

TRANSLOCATION OF LABELLED ASSIMILATES IN THE SOYBEAN

II. THE PATTERN OF TRANSLOCATION IN INTACT AND DEFOLIATED PLANTS

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Summary

The distribution of ^{14}C -labelled assimilate in the soybean has been studied, emphasis being placed on the expanding leaf as the major apical sink. The expanding leaf imports assimilate from leaves below, this import rising to a maximum and then falling to almost zero when the leaf is half-expanded. At this stage in the growth of the leaf, it has begun exporting assimilate to the younger leaves. Export, both to younger leaves and down the stem to the root, increases until the leaf is fully expanded.

The expanded leaves may also import assimilate to a very slight degree.

Export of assimilate from an expanded leaf to the apex and root is inversely proportional to its distance from these sinks. The concentration in the stem of labelled assimilate derived from one source leaf decreases with distance from the source leaf; if derived from four source leaves the concentration fluctuates with maxima at the nodes.

Defoliation between the source leaf and the root causes more assimilate to move to the root and less to the apex.

I. INTRODUCTION

This paper presents the results of experiments designed primarily to extend our knowledge of the pattern of translocation in normally growing soybean plants. The planning of the experiments allowed for the previously demonstrated large degree of variability between individuals of similar age and treatment (Thaine, Ovenden, and Turner 1959) and for the need to supplement radioautography by more precise counting methods. A second aim of the work was to determine the effects of partial defoliation upon the distribution of the radioactive assimilate.

It had been shown in the previous experiments that defoliation between the source leaf and the apex increased the amount of active material moving from the source to the apex. This investigation has been extended to show the effects of defoliation between source leaf and root.

In the majority of experiments presented here the age of the plants was standardized at 8 weeks. At this stage, the soybean plant consists of a simple unbranched axis with a well-developed root system. There are a pair of opposite, simple, primary leaves, four to five trifoliate leaves, which have expanded to their maximum size, and a series of young expanding leaves at the apex. It should be emphasized that the results obtained refer specifically to plants of a particular species and age.

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II. MATERIALS AND METHODS

(a) *Treatment of Plants*

Plants of soybean, *Glycine max* (L.) Merr. cv. Biloxi, were used. They were grown in vermiculite in 5-in. unglazed pots in the greenhouse and were irrigated twice weekly with the nutrient solution devised by Arnon and Hoagland (cf. Hewitt 1952).

The plants were used when they were 8 weeks old and had four or five fully expanded trifoliolate leaves. If five such leaves were present the lowest one was cut off on the day prior to the experiment so that all experimental plants had four leaves. The only exception to this was the experiment dealing with the effects of defoliation (expt. 11) where older plants (12 weeks old) with eight or nine leaves were used.

For the majority of the experiments the intensity of the sunlight in the glass-house during $^{14}\text{CO}_2$ assimilation was between 1000 and 2000 f.c. In experiment 1 the effect of a lower intensity (500–700 f.c., produced by shading) was investigated. In experiment 2 the plants were pre-illuminated for 4–6 hr. In all other experiments there was no pre-illumination. The plants were darkened at 5 p.m. on the day prior to the administration of $^{14}\text{CO}_2$, the dark period being approximately 17 hr. In all experiments except one (expt. 6) each plant received $5\mu\text{c}$ of $^{14}\text{CO}_2$. This was administered to one leaflet, the spot-feeding technique described by Thaine and Walters (1955) being used. The source leaflet was varied to suit the demands of each experiment.

The plants were allowed to assimilate $^{14}\text{CO}_2$ for 2 hr, in which time the amount of $^{14}\text{CO}_2$ supplied is completely used (Thaine, Ovenden, and Turner 1959). At the end of the 2-hr period the plants were cut into sections and air-dried under light pressure.

(b) *Analysis of Dried Material*

(i) *Conversion to BaCO_3* .—Plant parts were checked for radioactivity with a Philips electronic counter type PW 4035 and Geiger-Müller tube type PW 4100. Surface counts on the plant parts were obtained by placing the parts at a set distance from the end window of the Geiger tube and making a count of the radiation emitted from the surface of the tissue. This method gives information on the presence or absence of activity in the tissue and previous testing has shown it to be reliable. It has the advantages of being sensitive and time saving and, provided the same type of tissue is compared, it gives some indication of the relative amounts of activity present.

Those parts which showed activity were weighed, their lengths measured (stems only), and then they were ground to powder with a Wiley micro-mill fitted with a 40-mesh screen. The powdered samples were thoroughly mixed by stirring and shaking, and 10-mg aliquots taken and subjected to combustion. The Van Slyke-Folch wet oxidation method (Comar 1955; Aronoff 1956) was followed for this combustion process.

The CO_2 resulting from the combustion was absorbed in NaOH, precipitated as BaCO_3 by addition of BaCl_2 , and filtered off. The weight of BaCO_3 produced was calculated from the titration of residual NaOH in the filtrate against HCl. The

dried precipitates of BaCO_3 were counted with the electronic counter and a scaler of the predetermined count type. For counts of 1000, 3000, and 10,000 pulses the probable error is 2.12, 1.23, and 0.67%, respectively. In the majority of these experiments a count of 1000 pulses was used; where a low activity made it advisable the count was increased to 3000 or 10,000. A check was made on the homogeneity of the powdered samples after mixing, by counting 20 successive aliquots of one sample. The standard error of the mean of these counts was slightly less than 1%. The weight of the aliquot (10 mg) was chosen because it gave a precipitate sufficiently thin to avoid cracking during the drying process but still of "infinite thickness", which for BaCO_3 is 20 mg/cm^2 (Aronoff 1956).

Total activity for each plant part was calculated by means of the following expression:

$$T = S M' p / q,$$

where T = the total activity of each plant part;

S = the specific activity of the BaCO_3 produced by combustion of the plant sample. This is directly proportional to the number of counts/minute obtained when the activity of a sample of infinite thickness is measured;

M' = the mass per unit area of the BaCO_3 precipitate (M) minus the blank value for the apparatus (m). (This blank value is due to the pick-up of extraneous CO_2 during operations and can be calculated by direct titration of the NaOH .)

p = the mass of the original plant part; and

q = the aliquot mass (in all experiments reported below $q = 10$ mg).

(ii) *Powder Counting*.—In experiments 1–10, a method involving direct counting of the powdered material was used. O'Brien and Wardlaw (1961) have shown that this method gives a coefficient of variation of less than 3% provided that the following conditions obtain:

- (1) The material is thoroughly dry (comparable to being oven-dried at 110°C for 3 days).
- (2) The sample is ground to pass a 30-mesh or finer screen.
- (3) The distance of the sample from the counting window is constant and the height of the sample is kept constant (this is achieved by use of a planchet of adjustable depth).

These conditions were adhered to in the experiments reported here.

A comparison was made between the counts per minute per unit area of samples of the powdered material and of the same samples after combustion and conversion to barium carbonate. The following ratio was found to apply:

$$\frac{\text{counts on powdered material}}{\text{counts on barium carbonate}} = 2.86 \pm 0.07.$$

This expression of the standard error covers variation due to experimental factors. Counts were made over a period of time sufficiently long so that variation inherent in counting was minimal. With this ratio and the expression for total activity previously stated, it was then possible to calculate the total activity for each plant part.

(iii) *Radioautography*.—In experiments 5 and 9 radioautographs were prepared. The technique used here has been described in an earlier paper (Thaine, Ovenden, and Turner 1959).

III. RESULTS

(a) *Effect of Light on the Pattern of Translocation*

(i) *Light Intensity (Expt. 1)*.—Twenty plants were used, each 8 weeks old and with four expanded leaves. Each received $5 \mu\text{C}^{14}\text{CO}_2$ on the uppermost fully expanded leaf. Ten plants were illuminated at 1000–2000 f.c. and 10 at 500–700 f.c. for 2 hr.

TABLE 1
DISTRIBUTION OF LABELLED ASSIMILATE IN SOYBEAN PLANTS AFTER EXPOSURE TO
DIFFERENT LIGHT INTENSITIES FOR 2 HR
 $5 \mu\text{C}^{14}\text{CO}_2$ administered to both groups of plants

	High Light Intensity (1000–2000 f.c.)	Low Light Intensity (500–700 f.c.)
$10^{-4} \times$ total activity (counts/min minus background) in plants	105.5 ± 10.7	97.9 ± 5.0
Percentage of total activity:		
In plant parts above source leaf (A)	7.8 ± 0.5	$3.4 \pm 0.5^{**}$
In plant parts below source leaf (B)	36.3 ± 2.0	$23.5 \pm 1.9^{**}$
Which has moved out of source leaf	44.1 ± 1.9	$26.9 \pm 2.1^{**}$
Distribution ratio (B/A)	4.7 ± 0.6	$8.1 \pm 1.1^*$

* Significantly different from high light intensity results at $P = 0.05$.

** Significantly different from high light intensity results at $P = 0.01$.

After treatment each plant was analysed for total activity, percentage of total activity leaving the source leaf during the illumination period, percentage of total activity moving down the stem (B) and up the stem (A). The ratio B/A, termed here the distribution ratio, was calculated and shown to be a function of light intensity (Table 1).

The total activity in the plants is not significantly different in the two treatments. This is taken to indicate that at both 500–700 f.c. and 1000–2000 f.c. the leaf uses up all the labelled CO_2 supplied to it. The percentage of total activity moving out of the source leaf is lower at 500 f.c. than at 1000 f.c., and both ascending and descending components decrease. The distribution ratio, however, rises.

(ii) *Pre-illumination (Expt. 2)*.—Eighteen plants were used, each 8 weeks old and with four expanded leaves. Ten plants each received $5 \mu\text{C}^{14}\text{CO}_2$ (as in expt. 1) immediately following a dark period of 17 hr, and eight received $5 \mu\text{C}^{14}\text{CO}_2$ after a 4- or 6-hr light period. Light intensity was 1000–2000 f.c. Results are presented in Table 2 and show that the total activity in the plants was not significantly altered

by the pre-illumination. None of the values for the 4-hr pre-illumination period was found to be significantly different from those of the control plants. The values for the 6-hr period, in contrast, were markedly decreased in comparison with control plants. The percentage of the total activity moving out of the source leaf was diminished as were percentages for materials both moving up and down the plants. The distribution ratio was slightly increased.

TABLE 2
DISTRIBUTION OF LABELLED ASSIMILATE IN SOYBEAN PLANTS AFTER VARIOUS
PRE-ILLUMINATION PERIODS
Light intensity 1000–2000 f.c. $5 \mu\text{C}$ $^{14}\text{CO}_2$ administered for 2 hr to both control and test plants

	Controls (no pre-illumination period)	Plants Pre-illuminated for:	
		4 Hr	6 Hr
$10^{-4} \times$ total activity (counts/min minus background) in plants	105.5 ± 10.7	81.1 ± 3.4	106.8 ± 19.7
Percentage of total activity:			
In plant parts above source leaf (A)	7.8 ± 0.5	6.7 ± 4.7	$4.5 \pm 0.0^{**}$
In plant parts below source leaf (B)	36.3 ± 2.0	36.1 ± 3.6	$26.7 \pm 0.6^{**}$
Which has moved out of source leaf	44.1 ± 1.9	42.8 ± 1.3	$31.2 \pm 0.1^{**}$
Distribution ratio (B/A)	4.7 ± 0.6	11.2 ± 8.4	$5.9 \pm 0.1^*$

* Significantly different from control at $P = 0.05$.

** Significantly different from control at $P = 0.01$.

(b) Translocation in the Intact Plant

(i) *Import by the Expanding Leaf from the Leaf below It (Expt. 3).*—Twenty-five plants were used. The source leaf was leaf IV, the uppermost fully expanded leaf on the plant. Plants were chosen so that the expanding leaf immediately above the source leaf ranged in size from a length of 1.7 cm, corresponding to 1.0% of the adult area, to one of 14.7 cm, corresponding to 83.0% of the adult area. Activity in the young leaf and total activity in the plant were assessed, and the amount imported by the young leaf is expressed as a percentage of the total activity in the plant. Results of the experiment (see Fig. 1) show that young leaves of up to 50% of the adult area import assimilate from the leaf below it. Beyond this area import is inappreciable. A later experiment (expt. 8) shows that if a source of higher activity is used ($250 \mu\text{C}$ instead of $5 \mu\text{C}$), then some small import into mature leaves may be demonstrated. Thus, above 50% adult area import may be said to fall to an extremely low value rather than actually cease.

(ii) *Import by the Expanding Leaf from the Four Leaves below It. (Expt. 4).*—Forty plants were used and leaves I, II, III, and IV were in turn the source leaf. The percentage of total activity which moved into the expanding leaf in each case

was calculated. The results and their statistical analysis given in Table 3 and the confidence limits are based on the residual mean square in the analysis of variance for log imports. This analysis also confirms the significance of the differences between mean imports of the four types of leaf taken as a group. The analysis was carried out in terms of log imports rather than imports themselves because of the extreme

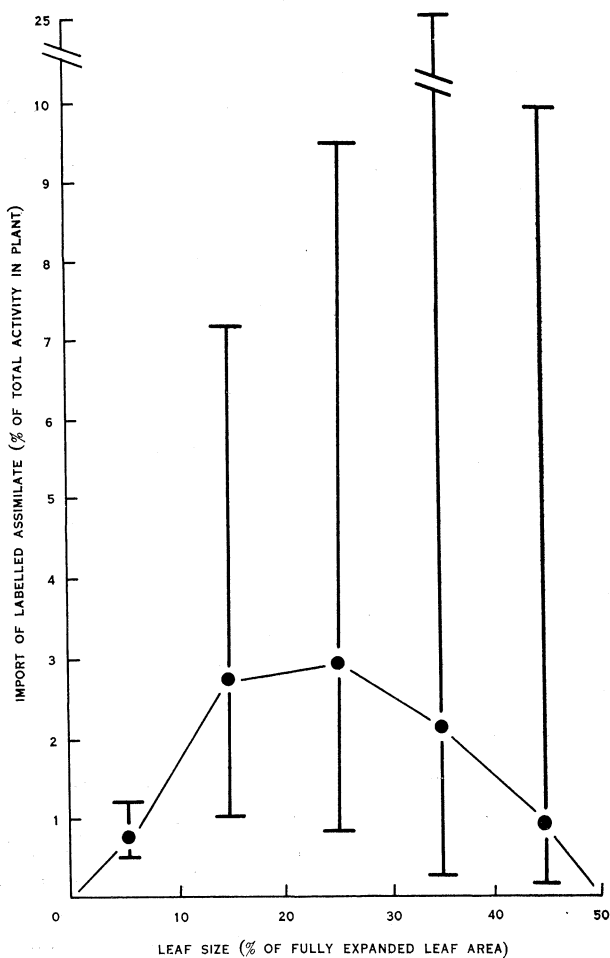


Fig. 1.—Changes in mean import of labelled assimilate by the expanding leaf from the leaf below as the expanding leaf grows to full size (expt. 3). The 95% confidence limits shown are limits for these geometric means (i.e. antilogs of mean logs) and not for variation of import in individual leaves.

skewness of the actual imports, and also the marked tendency of the variation to increase with the mean. The results show that the upper leaves are the major sources of supply for the expanding leaves, import of assimilate from the lower leaves is markedly less. The expanding leaf is shown to import more from the second leaf down than from the leaf immediately below it on the stem. The reason for this is not yet clear.

(iii) *Import by the Expanding Leaf: Distribution after 6 Weeks Growth (Expt. 5).*—This experiment gave results which confirm the relationship between import and leaf size shown in Figure 1. Plants with two expanded leaves were used, the upper leaf (II) being used as the source. These plants were left for 6 weeks between application of $^{14}\text{CO}_2$ and harvesting. At the time of harvesting nine expanded leaves were present. Plate 1 shows radioautographs of one of the experimental plants. The expanded leaves are numbered I to IX, the lowest being I. The expanding leaves are numbered *i*, *ii*, *iii*, and so on, number *i* being the largest and oldest. Table 4 gives morphological data on the young leaves from one plant. Dissection and measurement of a number of plants showed that these data were typical.

TABLE 3
AVERAGE IMPORT OF LABELLED ASSIMILATE IN SOYBEAN PLANTS BY THE
EXPANDING LEAF FROM THE FOUR LEAVES BELOW IT ON THE STEM

Source Leaf No.	Average Import*	95% Confidence Limits
IV (uppermost leaf)	1.43	0.91–2.32
III	3.98	2.55–6.21
II	0.87	0.56–1.36
I (lowest leaf)	0.50	0.32–0.77

Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Squares	Variance Ratio
Between leaf positions	3	1.1633	} 12.7136***
Between leaves in the same position	36	0.0915	

* Calculated logarithmically.

*** Significant at $P = 0.001$.

Expanding leaf *ix* is present only as a protuberance on the side of the domed shoot apex; at this stage the stipules have not yet formed. By the time the leaf is present as a definite single lobe (leaf *viii*) the stipules have formed as similar, lateral lobes. The growth rates of stipules, leaf hairs, and leaves differ markedly at this early stage in their development. Figure 2 shows the lengths of stipules, leaves, and hairs for each developing leaf in one series. Leaves *iv–viii* are smaller than their stipules which show rapid growth during this period. The hairs are formed at leaf *vii* (on the stipule) and show their period of active growth from leaf *vii* to *v*.

Reference to Plate 1 and Table 5 shows the relationship between these periods of active growth and movement of ^{14}C -labelled sugars into the plant part. Apparently no redistribution of the labelled material takes place, as hairs and stipules formed

after the time of application of $^{14}\text{CO}_2$ (e.g. on expanding leaf *iii*) show no activity. Similarly, hairs and stipules formed before the application of $^{14}\text{CO}_2$ (e.g. on leaf *III*)

TABLE 4
YOUNG, EXPANDING LEAVES OF SOYBEAN PLANTS: MORPHOLOGICAL DATA

Expanding Leaf No.	Leaf Length (mm)	Leaf Form	Length of Stipules (mm)	Length of Leaf Hairs (mm)	Area of Leaf (% of adult area)
<i>i</i>	59.0	Trifoliate	7.0	1.3	31
<i>ii</i>	16.0	Trifoliate	7.0	1.3	<2
<i>iii</i>	9.0	Trifoliate	7.0	1.3	<1
<i>iv</i>	6.0	Trifoliate	7.0	1.3	<1
<i>v</i>	2.3	Transitional	3.4	1.1	<1
<i>vi</i>	0.9	3-lobed	2.2	0.6	<1
<i>vii</i>	0.2	3-lobed	0.7	—*	<1
<i>viii</i>	0.1	Single lobe	0.1	—†	<1

* Hairs present only on stipule; hairs 0.2 mm long. † No hairs present.

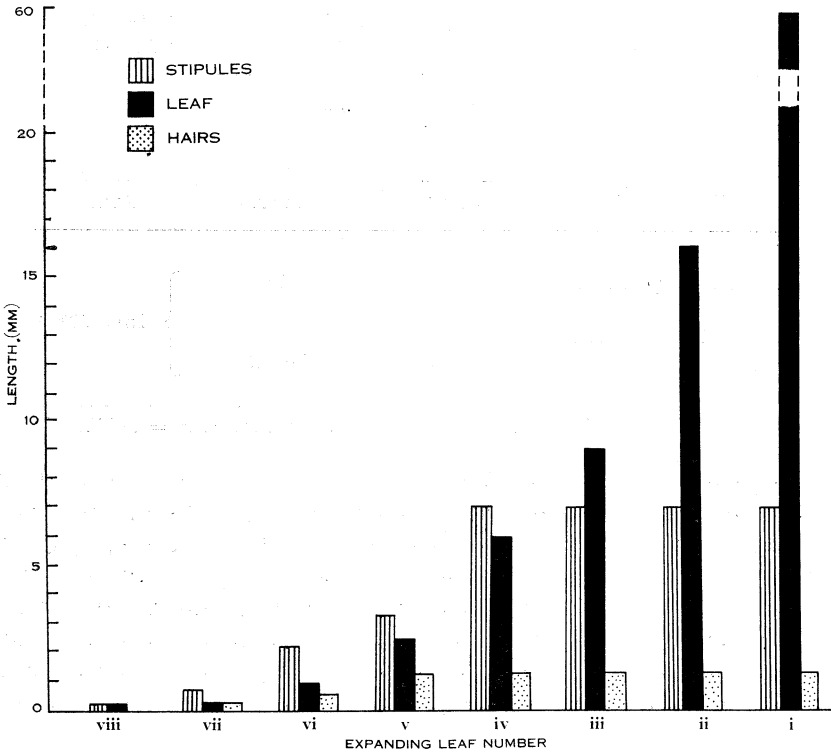


Fig. 2.—Length of stipules, leaf, and hairs of successive developing leaves on the shoot apex of soybean (expt. 5).

also show no activity. Only those which were actually expanding, or had just finished expansion show import of active material (cf. Plate 1). The fact that the leaf accom-

panying a stipule gives a paler print on the radioautograph than does the stipule is due to the dilution of labelled material by subsequent expansion of the leaf.

Leaf IV shows the highest activity (2148 counts/min). Reference to Table 5 shows that this was expanding leaf *ii* at the time of application of $^{14}\text{CO}_2$. Leaf *ii* is less than 2% of the adult area and is therefore in the phase of increasing import (cf. Fig. 1). On the other hand, leaf III, which was expanding leaf *i* at the time when $^{14}\text{CO}_2$ was applied, shows less activity (227 counts/min). This leaf was 31% of the

TABLE 5
DISTRIBUTION AND RELATIVE AMOUNTS OF ACTIVITY 6 WEEKS AFTER APPLICATION OF $^{14}\text{CO}_2$ TO AN UPPER LEAF OF SOYBEAN PLANTS

Leaf No. at Harvesting	Surface Counts on Leaf (counts/min minus background)	Activity in Stipules*	Activity in Leaf Hairs*	Expanding Leaf No. at Time of Application of $^{14}\text{CO}_2$
II (source leaf)	71,000	—	—	II (uppermost expanded leaf)
III	227	—	—	<i>i</i>
IV	2148	+	—	<i>ii</i>
V	376	+++	+++	<i>iii</i>
VI	188	+++	++	<i>iv</i>
VII	81	+++	+	<i>v</i>
VIII	85	++	—	<i>vi</i>
IX	82	++	—	<i>vii</i>
<i>i</i>	14	++	—	<i>viii</i>
<i>ii</i>	41	+	—	<i>ix</i> (primordium)
<i>iii</i>	21	—	—	<i>x</i> (primordium)

* Activity in stipules and hairs is expressed on an arbitrary scale of +, ++, or +++ and is based on a visual assessment of density on the radioautograph (Plate 1).

adult area and apparently in the decreasing phase of import. Leaves V, VI, and upwards show a progressive decrease in activity. These leaves at the time of administration of $^{14}\text{CO}_2$ were all very small and folded in the apical bud. Their small size at the time of application of $^{14}\text{CO}_2$ and their subsequent enlargement (which dilutes the activity) combine to give a pattern of progressively decreasing activity as distance from the source leaf increases. These results indicate that import into stipules, hairs, and leaves is linked with their period of active growth.

Plate 1 also shows that there is perceptible activity present in leaf I which was fully expanded at the time of $^{14}\text{CO}_2$ application to leaf II. It will be shown later that this activity is largely due to pick-up of $^{14}\text{CO}_2$ which has entered the immediate atmosphere from leaf II during and subsequent to the application period. Recently Okanenko, Grodzinskii, and Batyuk (1960) have shown that sugar-beet leaves which have received $^{14}\text{CO}_2$ subsequently give off $^{14}\text{CO}_2$ for at least 24 hr and it would appear that soybean leaves behave in a similar manner.

(iv) *Export by the Expanding Leaf (Expt. 6).*—The expanding leaf itself was the source leaf and the amount exported to the rest of the plant was determined,

No export occurs when the area of the leaf is below 30% of the adult size; thereafter export increases. Comparison of Figure 3 with Figure 1 shows that there is an overlap between 30% and 50% of the adult area where the expanding leaf is simultaneously importing and exporting assimilate.

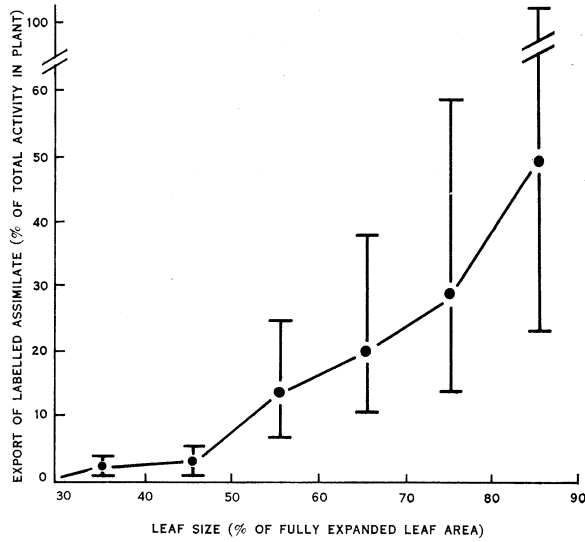


Fig. 3.—Changes in mean export of labelled assimilate by the expanding leaf as it grows to full size (expt. 6). The 95% confidence limits shown are as for Figure 1.

Ten plants were used for measurement of the growth rate of the leaves—which was estimated as the increase in area (and expressed as a percentage of the adult leaf area) per day. The leaf area was measured by weighing leaf tracings on standard

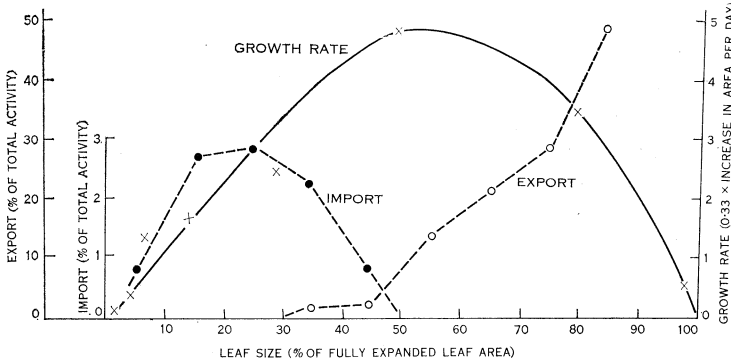


Fig. 4.—Comparison between import, export, and growth rate of the expanding leaf (expt. 6). Growth rate is given as daily increase in percentage of the adult leaf area.

graph paper. Adult leaf area was taken as that of the uppermost fully expanded leaf on a plant with four expanded trifoliate leaves. The results (Fig. 4) show a

maximum growth rate at approximately 50% of the adult area and comparison with import and export curves (reduced for convenience to comparable scales) shows that this maximum corresponds to diminishing import and (overlapping) increasing export.

Figure 5 shows the growth curves obtained for two neighbouring leaves and includes the data obtained from experiment 3. It can be seen that import to leaf *i* ceases some time before its full expansion and that leaf *ii* begins a rapid increase in area at this point. Leaf *ii* is at this time 8% of the adult area, and reference to Figure 1 shows that this is within the phase of sharply increasing import.

(v) *Export by the Mature Leaf (Expt. 7).*—In experiment 4 the leaves were used in turn as the source leaf. In experiment 7 the stem, mature leaves and roots below the source, and the stem and leaves above the source were analysed for activity.

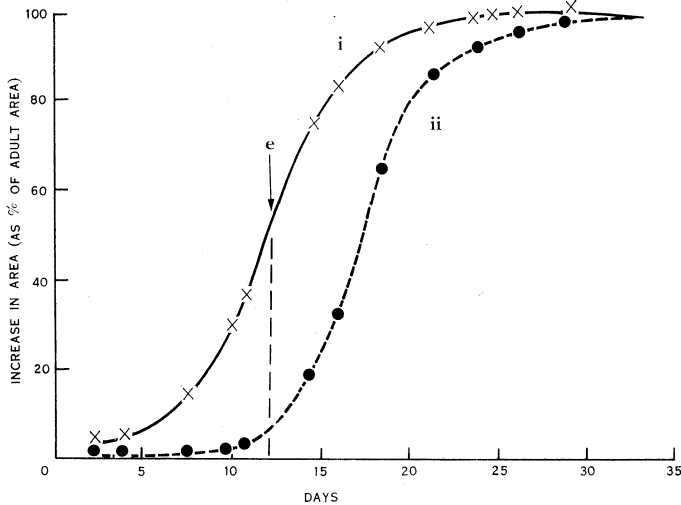


Fig. 5.—Increase in area of expanding leaf *i* and the next smaller expanding leaf *ii* with time (expt. 6). The arrow *e* indicates the point (at 50% of the adult area) where import to leaf *i* from leaves below ceases. The dotted extension of the arrow gives the corresponding size of leaf *ii*.

Figure 6 shows the distribution of material for the four source leaves. In all cases movement down to the root is markedly greater than movement up to the apex and developing leaves. Furthermore, movement to the apex is greater in the upper compared to the lower leaves, and, conversely, movement to the roots is more marked in lower than in upper leaves. Table 6 shows the distribution ratios calculated for the four source leaves, and demonstrates again the increased importance of the lower leaves in supplying the root.

(vi) *Import by the Mature Leaf (Expt. 8).*—Previously it has been shown that the adult leaves apparently import material in small quantities (Thaine, Ovenden, and Turner 1959). Canny (1960b) has shown that a plant which is receiving $^{14}\text{CO}_2$ through the leaf may give off respired $^{14}\text{CO}_2$. This $^{14}\text{CO}_2$ might then be assimilated by other leaves on the plant and give the appearance of translocation in radioautographic results.

Another source of possible error lies in the "spot-feeding" method. $^{14}\text{CO}_2$ entering the abaxial surface of the leaf from the vessel applied to it may diffuse through the air spaces in the leaf and leave the leaf surface via stomata outside the area covered by the vessel mouth. This labelled gas is then free in the atmosphere for photosynthetic uptake by older leaves of the experimental plant. A preliminary experiment (expt. 8) was designed to test these possibilities.

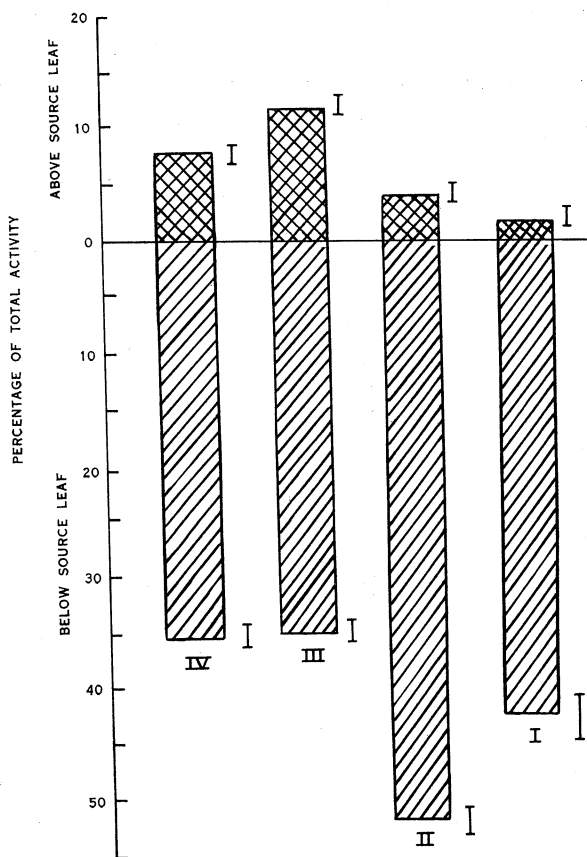


Fig. 6.—Percentage of total activity above and below the source leaf for source leaves I, II, III, and IV respectively (expt. 7).

Four plants were used in this experiment. The plants had two expanded trifoliate leaves and the primary leaves were still green. During the experiment the plants were placed side by side in close proximity to each other. Plants A, B, and C received $250\ \mu\text{c}$ of $^{14}\text{CO}_2$ on the abaxial surface of the uppermost fully expanded leaf. Plant D received no $^{14}\text{CO}_2$. Two leaves on plants A and C (a primary leaf and trifoliate leaf I) and a trifoliate leaf on plant B were enclosed in Erlenmeyer flasks and sealed in with "Silcote" self-vulcanizing silicone rubber prior to the liberation of $^{14}\text{CO}_2$. One leaf on plant D was similarly treated. Preliminary checks

showed that a ring of "Silcote" did not affect translocation through a stem over the period of the experiment, and that satisfactory gas-tight seals could be obtained with this material.

TABLE 6
AVERAGE DISTRIBUTION RATIOS FOR FOUR SOURCE LEAVES OF SOYBEAN PLANTS

Source Leaf No.	Average Distribution Ratio*	95% Confidence Limits†
IV (uppermost leaf)	4.71	3.24-6.84
III	3.92	2.30-5.69
II	13.09	9.01-19.01
I (lowest leaf)	14.95	10.30-21.72

* Calculated logarithmically.

† Based on residual mean square in analysis of variance for log distribution ratios.

The results of experiment 8 are given in Table 7 and from the results a pattern emerges for the leaves not enclosed in flasks. These leaves, both on the plants receiving $^{14}\text{CO}_2$ and the control plant, show a definite amount of activity, the amount

TABLE 7
DISTRIBUTION OF ACTIVITY IN LEAVES OF SOYBEAN PLANTS AFTER EXPOSURE OF UPPERMOST FULLY EXPANDED LEAF OF PLANTS A, B, AND C TO $250\ \mu\text{c}\ ^{14}\text{CO}_2$ FOR 2 HR

Leaf Tested	Activity (counts/min minus background)			
	Plant A	Plant B	Plant C	Plant D†
Expanding leaf	37×10^3	45×10^3	52×10^3	105
Leaf II				
Terminal leaflet (source)	83×10^3	77×10^3	67×10^3	1*
Lateral leaflets	190	276	344	7*
	228	488	303	4*
Leaf I				
Terminal leaflet	10*	11*	3*	57
Lateral leaflets	21	11*	7*	93
	44	7*	5*	63
Primary leaves	13*	901	8*	25
	27	301	118	178

* Leaf enclosed in Erlenmeyer flask.

† Control—no $^{14}\text{CO}_2$ administered to this plant.

being in general, directly related to the distance from the source leaf. For example, the lateral leaflets of the source leaf itself are more active than are the lateral leaflets of leaf I, which are further away from the source. Some of the primary leaves, on the

other hand, show a higher activity than would be expected. The reason for this is not known. The activity must be derived either from photosynthetically incorporated $^{14}\text{CO}_2$ or from translocated labelled sugars. No information is at present available to show whether primary leaves differ metabolically from trifoliate leaves in a way that could explain this unexpected activity.

The leaves on plant D could have become active only by photosynthesizing $^{14}\text{CO}_2$ from the atmosphere. From this it can be concluded that there is free $^{14}\text{CO}_2$ emanating from the source leaves by one or both of the two methods previously suggested.

Enclosed leaves on all plants show low activity. This activity can only have reached these leaves by translocation through the plant. In the control plant D which was not fed $^{14}\text{CO}_2$ directly, sufficient $^{14}\text{CO}_2$ has apparently been picked up from the atmosphere to label material moving into the enclosed leaf. It is of interest that the amount of activity shown by the enclosed leaves is uniformly low and of comparable amounts in both control and experimental plants.

The activity used in this experiment is 50 times that used normally in this series of experiments. Consequently the activities shown by the enclosed mature leaves would not be detected in the normal experimental situation.

A further feature of interest is that in these plants, which have the primary leaves and two trifoliate leaves expanded, the route of the major part of the assimilate moving from the uppermost leaf is to the apical expanding leaves. Some activity has moved to the roots (samples give counts of the order of 1000/min), but the predominant sink for this source leaf is the apex. This result is in accordance with that obtained for older plants in experiment 7.

(c) *Vascular Pattern in the Stem (Expt. 9)*

Earlier anatomical studies on the soybean stele (Thaine, Ovenden, and Turner 1959) have indicated that there are interconnecting phloem strands between the main stem bundles, and that the phloem of the stem resembles an open reticulum rather than separate, isolated bundles. The following experiment was designed to show whether the lateral connections in the phloem are functionally important in transport.

$^{14}\text{CO}_2$ was fed to a leaf of soybean which was allowed to assimilate for 2 hr. The tissues exterior to the cambium were then carefully peeled from the xylem cylinder. Transverse sections of the cylinder remaining after peeling showed that it comprised only xylem tissue and central pith. The peripheral tissues were air-dried under slight pressure to flatten them, then the inner surface was radioautographed. By this means it is possible to follow the vascular strands from a source leaf.

Plate 2, Figures 1 and 2, shows the tissues prepared in this way and the radioautograph obtained from them. The source leaf node is seen on the extreme left-hand side of each figure. It is seen that from this node one small strand goes upwards and one major and several lesser strands proceed downwards. The main strand may be followed down each successive strip to that on the extreme right, which ends at ground level. The lateral strands tend to fade out before this level is reached.

Plate 2, Figures 3 and 4, illustrates the same pattern occurring in the stem transection. Figure 3 shows a section of a young stem of soybean. The three bundles indicated by *x*, *y*, and *z* are those which are derived from the leaf immediately above. Figure 4 shows a radioautograph of a similar stem after the leaf above had assimilated $^{14}\text{CO}_2$ applied to the terminal leaflet. Activity is concentrated in the bundle *y*, which corresponds to the strand from the terminal leaflet (see also Plate 2, Fig. 6). Slight activity can also be detected in the bundles *x* and *z*, which derive from the lateral leaflets of the source leaf. This corresponds to the slight activity in the lateral bundles in Plate 2, Figure 2. The origin of this activity is assumed to be from the slight activity picked up by the lateral leaves. These have already been shown (expt. 8) to take up $^{14}\text{CO}_2$ lost to the atmosphere by the source leaf.

In the soybean the petiolar bundles condense to an annulus of vascular tissue at the pulvinus, before entering the stem stele as three separate bundles. It would seem possible that some slight lateral movement of active material from the strand derived from the terminal leaflet might occur at the pulvinar annulus.

In Plate 2, Figures 5 and 6, the effects of multiple source leaves are shown. In Figure 5 two successive leaves each received $^{14}\text{CO}_2$ on the terminal leaflet. The main activity is concentrated in the two bundles which proceed separately down the stem. In Figure 6 $^{14}\text{CO}_2$ was applied to each of the three leaflets of one trifoliate leaf. The three strands can be readily followed throughout the stem. The central bundle appears to fork, but there is no evidence of lateral exchange between the strands.

The predominance of downward movement in these radioautographs is due to the fact that the leaves are low down on the stem, as the older stem is the most satisfactory for stripping.

These results indicate that the phloem strands from each leaflet are functionally far more independent than a purely anatomical study of the vascular system would suggest.

(d) Concentration Gradients in the Stem (Expt. 10)

Reference to Figure 6 shows that, in a soybean plant with four mature expanded leaves, each of the leaves contributes assimilate to both apex and root. The distribution ratios differ for each leaf, increasing as the source leaf is nearer to the root. It therefore appears that a section cut from an internode between any two leaves would include material (assumed to be sucrose) moving both downwards and upwards in the stem, as well as substances derived from sucrose and incorporated into the structure of the cells of the phloem. The results of experiment 9 show that assimilate moving from any one leaf would be, for the most part, but not entirely, confined to one of the bundles of the internode.

When the assimilate from a single leaf on the stem is labelled it is now well established that the concentration of labelled assimilate tends to fall logarithmically as the distance from the source node increases (Canny 1960a). If the assimilate from every leaf on a stem were labelled simultaneously, then the amount of labelled assimilate at any point along the stem should be the sum of material provided in four downward- and four upward-moving "streams"—in at least four separate bundles. Assuming that the amount incorporated into phloem cells was small in

comparison to that moving in the phloem, then it would be expected that the concentration of labelled assimilate would fluctuate, with the maxima at the nodes.

This conclusion is substantiated by the results of experiment 10. Two treatments were used: in one, each of the four leaves received $5 \mu\text{c}$ of $^{14}\text{CO}_2$, in the other, one leaf only (leaf III) received $20 \mu\text{c}$ of $^{14}\text{CO}_2$. After 2 hr, free-hand sections of the fresh material were cut at successive nodes and internodes and the activity of these

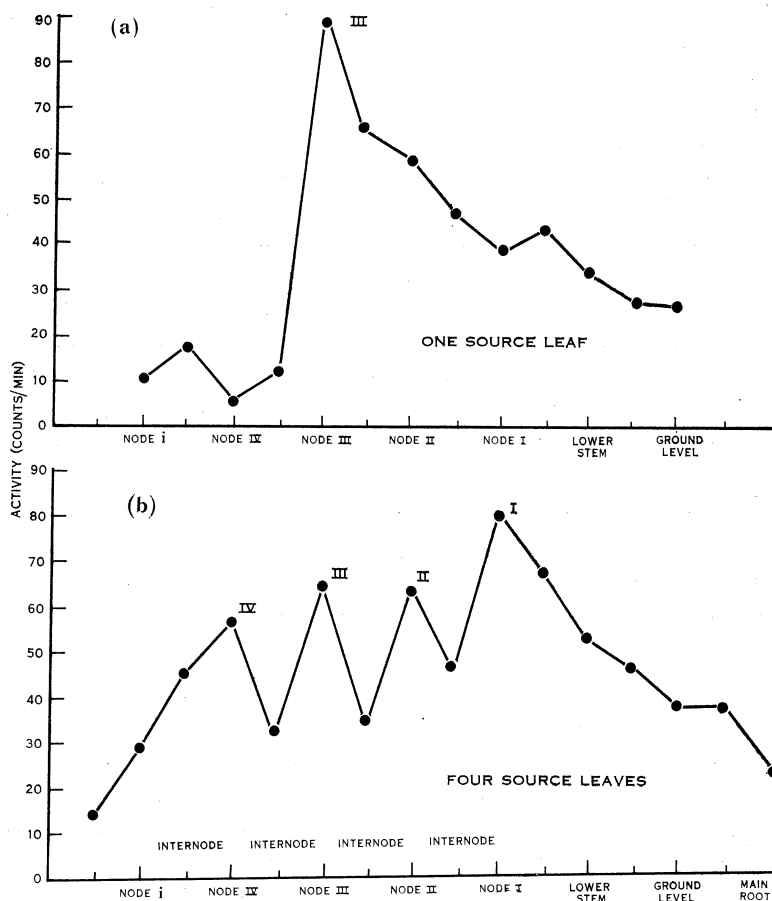


Fig. 7.—Activity of stem transections at successive nodes and internodes down the stem for (a) one source leaf (leaf III), and (b) for source leaves I, II, III, and IV (expt. 10).

sections was measured by surface counting. Figure 7 shows the results of a typical experiment. Each graph refers to measurements on one plant; three replications of the experiment gave similar results.

These results make it clear that the only concentration gradients relevant to a discussion of the mechanism of translocation are those which apply to a single vascular unit joining source leaf to sink. Even the gradient shown in Figure 7(a)

refers to more than one bundle (and certainly to numerous sieve tubes). Measurements of total sucrose content of node and internode would show smooth gradients only in the regions below and above the internodal region of the stem which carries mature leaves. The position with regard to the stem region above the mature leaves is further complicated by the fact that here the stem is usually still elongating and is itself a sink for assimilate.

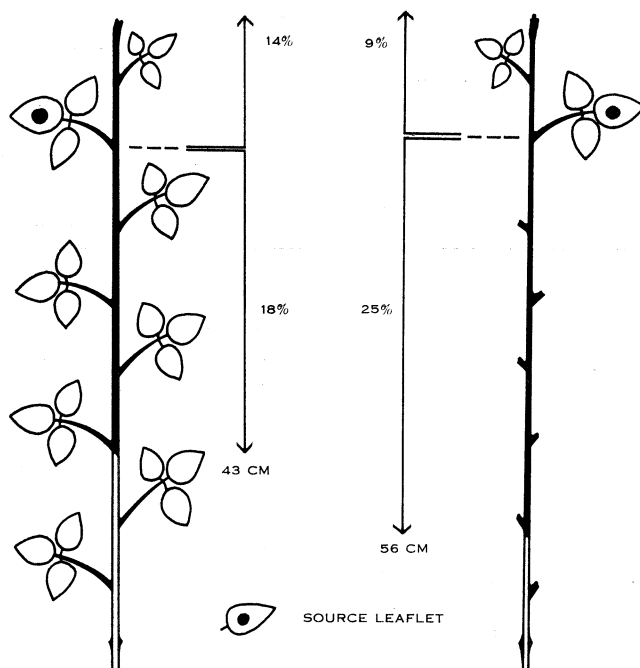


Fig. 8.—Distribution of labelled assimilate in defoliated and intact plants (expt. 11).

(e) *Translocation in the Defoliated Plant (Expt. 11)*

In this experiment 25 plants had all but the uppermost expanded leaf cut off 24 hr before $^{14}\text{CO}_2$ administration. 25 matched plants were left with all leaves intact. The plants used in this experiment were 12 weeks old and had either eight or nine expanded leaves. $^{14}\text{CO}_2$ was applied to the uppermost fully expanded leaf in all 50 plants. The plants were subsequently analysed for activity in parts above and below the source leaf, and for total activity.

Preliminary checking (using surface counts) showed that there was no activity present in the roots, nor in the petioles and mature leaves other than the source leaf. Comparison between surface counts and counts on the same material subjected to combustion and converted to BaCO_3 showed that the surface counts were the more sensitive; consequently, material which showed no activity on surface counting was discarded.

Results show that approximately 33% of the total activity moved out of the source leaf in the 2-hr period, irrespective of the presence or absence of the lower leaves (see Table 8 and Fig. 8). Surface counts on stem sections gave the lower limits at which activity could be detected down the stem. Mean distances are recorded in Table 8 and Figure 8.

Canny (1960a) has shown that the distance measured from a source to such a "front" of activity in a stem will depend on the sensitivity of the detector and the activity of the source. In this experiment the same detector was used throughout, and activity was constant for each source leaflet. As shown in Figure 8, defoliation appears to increase the distance moved in a given time.

TABLE 8
DISTRIBUTION OF LABELLED ASSIMILATE IN DEFOLIATED AND INTACT PLANTS

	Intact Plants	Defoliated Plants
Percentage of total activity:		
In parts above source leaf (A)	14.7 ± 1.5	$9.0 \pm 1.0^{***}$
In parts below source leaf (B)	18.6 ± 2.0	$25.0 \pm 2.1^*$
Which has moved out of source leaf	33.4 ± 2.7	32.9 ± 2.7
Distance (cm) down stem from source leaf at which activity can be detected	42.8 ± 3.4	$56.5 \pm 4.1^{**}$
Distribution ratio (B/A)	1.5 ± 0.2	$3.7 \pm 0.5^{***}$

* Difference significant at $P < 0.05$.

** Difference significant at $P < 0.02$.

*** Difference significant at $P < 0.01$.

IV. DISCUSSION

The results of experiments 3-11 provide some details of the translocation pattern in a normal soybean plant. This pattern is a most complex one, changing continually as the shoot and root develop. Each leaf in turn passes from the primordial stage to the mature leaf through the stage of slow growth and slight import to that of rapid growth and increased import typical of the outermost expanding leaf of the apical group. The rate of import then falls to a very low value before the leaf reaches full size. Near the end of the import phase, export begins and increases in extent to the value found in the fully expanded leaf.

Biddulph (1959), in a discussion of bidirectional movement in the vascular tissue, states that the young leaf imports material until it is functionally mature, then begins to export carbohydrates and excess minerals. In soybean it has been shown that the changeover from import to export occurs when the leaf is but half expanded and that there is a short stage where both import and export proceed side by side. If "the phloem of the leaf experiences a reversal in the predominant direction of flow" as Biddulph suggests, it would seem from the results presented here that this changeover does not occur simultaneously in all sieve tubes.

Changes in the economy of the leaf are accompanied by the passage of the leaf from its apical position to one lower on the shoot and this in turn by a change in the destination of the exported assimilates. The uppermost expanded leaves mainly supply the apex and young leaves. The lowermost leaves mainly export to the roots. Mature leaves midway on the stem supply both apex and root, the assimilate moving from them both upwards and downwards in different channels in the phloem.

This general distribution pattern is in close agreement with that described by Swanson (1959) for ^{14}C -labelled translocate and with that figured by Linck (1959) for ^{32}P -labelled translocate. There is little significant import by the fully mature leaves at any position on the stem.

It is of interest here to refer to determinations of concentration gradient made by Mason and Maskell (1934). Their main experiments were performed on the defoliated lower stems of cotton plants. Here, as might be expected, they found a positive concentration gradient between the lowest leaf and the root. They then attempted to determine the gradients in whole plants with up to 28 nodes analysed in 7-node sections. Here their results immediately become less consistent. In two cases they were able to demonstrate a positive gradient from top to base of the plant, in other cases the gradients showed fluctuation.

The demonstration with radiocarbon of both upward- and downward-moving material deriving from any one source leaf also allows of re-interpretation of another experiment of Mason and Maskell (1928). This experiment has often been quoted as showing that the direction of translocation can be reversed by defoliation and ringing. It now becomes apparent that these workers were measuring the downward flow from upper leaves (having eliminated any upward flow by defoliation and ringing) and the upward flow from lower leaves (again having eliminated the possibility of downward flow by this treatment). The treatment thus did not reverse the translocation stream, it merely separated and perhaps strengthened one component of the bidirectional streams normally present by eliminating the other component in each case.

Mason and Maskell's early conception of translocation as related to the concentration gradients of the stem implied that the phloem of the stem functions as one channel, and that the concentration of sucrose is the same in all the sieve tubes at any particular level in the stem and is measurable as the total concentration of sucrose at that level in the stem. Furthermore, they attempted to relate the concentration gradients of the stem as a whole to the movement of sucrose in the stem.

The experiments presented here have shown that this simplified concept is untenable, and that concentration gradients must be determined in the individual channels of the phloem bundles.

The effect of defoliation between source leaf and the root is to alter the distribution ratio of assimilate, so that a greater proportion of it is translocated to the root. This cannot be due to the removal of leaf sinks competing with the roots because it has been shown that the mature leaves import very little assimilate. It is interpreted rather as due to the defoliation causing a greater sugar deficit in the roots. The lower leaves normally export mainly to the roots and their removal may be expected to

deprive the roots of (unlabelled) assimilate. However, defoliation should also lower (although to a much smaller extent) the concentration of total assimilate in the shoot apex. One would therefore expect the source leaf to export more assimilate to both shoot and root in the defoliated plant. Somewhat surprisingly, this does not occur. Although the source leaf continues to supply the apex, the additional supply to the roots is apparently at the expense of movement to the apex. Within the 2 hr of the experiment, defoliation does not alter the total amount exported by the source leaf. It is possible that this anomaly could be resolved by longer experiments in which we might expect the distribution ratio to return towards the normal value. The results emphasize once again the difficulty of analysing the translocation pattern without data on concentration gradients in the phloem itself.

The results of the preliminary experiments (1 and 2) may also be interpreted provisionally in terms of concentration gradients (or of related hydrostatic gradients) in the phloem. For plants with similar total activity in ^{14}C it was shown that the amount translocated from the source leaf varies with both the light intensity during CO_2 uptake and the length of the pre-illumination period. The greatest export occurs when light intensity is high and the 2-hr exposure to $^{14}\text{CO}_2$ follows immediately on a 17-hr dark period. This is presumably because the sinks (including the source leaf) are relatively unsaturated and the source leaf itself becomes inefficient as a sink after a short period of high light intensity. Similarly, there is less translocation from the source leaf when the light intensity is low or when the whole shoot has been exposed to light for 6 hr before the experiment proper begins.

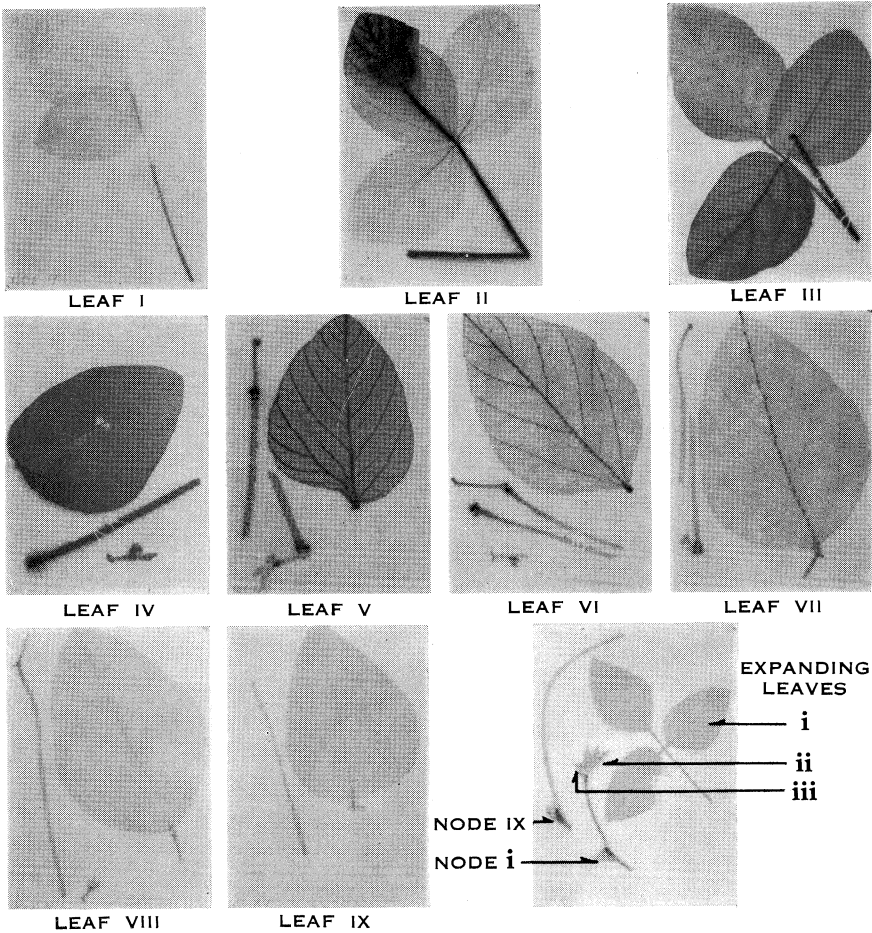
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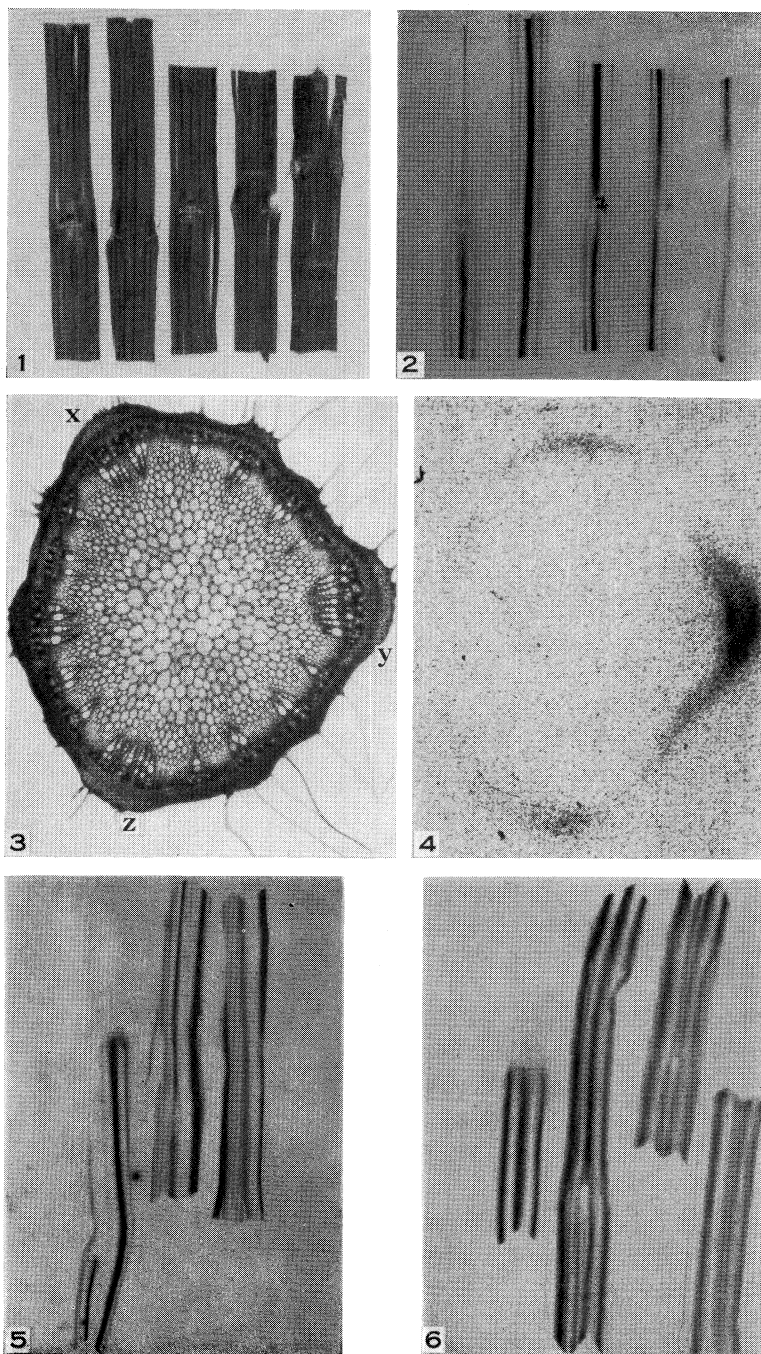
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TRANSLOCATION IN THE SOYBEAN. II



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EXPLANATION OF PLATES 1 AND 2

PLATE 1

Radioautographs of successive leaves of a soybean plant which had received $^{14}\text{CO}_2$ on leaf II 6 weeks previously (expt. 5). Exposure time for all radioautographs was 7 days. Leaf I is the lowest leaf, leaf IX is the highest leaf.

PLATE 2

- Fig. 1.—Pieces of flattened bark stripped from the stem of a soybean plant which had received $^{14}\text{CO}_2$.
- Fig. 2.—Radioautograph of the inner surface of this bark.
- Fig. 3.—Transection of a young soybean stem. *x*, *y*, and *z* mark the vascular bundles from the leaf above.
- Fig. 4.—Radioautograph of a section of soybean stem which had received $^{14}\text{CO}_2$ on the terminal leaflet of the leaf above.
- Fig. 5.—Radioautograph of the inner surface of bark when two successive leaves had received $^{14}\text{CO}_2$ each on the terminal leaflet.
- Fig. 6.—Radioautograph of inner surface of bark when the three leaflets of one trifoliate leaf had each received $^{14}\text{CO}_2$ (expt. 9).