf effect was marked in this experiment, i.e. the amount of ^{14}C in the stressed leaf blades was many times the control value. Column 2, on the other hand, shows that only about 40% of the ^{14}C

in the leaf was found in the 3-mm ring surrounding the application site, and that this percentage was approximately the same for both stressed and control plants. It thus appears that the stressed leaf effect cannot be explained on the basis of enhanced entry of ^{14}C into a band of tissue a few millimetres wide surrounding the punched area.

(*b*) *Effect of Prior Steam Girdling on* ^{14}C *Movement*

Table 3 gives the results of two experiments carried out to investigate the effect of steam girdling the stem on ^{14}C movement. In this table the absolute radioactivity detected in each fraction of the girdled plants is expressed as a percentage of that in the appropriate non-girdled control. It will be seen that the girdle drastically reduced translocation, not only to those plant parts below the girdle (columns 7 and 8), but also to all the plant parts analysed, including the treated leaf blade. A similar pattern of results was obtained whether the translocation period was 15 min, 30 min, or 3 hr, though the effect of the girdle was less marked after 3 hr. The fact that movement away from the treated spot into the rest of the leaf blade was inhibited to the same or to an even greater extent than was transport in the stem suggests that the mechanism of movement within the leaf resembles that in the stem and is probably phloem transport. It may be noted that the girdle was apparently even more effective under conditions of water stress.

(*c*) *Simultaneous Movement of THO and of* [^{14}C]*Sucrose*

A further approach to the problem of the nature of ^{14}C movement in the leaf involved the use of tritiated water as solvent for the labelled sugar. If the stressed leaf effect on sugar movement were due to some form of mass flow, the amount of THO, like that of ^{14}C, should be greater in stressed leaves. In a preliminary experiment the interesting result was obtained that while the values for ^{14}C were 64,200 and 813 for stressed and control leaves respectively (S.E. 18,600), the THO analyses showed exactly the opposite effect of stress: the values for specific activity of tritium were 16,900 and 64,900 respectively (S.E. 10,500). The possibility suggested itself that the lower value for THO in the stressed leaves might be due, not to slower movement of water out of the treated spot, but, on the contrary, to accelerated translocation out of the treated leaf. A further experiment was therefore performed which included complete analyses of the other parts of the plant (Table 4).

Columns 3 and 4 (Table 4) show that again considerably more THO was recovered from the blades of control leaves, while as usual the opposite effect was seen for ^{14}C (column 3). Total THO in the fractions midvein to root (column 11) was slightly higher in the stressed plants, but the difference was not significant and was in any case too small to account for the very large discrepancy between the values for stressed and control leaf blades, a discrepancy of 49,000 counts/min (see columns 3 and 4). This discrepancy would be even greater if exchange with the atmosphere were taken into account, since the transpiration rate of the control plants was 7–10 mg water/cm^2 leaf area/hr, as compared with less than 1 mg water in the case of stressed plants. While translocation of ^{14}C to most other plant parts was inhibited by stress (see columns 5–10 and especially column 12) the translocation

TABLE 4

INFLUENCE OF WATER STRESS ON THE DISTRIBUTION OF ALCOHOL-SOLUBLE ^{14}C AND OF THO THROUGH BEAN PLANTS 30 MIN AFTER APPLICATION OF [^{14}C]SUCROSE DISSOLVED IN TRITIATED WATER TO PRIMARY LEAF

The experiment was performed in quadruplicate

1	2	3	4	5	6	7	8	9	10	11	12	13
		Radioactivity (counts/min)* in:										
Isotope Analysed	Treatment	Treated Half of Leaf Blade	Opposite Half of Leaf Blade	Midvein	Petiole	Opposite Leaf	Second Internode and Growing Point	First Internode plus Hypocotyl	Root	Total Radio-activity Midvein to Root (counts/min)	^{14}C or ^{3}H Trans-located (as % of total ^{14}C or ^{3}H in plant)	Soil Moisture Tension (atm)
^{14}C	Stressed	44,700	140	43	6	150	78	31	106	265	4·0	15·0
	Control	555	190	215	67	124	79	112	198	669	47·2	0·2
Standard error		14,700	52	54	17	56	17	21	52	94	9·6	
^{3}H	Stressed	5,700	3,200	5,160	2,590	1,300	530	850	970	11,400	38·6	15·0
	Control	48,400	9,340	5,410	1,590	770	250	250	230	7,750	14·4	0·2
Standard error		9,600	2,020	1,200	615	330	125	129	295	1,380	2·6	

* Expressed as counts/min/ml water in tritium assay.

of THO was if anything stimulated (column 12). Also the gradient in specific activity of THO from the treated to the opposite half of the blade was steeper in the case of control plants (columns 3 and 4). As a consequence the ratio of THO in stressed to that in control plants fell from 1 : 8 for the treated half blade (column 3) to 1 : 3 for the opposite half (column 4). In the midvein (column 5) the ratio was 1 : 1.

The effect of steam girdling the stem on the simultaneous movement of THO and of [^{14}C]sucrose in the leaf may be seen in Table 5. Girdling again drastically inhibited ^{14}C movement in the leaves of both stressed and control plants. On the other hand only a slight effect was observed on THO movement. Apart from yielding the information summarized in Table 5, this experiment again confirmed the opposite effects of stress on ^{14}C and on THO recovery as indicated in the following tabulation:

^{14}C, 17,300 counts/min for stressed leaves, 8,400 for control, standard error 4,000;
THO, 5,200 counts/min for stressed leaves, 58,400 for control, standard error 9,400.

The earlier finding with regard to the steeper gradient in specific activity of THO in control plants was also confirmed, the ratios of stressed to control being 1 : 11, 1 : 9, and 1 : 3 for treated half of blade, opposite half, and midvein respectively.

TABLE 5

EFFECT OF A PREVIOUSLY APPLIED STEAM GIRDLE ON THE DISTRIBUTION OF ALCOHOL-SOLUBLE ^{14}C AND OF THO WITHIN TREATED LEAVES OF STRESSED AND NORMAL BEAN PLANTS 30 MIN AFTER APPLICATION OF [^{14}C]SUCROSE DISSOLVED IN THO

The experiment was performed in quadruplicate

Isotope	Treatment	Radioactivity in Girdled Plants as % of Value for Non-girdled Plant		
		Treated Half of Blade	Opposite Half of Blade	Midvein
^{14}C	Stressed	29·1	12·3	1·6
	Control	15·8	11·4	17·5
^{3}H	Stressed	92·2	134·2	63·2
	Control	84·4	98·0	105·0

(*d*) *Evolution of $^{14}CO_2$ by Stressed and Control Plants*

The possibility that respiration of $^{14}CO_2$ was playing an important part in the stressed leaf effect was investigated directly in several experiments. Respiratory CO_2 was collected over a 30-min period as described in Section II(*f*) and precipitated as $BaCO_3$. If the discrepancy between ^{14}C content of control and stressed leaves had been due in large measure to the respiration of $^{14}CO_2$ by the former, a count of several thousand per minute would have been expected for the $BaCO_3$ precipitate in the case of control plants, since precipitates were near "infinite thinness". In fact, counts of maximum 50% above background were obtained. It is therefore unlikely that respiration was a major factor in the stressed leaf effect.

IV. Discussion

Movement of sugar away from the ringed supply area on the leaf might be envisaged as resulting from two types of process: purely physical, e.g. by capillary movement within the cell walls and intercellular spaces; or physiological, e.g. by some specific transport mechanism. The dark ring surrounding the punched area on the radioautographs suggests that there may in fact have been capillary movement in stressed plants, but that this only occurred over a very short distance (about 2 mm) possibly due to the fact that only a very small volume of solution was supplied.

Most of the results obtained in this investigation argue against passive capillary movement and suggest that the principal processes involved were physiological. When, for instance, tritiated water was used as the solvent for the labelled sugar, the effect of stress on water movement was seen to be the opposite of that on the movement of sugar. While the amount of ^{14}C recovered from the leaf blade, after removal of the ringed area, was as usual higher in stressed plants, the opposite was true for THO [Table 4; Section III(*c*)]. Accelerated translocation of THO out of the leaf did not account for this discrepancy.

Furthermore, movement of sugar out of the treated area was not random in all directions, but was clearly directed towards the midvein (Table 1). Supporting evidence is provided by the radioautographs (Plate 1), which show that labelled sugar moved from the application site principally in one or two veins in the direction of the midrib. As for the dark ring suggesting a higher concentration of ^{14}C in a 3-mm band surrounding the punched area in stressed leaves, analyses showed that only 40% of the ^{14}C in the leaf blade was located in this band, and that this proportion was the same for both control and stressed leaves, and thus cannot be responsible for the stressed leaf effect.

The fact that ^{14}C was detectable in veins on the opposite side of the midrib in radioautographs of stressed leaves raises the possibility that the effect was due to xylem movement. The opposite effects of stress on ^{14}C and on THO movement, however, seem to dispose of the possibility, since both substances would presumably move together through the xylem.

Strong supporting evidence that the passage of sugar out of the treated area of the leaf is indeed by phloem transport may be seen in the fact that a steam girdle round the stem reduced ^{14}C movement in the leaf to at least the same extent that it inhibited movement into the plant parts below the girdle (Table 3). The girdling effect was even more drastic in stressed than in control leaves, again indicating that the stressed leaf effect was not due to passive flow under capillary forces. It is of interest that THO transport was not reduced by the steam girdle (Table 5).

The conclusion thus appears to be that movement of ^{14}C in the leaf, even over short distances from the site of application, is phloem transport; and that one or more steps in this transport are stimulated by water stress. The stimulated step might be uptake of sugar by the phloem parenchyma, or its secretion into the sieve tubes. Assuming that the pressure flow hypothesis for phloem transport is valid, the fact that translocation occurs even under wilting conditions (Plaut and

Reinhold 1965) indicates that vigorous sugar secretion must take place in stressed leaves in order to build up the necessary osmotic pressure in the sieve tubes. Weatherley, Peel, and Hill (1959) showed that secretion of sugar into the sieve tubes can be maintained even when the vacuoles of the latter contain 50% sucrose.

On the other hand, the stressed leaf effect might be due to a stimulation of transport within the sieve tubes themselves. It would be reasonable to connect such an effect with the stimulating influence of stress on ^{14}C transport to the lower plant parts previously observed when translocation periods were shorter than 45 min (Plaut and Reinhold 1965). It is of interest that in the case of the leaf effect stimulation is also observed when the translocation period is well in excess of 45 min (see Plaut and Reinhold 1965).

Wilson and McKell (1961) reported a decreased uptake of ^{32}P by sunflower leaves under water stress. Since they did not punch out the area where the radioactive droplet was applied, as we did, but determined uptake after washing the leaf surface, a very large proportion of the ^{32}P recovered from the leaf will have been in the area to which the drop was applied and any effect on movement away from this area will thus have been masked.

With regard to the effects observed for tritiated water, a considerable amount of THO would be expected to leave the leaf in the course of transpiration. The transpiration rate, however, was 7–10 times higher in the control plants. If transpiration were taken into account, therefore, the inhibiting effect of stress on THO movement in the leaf would be even more marked.

Water movement through the leaf may take place largely in the cell walls (see Weatherley 1963). The reduced movement of THO in stressed leaves might thus be due to the lower water conductance of dry cell walls owing to the interruption of water columns in the capillaries, or to the shrinkage of capillary space. Moreover, adsorption of water by dry cell walls (or absorption by dehydrated protoplasts along the path) would leave less THO free to move through stressed leaves. Whether the applied THO did in fact move largely through the cell walls must remain in doubt, however, because of the opposite effect of stress on sugar movement. Capillary movement through cell walls would be expected to involve the whole solution.

The fact that eight times as much THO was detected in the treated halves of control leaves as compared with stressed, and only three times as much in the opposite halves of the blades [see Section II(*c*)] suggests that movement over long distances through the blades was less affected by stress than movement over short distances. This may indicate that more than one type of water movement was involved.

V. Acknowledgment

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