TRANSLOCATION OF LABELLED ASSIMILATES IN THE SOYBEAN

By R. Thaine,* Stella L. Ovenden,† and J. S. Turner†

[Manuscript received July 3, 1959]

Summary

When $^{14}$CO$_2$ was supplied to a limited area of a mature illuminated leaf of soybean, the radioactive compounds appeared rapidly in the root, the apex of the shoot, and in young unexpanded leaves. Translocation to these "sinks" took place in the light, during photosynthesis at the source.

Translocation also went on in darkness subsequent to fixation in the leaf, and after 24 hr the concentration of mobile tagged compounds in the source leaf was very low. Considerable activity, however, remained at the source in the form of insoluble compounds which were not starch. It is concluded that masking lowers the concentration of mobile assimilates in leaves.

In the soybean the vascular pattern is such that movement of assimilate from the source leaf to any other leaf can occur. Movement into mature leaves was very small in extent when compared to movement into growing points or expanding leaves. Nevertheless, movement into primary and trifoliate mature leaves, or from one leaflet to another of a mature leaf, can be demonstrated if $^{14}$CO$_2$ of sufficiently high activity is applied to the source leaflet, and especially if other sinks for assimilate are removed.

Movement of tagged assimilate into a mature leaflet was clearly demonstrated, even when applied at low activity, when the leaf showed necrotic spots (presumably due to nutrient imbalance). Radioactivity then appeared at a high level around the necrotic areas.

Movement into the apical halves of source leaves is much smaller in extent than is basipetal movement. Acropetal movement may, however, be demonstrated and its extent is increased by shading. In general, the shading of a mature leaf increases the tendency of tagged assimilate to move into it, presumably because shading lowers the total concentration of assimilates.

The removal of all mature leaves between source and apex significantly increased movement to the apex. As the leaves removed normally import little, it is presumed that this result is due to the lowering of the concentration of total assimilate at the apex resulting from the removal of leaves which would normally export untagged assimilate.

Shading of the green apex, in many but not in all experiments, led to a decreased movement to the apex from the mature source leaf. This anomalous result was not established as statistically significant and needs further investigation.

The direction of movement from a given leaf depends upon the age and position of the leaf on the stem. A young leaf forms part of the major apical sink for upward-moving assimilate and for its own photosynthetic products, exporting nothing to the rest of the plant. When it has reached 60 per cent. of its maximum size, it begins to lose this function and increases in importance as an exporting organ. The destination of material moving from mature leaves is primarily the

* Botany School, University of Melbourne; present address: Farm Lane, Send, Woking, Surrey, England.
† Botany School, University of Melbourne.
shoot apex and the root. It is predominantly the apex when the source leaf is close to the apex, predominantly the root as growth takes the apex further from the source leaf.

In general, it is concluded that the radioactive compound moves along a concentration gradient of the whole assimilate, labelled and unlabelled. It does not move uniformly along its own concentration gradient.

I. INTRODUCTION

This paper is concerned with the movement of compounds containing $^{14}C$, produced in photosynthesis by the soybean (Glycine max. (L.) Merr.). Emphasis has been placed on the investigation of the spatial distribution of assimilate in plants bearing several mature trifoliate leaves, and on changes in the pattern of distribution when the plant is subjected to treatment such as partial defoliation or masking from light.

II. METHODS

Plants of soybean (cv. Biloxi in most experiments) were used because the large thin leaves and long internodes are satisfactory for radioautography. They were grown in pots in a greenhouse under variable environmental conditions. The labelled assimilate was produced by allowing part of a leaf to photosynthesize in an atmosphere containing $^{14}CO_2$. The isotope was supplied as barium carbonate from the Radiochemical Centre, Amersham, England, appropriate mixtures being obtained by further dilution with inactive barium carbonate. Great care was exercised to obtain a uniform mixture. The carbonates were mixed in small amounts at a time, and ground together with a glass rod on a ground-glass surface; the final mixture was given prolonged grinding in a mortar and pestle and samples of 1 mg checked for similarity by counting. The specific activity of the mixture used is stated for each experiment.

The carbon dioxide was usually supplied locally to part of a leaflet only, in an assimilation chamber made from a 10-ml weighing bottle with an open end usually of cross-sectional area 1 sq. cm. The initial carbon dioxide concentration in the assimilation chambers was calculated to be 1 per cent. A 1-mg sample was weighed into a chamber after the ground edges of the open end had been coated with porometer luting wax (a mixture of colophony resin, "Vaseline", and small pieces of rubber tubing, heated to melting, stirred, and cooled). The chamber was held in a clamp just under the leaf surface.

An excess of 1x HCl was delivered to the bottom of the exposure chamber with a hypodermic syringe and the abaxial side of the leaf immediately sealed to the exposure chamber. The whole plant (with the exception of any masked region) was exposed to sunlight for a specified period, usually either 1 or 2 hr. On dull days the daylight was supplemented by light from a bank of white fluorescent discharge tubes. In most experiments the intensity ranged from 500 to 2000 f.c. Under these conditions it was shown (by transferring the chamber from leaf to leaf at intervals of 20 min) that most of the $^{14}CO_2$ in the chamber was lost in 40 min, and that insignificant amounts remained after 2–2½ hr.
It was necessary to find a method of tissue drying which would minimize the movement of assimilate after the experiment proper had ceased. Preliminary tests showed that freeze-drying is a satisfactory method when the detailed distribution of isotope within a single organ is required. It is, however, inconvenient for experiments using many large plants, and it often leads to distortion of the leaves. Even when light pressure is applied to the leaf during freeze-drying, the distortion is such that intimate contact of a leaf with the X-ray film is not possible. For the purposes of this work we relied mainly on the immediate dissection of the plant, after its illumination, into suitable pieces (e.g. half leaves, leaf segments, internodes) and the air-drying of the separate pieces between clean sheets of absorbent paper maintained under light pressure. This method is simple and satisfactory for the study of the movement of assimilate from one organ to another, or movement within an organ which is dissected before drying (Thaine and Walters 1955).

In the preparation of radioautographs the segments of dried leaves, stems, and roots were placed on sheets of paper 5 by 7 in., their outlines traced, and other identifying details noted. The paper with the specimens was then placed between two sheets of \( \frac{1}{4} \)-in. glass of the same dimensions. Finally, in the darkroom, using a No. 6B Wratten filter, a piece of "Kodirex" (no-screen) duplitized X-ray film was placed in contact with the tissue and the whole held firmly with spring-back clips. These plates were kept at \( 1^\circ \text{C} \) for 14 days in a light-tight tin. Development was with the manufacturers' developer for 3 min at \( 20^\circ \text{C} \), and an acid fixing solution was used. Contact prints were made from the radioautographs, using lightweight glossy paper of high contrast, exposed for 1–3 sec, 5 ft from an unshaded frosted 300-W incandescent globe. With perfectly dry clean tissue no difficulty was experienced from pseudophotographic effects.

The absorbent paper used for drying was checked for activity by placing it in contact with X-ray film for 14 days. In no case could any activity be shown to have moved from the active soybean tissue into the drying paper.

In some experiments we have supplemented the information given by radioautographs by measuring radioactivity by means of a Geiger–Muller counter and scaler. For the experiments described here we have not measured total or soluble radioactive material in any tissue. Counts have been made on known areas of thin dried tissue. Owing to self-absorption, the values obtained are assumed to give only the same information as would accurate measurement of the density of the radioautograph. They thus give quantitative expression to the radioautograph and allow tabling and the statistical treatment of the results.

The vascular anatomy of the soybean stem and petiole was studied by serial hand-sections, examined fresh, and stained.

III. The Vascular Anatomy of the Soybean

All the leaves in the soybean plant are linked by vascular connections. Transverse sections of the stem show that, whilst there are usually about 10 xylem bundles, there are 50–55 small phloem strands fairly evenly spaced around the stem. Longitudinal sections of the stem show these phloem strands to be connected by
numbers of sieve-tube bridges passing diagonally between them. Sections of the petiole show a similar construction throughout most of its length. In the pulvinar region, the vascular strands of the petiole come together to form one large central contracted bundle, the outer part of which is a continuous annulus of phloem tissue. This bundle divides into three and the three petiolar strands enter the axis stele at three points, one immediately opposite the petiole and the other two at points 90° to each side of it.

Thus, in the stem the phloem tissue does not run in strictly defined longitudinal bundles, but rather is a loose anastomosing network of sieve-tube strands, the three strands of petiolar phloem entering this network at three points well separated on its circumference.

IV. RESULTS AND CONCLUSIONS

(a) The Redistribution of Labelled Translocate in Whole Plants

When part of a green leaf is exposed to light, for at least 1 hr, in the presence of $^{14}$CO$_2$ (specific activity 0·6 mc/m-mole), the radioautograph of the site of application appears in the negative as a black circle or square with a diffuse margin (c.f. Plate 2, Fig. 3). A considerable proportion of the labelled material is fixed at the site as immobile compounds, but some of it is mobile in the veins and moves out of the leaf during the experiment. Movement within the source leaf will be discussed later. During the course of photosynthesis for 1 hr, isotope moves from its site of application to the root tips, stem, apex, and youngest unfolded leaves. In the growing organs (stem and root tips and young leaves), the translocate appears to enter all the cells. In the mature source leaf, the stem, and the root it is located mainly in the veins, which usually show clearly on the radioautograph. However, there is always some movement from the veins to the surrounding tissue during air drying. Sometimes the radioautographs also show an accumulation of isotope at the cut ends of the veins, indicating some mass flow of material on cutting the phloem or during the early stages of drying.

(i) Experiment I-1 (specific activity 0·6 mc/m-mole).—Eight soybean plants were used, each with two fully expanded trifoliate leaves. $^{14}$CO$_2$ was applied to the uppermost fully expanded leaf, in four cases to the terminal leaflet, and in four cases to one or other of the lateral leaflets (Fig. 1). The period of exposure was 2 hr, the light intensity 700–1000 f.c. At the end of the exposure plants were immediately dissected, air dried, and radioautographed. This experiment was repeated twice, and for a total of 24 plants the radioautographs showed an identical pattern of distribution (Plate 1). The labelled assimilate moved to the apex and to the root (where it was concentrated in the root apices) and to the immature leaf above the source leaf, but not (in amounts necessary to produce a radioautograph) to the primary leaves or to the fully developed trifoliate leaves, or to the other leaflets of the source leaf. It is concluded that, for the purposes of these experiments, it is immaterial which leaflet of a source leaf acts as the site of $^{14}$CO$_2$ application.

During the course of the experiments described in later sections of this paper it became apparent that the age, size, and position of each source leaf in relation
to apex and root were important factors concerned in the direction of the movement of labelled assimilate.

The pattern of redistribution of assimilate in whole undamaged plants was clarified by the results of the following experiment.

(ii) Experiment I-2 (specific activity 0.6 mc/m-mole).—Twelve similar soybean plants were used (Fig. 1, A–D). Each carried two simple primary leaves (1), two fully expanded trifoliate leaves (2, 3), and one expanding leaf (4), whose length varied between 20 and 90 per cent. of the mean adult leaf length. The apex and each type of leaf served as application sites for $^{14}$CO$_2$ and each site (including the apex) subsequently gave dense radioautographs. It was necessary to use an exposure chamber of modified shape for application of the gas to the apex. Exposure to $^{14}$CO$_2$ was for 2 hr at a light intensity of 2000 f.c., the remainder of each plant being similarly illuminated in air. At the end of the exposure period the plants were dissected, air dried, and radioautographed. The results are summarized in Table 1.

These clear but qualitative results were supplemented, in the same experiment, by counts made on approximately equal areas (1 in. sq.) of the dried plant tissue taken from different organs. The values obtained showed that the total amount of labelled assimilate formed and translocated under the same conditions varied according to the age of the tissue used as the site of application (Table 2(a)).
Distribution of the translocate between different parts of the plants was, therefore, calculated as a percentage of the total translocate for each plant (Table 2(b)). The results support those summarized in Table 1, and serve to indicate still more clearly the pattern of distribution.

The results of this experiment are substantiated by those of numerous other experiments carried out with different objects in view. Clearly, the radioactive fraction of the assimilate does not diffuse through the plant along its own concentration gradients. If we make the assumption that it acts as a tracer for the untagged assimilates, the results lead to the following conclusions.

For the lowermost leaves the major “sink” for assimilation is the root; for the uppermost leaves it is the apex and young expanding leaves. Leaves in a median position supply both apex and root. The apex and young expanding leaf of below 60 per cent. adult length show no export of active assimilate, the expanding leaf of greater size exports an increasing amount of assimilate, primarily to the apex.
TRANSLOCATION IN THE SOYBEAN

TABLE 2
RESULTS OF EXPERIMENT I-2 AND COMPARISON WITH OTHER DATA

(a) Relative amounts of total translocate provided by leaves of different age and type (see Fig. 1). The values are based on mean counts per min in all organs other than the source leaf.

<table>
<thead>
<tr>
<th>Leaf 1</th>
<th>Leaf 2</th>
<th>Leaf 3</th>
<th>Leaf 4: Degree of Expansion</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>90</td>
<td>100</td>
<td>85</td>
</tr>
</tbody>
</table>

(b) The distribution of labelled assimilate in the whole plant (see Fig. 1).

<table>
<thead>
<tr>
<th>Leaf No.</th>
<th>Percentage of Total Translocate Counted Found in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(site of application of $^{14}$CO$_2$)</td>
<td>Root</td>
</tr>
<tr>
<td>1</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
</tr>
</tbody>
</table>

(c) The distribution of labelled assimilate in the whole plant of soybean (data of Belikov 1958a). Values are $^{14}$C activity of plant part (counts/min/disk) after photosynthesis for 20 min expressed as percentage of total assimilate.

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Mature Source Leaf:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf 1 (primary)</td>
</tr>
<tr>
<td>Stem above source leaf</td>
<td>20</td>
</tr>
<tr>
<td>Stem and root below source leaf</td>
<td>80</td>
</tr>
</tbody>
</table>
Thus, a leaf, in passing from its position as a small folded leaf at the apex, through the stages of expansion to a fully expanded leaf, and possibly (by abscission of lower leaves) to the position of lowest leaf, shows also a steady change in its economy.

As a young leaf, up to 60 per cent. of adult length, it forms part of the major apical sink for upward-moving assimilate and for its own photosynthetic product; it exports nothing to the rest of the plant. Above this size it steadily loses its function as part of the apical sink and increases in importance as an exporting organ. The destination of material moving from such a leaf once it is fully expanded is both apex and root, predominantly the apex when the leaf is in a position close to the apex, predominantly the root as growth takes the apex further from the leaf.

In experiment I-2 there was no evidence of the import of labelled assimilate into mature leaves. Although a major role of the fully expanded leaf is that of an exporting organ, it will later be shown that it may import small amounts of assimilate from other leaves on the plant.

These conclusions are consistent with and supplement other results. Belikov (1955) found that labelled assimilates accumulate in the growing tips of stems and in young leaves of the soybean. Aronoff (1955) stated that for the same species the "movement of labelled photosynthate is greatest to the growing regions." Nelson and Gorham (1957a) showed that the labelled photosynthate produced in the primary leaf moved mainly downwards towards the roots. Vernon and Aronoff (1952) applied $^{14}$CO$_2$ to the oldest trifoliate leaf of soybean and found that, while it moved both to apex and base, "the predominance of activity moved basipetally."

Crafts (1956) states that, in cotton and Cucurbita, the upper leaves exported applied radioactive 2,4-D mainly to the apex, the lower leaves mainly to the base (roots). He assumed (without direct evidence) that this was also the pattern of redistribution of the assimilates. In earlier work, Goodall (1946) measured dry weight changes in tomato leaves, attached and excised. The youngest leaves provided little assimilate and acted as sinks for material coming from the older leaves. Translocation went on during photosynthesis; and in summer one-half of the translocate from the older leaves moved to the root and only one-eighth to the youngest leaves. In his experiments all the leaves were exposed to a CO$_2$ concentration of 0.03 per cent. In our experiment the site of application received much higher CO$_2$ concentration than did the rest of the plant. The general similarity of the two results suggests that the technique we used was not responsible for the pattern found.

In work published after the completion of our own experiments, Belikov (1958a, 1958b) also applied $^{14}$CO$_2$ to successive leaves of soybean plants and subsequently measured the radioactivity in parts above and below the source leaf. His results are summarized in Table 2(c). His conclusions are closely similar to ours, that the distribution of assimilate is localized, that mature leaves do not import, that from the upper leaves assimilate moves to the apex and young leaves, from the lower leaves it moves to the root, from the middle leaves it moves both up to the apex and down to the root.
These results all indicate the complexity of the problem of translocation in whole plants, and the need for the close matching of replicate plants used for experiments with radiocarbon.

(b) Transport to the Apical Bud

Experiments on the translocation of food materials from mature leaves to the apical bud seldom appear in the literature of translocation. Nevertheless, such movement must be one of the major pathways of the assimilated compounds. Our experiments have shown that when $^{14}$CO$_2$ is supplied in the light to a mature leaf of a normal soybean plant, tagged assimilate moves rapidly to the apical bud. We assume that such leaves, when illuminated normally in air, supply carbohydrate to

![Diagram](Fig. 2.—Plan of experiment II-2.)

the apex, which also presumably synthesizes additional carbohydrate in its own green cells. The movement of tagged assimilate to the apex was, therefore, investigated under conditions in which the green apical bud was either masked or illuminated, and in plants in which the leaves between apical bud and source leaf were either masked or removed before the experiment began. All three treatments (masking of apex, masking of leaves, removal of leaves) could be expected to lower the concentration of untagged carbohydrate in the apical region.

(i) Experiment II-1 (specific activity 0·04 mc/m-mole).—In this preliminary experiment, with only two plants, visual inspection of the apex radioautographs suggested that masking the apical region of a defoliated plant (as in Fig. 2, B) reduced rather than increased the import of tagged assimilate into the apex. Further experiments were designed to investigate this unexpected result.

(ii) Experiment II-2 (specific activity 0·04 mc/m-mole).—Eight soybean plants were used (Fig. 2), four with two fully expanded trifoliate leaves (A, B, C, D) and four with two fully expanded and one expanding leaf (E, F, G, H). In each group the plants were carefully selected so as to have apical leaves of uniform size. The plants were defoliated immediately before the exposure to $^{14}$CO$_2$ began, leaving only the apical bud and one primary leaf. Four plants (B, D, F, H) were masked above the primary leaf, the mask covering stem and apex, as shown in Figure 2. Exposure to $^{14}$CO$_2$ began at 3 p.m. and ended at 5 p.m. Light intensity was approximately
600 f.c. The application sites on the radioautographs showed uniformly high activity, much of which was probably due to radioactive starch and other insoluble materials. As might be expected from the position of the leaf of application, comparatively small amounts of tagged isotope moved to the apical parts of the stems. Radioautographs of the apices and of the upper parts of the stem clearly confirmed the result of experiment II-1. Those of the illuminated apices and stems gave much clearer radioautographs than those in which these parts had been masked from light during the experiment (e.g. see Plate 2, Fig. 3).

![Diagram](image)

Fig. 3.—Plan of experiment II-4.

(iii) Experiments II-3, II-4, and II-5 (specific activity 0.04 mc/m-mole).—In these three experiments the leaves between apex and source leaf were neither removed nor masked. In all there were 20 plants, and in half of them the apical bud was masked. Figure 3 shows the plan of experiment II-4. In all 20 plants (except Fig. 3, G) there was at least one expanded trifoliate leaf remaining between the site of $^{14}$CO$_2$ application and the stem apex. The application sites were on one or other of the two oldest trifoliate leaves. No consistent differences were found in the intensities of the radioautographs of the 20 apices.

(iv) Experiments II-6, II-7 (specific activity 0.04 mc/m-mole).—In experiment II-6 there were eight plants, each originally with two fully expanded trifoliate leaves. In all of these the application site was a primary leaf. In one group (A) the four plants were normal, in group B they were defoliated except for the expanding leaf and the source leaf. Immediately prior to the period of exposure to $^{14}$CO$_2$ (3 hr) three plants from each group were apically masked.

Experiment II-7 was a repetition of II-6, except that all eight plants originally had only one fully expanded trifoliate leaf, and the exposure period was for 2 hr. Light intensity in both experiments was approximately 700 f.c.

Comparison of the radioautographs obtained in both experiments showed that the removal of the fully expanded leaves undoubtedly increased the amount of tagged assimilate moving into both the stem and the apex from the site of application. In those plants with all the leaves attached, masking of the apex caused no detectable change in the import to the apex. This result confirmed those of
experiments II-3, II-4, and II-5. On the other hand, a clear confirmation of experiments II-1 and II-2 was not obtained. In defoliated plants, masking of the apex clearly decreased import to the apex in experiment II-6, but in II-7 the differences in the radioautographs were too slight to allow any distinction to be drawn between them.

(v) Experiment II-8 (specific activity 0.04 mc/m-mole).—Seventy-two soybean plants were used, each of which had three fully expanded trifoliate leaves. The experiment was carried out in a greenhouse over a period of 9 days, at a mean light intensity of 1500 f.c. The plants were divided into three groups \((L_i, L_m, \text{ and } L_o)\), and for convenience of operation, eight members of two of these groups were dealt with at one time in a so-called "block". There were nine blocks, each consisting of eight plants arranged in pairs. The treatments are set out in Table 4. The \(^{14}\text{CO}_2\) was applied for 3 hr to the lowest trifoliate leaf of each plant in a block. In groups \(L_m\) and \(L_o\), the two mature leaves between source leaf and apex were masked from the light \(L_m\) or cut off \(L_o\) 24 hr prior to the application of the \(^{14}\text{CO}_2\). Masking was continued during the 3 hr of the experiment. The apices were masked 1 hr prior to and during the assimilation of the \(^{14}\text{CO}_2\). At the end of the 3-hr period the apices were cut off, air dried under pressure, radioautographed, and the radioactivity counted. The results are set out in Tables 3 and 4. The results were submitted to a statistical analysis (see Appendix I).

(vi) Experiments II-1–II-8: Conclusions.—In experiment II-8, the radioautographs (Table 3) and the mean apical counts of \(^{14}\text{C}\) (Table 4) for groups \(L_i\) (all leaves attached and illuminated) and \(L_o\) suggest that the removal of the mature leaves, between source leaf and apex, results in a greatly increased movement of the tagged assimilate to the apex. There were considerable variations in the counts; nevertheless, the analysis of variance showed that this effect is highly significant (see Appendix I). It was concluded that the removal of the mature leaves increased the amount of radioactivity in the apex by about 60 per cent. This result confirms that obtained qualitatively in experiments II-6 and II-7.
Mature illuminated leaves have been shown to import negligible quantities of $^{14}$C from a source leaf under the conditions of this experiment. Hence the results just described cannot be due to the receipt by the apex of $^{14}$C which was prevented from entering the mature leaves because these were excised. We have adopted the hypothesis that the increased export of $^{14}$C to the apex was due to the lowering of the concentration of total assimilate in the apex, consequent upon the removal of

---

**Table 4**

**Plan and results of experiment II-8**

Values given are apical counts of $^{14}$C, expressed as counts/minute corrected for background

<table>
<thead>
<tr>
<th>Block No.</th>
<th>Group $L_i^*$</th>
<th>Group $L_m^+$</th>
<th>Group $L_o^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apex Masked</td>
<td>Apex Illuminated</td>
<td>Apex Masked</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>6</td>
<td>44</td>
</tr>
<tr>
<td>Mean all plants</td>
<td>4.9</td>
<td>6.4</td>
<td>11.2</td>
</tr>
</tbody>
</table>

$L_i^*$ all leaves attached and illuminated.

$L_m^+$ all leaves attached, upper two (between source leaf and apex) masked.

$L_o^+$ upper two leaves (between source leaf and apex) removed.
the two mature leaves. For in the control plant such leaves, illuminated in air, would be expected to export untagged assimilate to the apex.

In experiment II-8 (Table 4) the means of counts for groups \(L_1\) and \(L_m\) suggested at first that masking of the leaves between source and apex also increased movement to the apex. However, the differences, although large, did not reach statistical significance. One would expect masking to have a similar effect to defoliation, but it may be that the effects of masking are two-fold and counter-balancing. While the darkened leaf (like the excised one) could export little assimilate to the apex, the possibility exists (see Section IIIc) that it could import \(^{14}\text{C}\) from the source and thus divert it from the apex.

If the hypothesis set out above is correct, then one would expect that masking of the green apex alone would increase its import of \(^{14}\text{C}\) from the source leaf. No such increase has been detected. Rather the sum total of numerous experiments suggests the opposite—that masking the apex decreases the import. Qualitative support for this was given by the radioautographs of experiments II-1, II-2, II-6, and II-8 (but not II-7), in plants of which the leaves between source and apex were removed. On the other hand, in experiments II-3, II-6, II-7, with all leaves present, there was no such decrease due to masking. Finally, when the matter was put to quantitative test (expt. II-8, Table 4), while the mean counts were smaller for the apically masked plants in all three groups, the differences were not statistically significant. It appears that the apical masking effect, if present at all, is too small to be detected except possibly by precise counting methods applied to a simplified biological system, i.e. largely defoliated plants in which downward movement of assimilate to the root is prevented. Vernon and Aronoff (1952) have reported that light has no direct effect on translocation. Nelson and Gorham (1957a) have noted that a smaller amount of \(^{14}\text{C}\) is transported to the stem apex in the dark than in the light, but in their single experiment the whole plant was darkened after a 15-min light period. Moreover, the two plants used were not evenly matched.

(c) Translocation in the Leaf

(i) Export from Masked Leaves

Two experiments were designed to test the assumption that leaf masking decreases the concentration of the mobile products of photosynthesis in the leaves and in the phloem of the leaf veins.

(1) Experiment III-1 (specific activity 0·04 me/m-mole).—Eight similar plants were selected, each with two fully expanded trifoliate leaves. On each the terminal leaflet of the first leaf was masked for 24 hr. It was then illuminated for 3 hr, at 300 f.c. and while the \(^{14}\text{CO}_2\) was being applied to a central area. The exposure chambers were then removed and the leaves immediately masked again. Leaves were harvested at 0, 1, 2, 3, 4, 5, and 6 hr from this second masking. Each leaflet was severed from its petiole and cut transversely just below the site of application of \(^{14}\text{CO}_2\). The upper segment was then air dried and radioautographed. In Plate 2, Figure 1, the appearance of the eight central brighter areas (application sites) gives us no information about total export during the second masking. There is no
regular decrease in the activity with time—possibly there was uneven uptake. On the other hand, there is radioactivity in the veins passing out from the central areas in all eight figures. Although this was weaker in the samples masked for 6 hr, it is clear that the export of readily mobile material was not complete even after this period.

(2) Experiment III-2 (specific activity 0.04 mc/m-mole).—The procedure was similar to that described above, but the photosynthetic period was 2 hr at 500 f.c. and a longer period was used for the second masking, leaves from two plants being harvested at 4, 24, 48, and 72 hr. Before air drying, each leaflet was cut transversely into three segments and in Plate 2, Figure 2, the radioautographs of the central segments only are shown.

Lateral spread of the isotope was detectable (Plate 2, Fig. 2) in radioautographs of leaves harvested after masking for 4 hr. There was no activity in the peripheral veins of the leaf segments harvested after masking for 24 hr or longer, this suggesting that, after this period, the readily mobile or mobilizable products of a 2-hr photosynthesis period had been exported from the application site. This conclusion is valid even if some of the movement takes place during air drying, and in spite of the fact that the activity of the central area (site of application) showed no consistent decrease with time. The essential features that emerge from these two experiments are that (a) in leaf segments darkened for 24–72 hr and still with high radioactivity at the site, there is no activity detectable by radioautography in the veins leading from this site; (b) there is such activity in the peripheral veins of such segments darkened for only 0–6 hr. We know from iodine tests of this and parallel material that starch is all lost from the site after darkening for 24 hr. It may, therefore, be presumed that the activity shown in the central areas of the leaflets darkened for 24–72 hr is from relatively insoluble (or immobile) material which is not starch.

It is concluded that the concentration of readily mobile assimilate in the veins of a soybean leaf masked for 24 hr is lower than the concentration in the veins of an illuminated part; the magnitude of the difference is unknown.

(ii) Transport into Leaves

Carbohydrates synthesized in mature leaves pass from the leaf to the regions of growth and development. If the movement is necessarily along a positive concentration gradient in the phloem, reversal of the normal gradient might be expected to cause reversal of movement, e.g. import into a mature leaf. The question of import by mature leaves is relevant in any consideration of translocation theory and the results of a preliminary study of it are now presented.

(1) Experiment III-3 (specific activity 0.04 mc/m-mole).—Six soybean plants (A–F) were prepared according to the diagram (Fig. 4). The plants had four fully expanded trifoliate leaves and one very small expanding leaf. In plants A, B, and E, these young leaves, together with the stem apices, were removed just prior to the application of the $^{14}$CO$_2$. This eliminated the major sink for material coming from the uppermost mature leaf, which was the source. In B, D, E, and F, all the
other leaves were masked for 48 hr before the application of the $^{14}$CO$_2$ (light intensity c. 500 f.c. for 4 hr). All leaves were harvested separately after another 20 hr had elapsed, so that the total time of shading was 72 hr.

Radioautographs of the harvested leaves showed that in five of these plants ($E$ was damaged during the experiment) there was no detectable import of tagged assimilate into either masked or illuminated fully expanded leaves, irrespective of the presence or absence of the apex and expanding leaf. Judging visually from the radioautographs, it could be assumed that the amount of $^{14}$CO$_2$ fixation was relatively uniform in the five plants, and in $A$ and $B$ removal of the apical parts of the stem led to an increased transport of tagged assimilate to the root. There was no $^{14}$C activity detectable in either the mesophyll cells or the veins of the harvested “receptor” leaves of any of these plants. Nor was there any activity detectable in the lateral (illuminated) leaflets of the “source” leaf.

This result is typical for experiments of this type, in which the specific activity at the source was low ($0.04$ mc/m-mole). In experiment I-1 (Plate 1), no leaves were masked, but 25 plants have been used in experiments in which mature leaves (usually two) were masked for 24 hr, the $^{14}$CO$_2$ being applied to another mature leaf for the last 2 hr of this period. None of the masked leaves gave a radioautograph, whereas in all cases there was very marked activity around the $^{14}$CO$_2$ application site, and tagged assimilate was shown to move to the apex and expanding leaf (if present) and to the main stem and root.

The apparent failure of these mature leaves to import assimilate could have been due to the absence of vascular connection between the illuminated leaf and the masked leaves. This was considered improbable for the following reasons:

(a) The movement of tagged assimilate from mature leaves to young expanding leaves (e.g. expt. I-1). The translocation pathway thus demonstrated (and presumed to be phloem) is not likely to be broken as the leaf
matures, for mature leaves can all export assimilate to the stem, apex, and root.

(b) The lack of import shown by all five masked leaves of plants such as Figure 4, D; at least one of these could be expected to be linked via the phloem to the exporting leaf.

(c) Experiments with aqueous eosin showing that there are vascular (xylary) connections between all the leaves of a plant with three fully expanded trifoliate leaves. Regardless of which leaf was cut under eosin solution, the dye moved in the xylem to the veins of all other leaves.

(d) The anatomical peculiarities of the phloem tissue in the soybean, briefly described in Section III.

In the experiments already described, the specific activity of the barium carbonate used was low (0.04 mc/m-mole). It was adequate to demonstrate movement of assimilate from the leaf to the veins of the stem, to the root, the apex, and to leaves which had expanded to less than 85 per cent. of their full size. If there is some movement to the mature leaves, it should be demonstrable by raising the activity at the source. In the following experiment this was tested by using barium carbonate mixtures of three different specific activities, the total carbon dioxide concentration in the exposure chamber remaining at 1 per cent. in each case.

(2) Experiment III-4 (specific activities 0.04, 0.6, and 17 mc/m-mole).—Twelve plants were used, each having two mature trifoliate leaves and two primary leaves, the $^{14}$CO$_2$ being applied to a leaflet of a mature trifoliate leaf. No masking treatment was given these plants, and the light intensity was 700 f.c. The plants were dissected at the end of the experiment and the whole plant radioautographed. The radioautographs of source leaflet, apex, root, and stems showed increasing density with the increasing specific activity at the source; those with the highest activity were overexposed.

No indication of activity in the mature leaves could be seen in the group which had received the $^{14}$CO$_2$ at the lowest specific activity. In the medium activity group (0.6 mc/m-mole) very faint prints of the lateral leaflets of the source leaf could be seen, and scarcely perceptible prints of the basal parts and midrib in other mature leaves (trifoliate and primary).

In the group receiving the highest activity (17 mc/m-mole), the densest radioautographs were still those of the apex, but all the mature leaves now gave faint prints, the activity being greater in the veins than in the mesophyll (Plate 2, Fig. 4).

The total concentration of carbon dioxide at the source leaf was the same in this as in earlier experiments, and there is no reason to suppose that the import into these mature leaves is via the xylem. The result is consistent with the view already expressed, that there is phloem connection between individual leaves in the soybean. The extent of import into mature leaves now demonstrated is still small in comparison with that into apex or root. The radioautograph of Plate 2, Figure 4, suggests that the tagged material moves not only into veins, but also into the mesophyll cells of mature leaves. This latter, however, could be movement during
(iii) Movement within Single Leaves: Leaves Attached to Whole Plants

(1) Experiment III-5 (specific activity 0·04 mc/m-mole).—The plan and results of this experiment are shown in Figure 5. Two growing plants were used and certain leaf areas were masked for 24 hr. The $^{14}$CO$_2$ was then applied for 4 hr in the usual way to each of three locations on plant $A$ and five locations on plant $B$. During this period the unmasked parts were illuminated in sunlight (approx. 500 f.c.). Leaves or leaflets bearing masks were then excised, freeze-dried, and radioautographed. In this experiment there was considerable distortion of the leaves during freeze-drying, and diagrams are used instead of photographs to summarize the results.

As Figure 5 shows, under conditions in which there is always extensive movement from the source leaves to roots and stem apices, there was no movement of tagged assimilate into the masked primary leaves $A_1$, $B_1$, or into the upper halves of masked leaves $A_2$, $B_2$, $A_3$, $A_4$. In plant $B$, such movement as occurred into the central masked area appeared to be from the apical application site downwards towards the petiole. The outer margins of the masked areas were sharp, indicating that movement into these areas, if it occurred, was via the veins, not the mesophyll cells. On the other hand, the application sites within the illuminated areas were not so clearly defined, and we assume that this was due to some photosynthesis of $^{14}$CO$_2$ diffusing outwards in the leaf from the site of application (see expt. III-7).

(2) Experiment III-6 (specific activity 0·04 mc/m-mole).—This experiment was similar to III-5, but the $^{14}$CO$_2$ was applied to one leaf only (as in Fig. 5, $A_4$ only), just below the masked area. Total period of masking was 49 hr, the $^{14}$CO$_2$ being applied for the last 3 hr of this period at a light intensity of 500 f.c. The leaves were air dried after having been cut into halves along the line of the masks and then...
severed from their petioles. Typical radioautographs of two of five plants used are shown in Plate 3, Figures 1 and 2.

In plant A (Plate 3, Fig. 1), in which all the leaves were green and healthy, there was no movement of isotopic material into the unmasked primary leaves, the unmasked lateral leaflets, or into the masked upper half of the source leaf. This was in agreement with experiment III-5. In plant B (Plate 3, Fig. 2) again there was no movement into the unmasked primary leaves, and very little into the unmasked lateral leaflets (the slight basal activity here could have been due to leakage of $^{14}\text{CO}_2$ from the nearby source). However, there was in the terminal-source leaflet marked acropetal movement into the masked apical half and accumulation of activity around small chlorotic spots, believed to be caused by nutrient imbalance in the potting soil.

(3) Experiment III-7 (specific activity 1.06 mc/m-mole).—Eight healthy, mature, attached leaves (on three plants) were used. The specific activity at the source was high and the experiment was done on a bright sunny day in midsummer (light intensity 2000 f.c.). The application sites were on opposite lateral leaflets, one fully illuminated, the other completely masked except for the application site (Fig. 6(a)). Four leaves were dried before, and four after, being cut into segments transversely across the edges of the application site.
Unless the leaf is both masked as shown and segmented before drying, the radioautographs give a misleading picture of translocation, suggesting that the tagged assimilate moves both acropetally and basipetally to the same extent, and also crosses the main vein at least in the distal parts. A great deal of this movement, however, takes place during air drying (and even freeze drying), while a consider­able part of the activity outside the application area proved to be due to photosynthesis of $^{14}\text{CO}_2$ diffusing from this area into the rest of the leaf. In the only radioautograph shown (Plate 3, Fig. 3) both artefacts have been avoided (by masking and cutting) and the major movement has clearly been basipetal in the major veins. Movement across the main vein was inconsiderable. There was still considerable lateral movement during air drying in half of the central sector.

(4) Experiment III-8 (specific activity $0.04 \text{mc/m-mole}$).—Six large plants were used, each with five fully developed trifoliate leaves, all of which were illuminated in air during application of $^{14}\text{CO}_2$ to the apical leaflet of the youngest mature leaf. The site was central (Fig. 6(b)) or lateral, and the leaflet was masked in its upper half. In addition, the stem apex was removed 48 hr before the beginning of the experiment. Light intensity was high, 1000 f.c., and the period of $^{14}\text{CO}_2$ application was 4 hr. Under these conditions it was considered that the gradient of mobile assimilate between the lower and the masked halves of the terminal leaflet would be steeper than in the experiments already recorded, although the root still existed as a sink. At the end of the experiment the terminal leaflet was removed and cut into two just above the line of the mask; the parts were air dried, radioautographed, and the result shown in Plate 3, Figure 4, is typical of those obtained from the six plants.

It is clear that under these conditions tagged assimilate moved acropetally into the veins of the masked leaf tip. There was little movement across the main vein into the other half of the leaf.

(iv) Movement within Single Leaves: Leaves Detached from the Plant

(1) Experiments III-9—III-14 (specific activity $0.04 \text{mc/m-mole}$).—In each experiment there were eight trifoliate leaves, four partially masked, four unmasked, as shown in Figure 6(c). While still attached they were pre-illuminated in air (except for the masked areas) for periods stated in Table 5. At the end of this period tests showed that the illuminated tissues contained starch, while the masked portions were free of starch. After this pretreatment the petioles were cut under water and the detached leaves were placed with the cut ends of the petioles dipping into water. $^{14}\text{CO}_2$ was applied to the lower surface of the lower half of each terminal leaflet, and after 2 hr exposure the leaflets were separated, the terminal leaf cut into two just above the application site (and along the line of the mask when present). The pieces were air dried and radioautographed. Six of these experiments were carried out, the conditions and results being summarized in Table 5.

It is shown, therefore, that under conditions where the normal sinks have been removed, tagged assimilate can move acropetally within a single leaflet and from the apical leaflet into a lateral leaflet of a trifoliate leaf. This latter demonstrated again that there is a phloem connection between the leaflets. Such acro-
petal movement, however, was only rarely obtained when the importing leaf part was illuminated in air (a total of six cases out of 48, and in these six the movement was slight, the radioautograph being faint). Acropetal movement into the masked apical halves of leaves or into masked leaflets was clearly shown in 27 out of 48 cases.

The considerable variation in the behaviour of comparable samples appears to be characteristic for experiments on translocation. It is known that the contents of the sieve tubes are under pressure: the possibility, therefore, exists that the failure of movement into nearly half the masked lateral leaflets was due to damage to the phloem at the nodal region consequent upon cutting the petiole.

### Table 5
**Detached Leaves of Soybean: Distribution of Labelled Assimilate**

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Period of Pre-exposure in Air of Unmasked Leaves to Light (hr)</th>
<th>Light Intensity during $^{14}$CO$_2$ Application (f.c.)</th>
<th>Proportion of Leaflets in each Experiment showing Acropetal Movement of Labelled Assimilate into:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leaflet Tips</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Masked</td>
</tr>
<tr>
<td>III-9</td>
<td>5</td>
<td>1500</td>
<td>4/4</td>
</tr>
<tr>
<td>III-10</td>
<td>7</td>
<td>750</td>
<td>4/4</td>
</tr>
<tr>
<td>III-11</td>
<td>24</td>
<td>1500</td>
<td>3/4</td>
</tr>
<tr>
<td>III-12</td>
<td>24</td>
<td>1000</td>
<td>2/4</td>
</tr>
<tr>
<td>III-13</td>
<td>24</td>
<td>1200</td>
<td>2/4</td>
</tr>
<tr>
<td>III-14</td>
<td>24</td>
<td>500</td>
<td>2/4</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>17/24</td>
</tr>
</tbody>
</table>

(2) *Experiments III-1–III-14: Conclusions.*—Following early experiments with whole plants and with $^{14}$CO$_2$ supplied at low activity, it was concluded that import into mature leaves was negligible (e.g. expts. I-1, III-3, III-5, III-6). A similar conclusion has been drawn by others. Thus, Aronoff (1955) stated that no flow occurs within a leaflet or to an adjacent mature leaflet merely because of a concentration gradient. “Darkening of leaves for 48 hr prior to $^{14}$CO$_2$ application never has a greater effect than the normal variation”. Vernon and Aronoff (1952) could not demonstrate any movement of radiocarbon into either the primary leaf or the cotyledon. Nelson and Gorham (1957b) showed that “only a trace of $^{14}$C was translocated to the primary leaf opposite to the source leaf”. Belikov (1955) also reported that mature leaves do not import, even if they are deficient in assimilates, and again (1958a, 1958b) that no movement occurs into the shaded part of a source leaf “even although it was dying from a deficiency of light.” All these workers used the soybean. Mason and Maskell (1928) measured the carbohydrate...
content of cotton leaves and concluded—"We infer an import of sugar by darkened (mature) leaves over a period of 24 hours . . . the correlation coefficients are, however, too small to make the inference certain . . . They establish a rather strong presumption that sugar was actually imported into the darkened leaves." In a more direct experiment with sugar-beet (Leonard 1939), plants were darkened for 5 days then transferred to sunlight with some leaves masked. He concluded "If there was any reversal of translocation from the illuminated to the darkened leaves, it was not measurable".

While, therefore, it is generally agreed that mature leaves import little or no sugar, even when darkened, we have now shown that there is no bar to such import. It may be demonstrated even in illuminated leaves or leaflets if the specific activity of the radiocarbon source is raised (expt. III-4). Its extent is usually increased if the leaf is darkened, or if other sinks are removed. Acropetal movement within the source leaf (attached or excised) may also be demonstrated under appropriate conditions, and marked import to necrotic areas suggested (expt. III-6) that high metabolism within a leaf could convert an area of mature leaf tissue into an efficient sink for assimilate. The darkening of healthy leaves lowers the concentration of mobile assimilates (expt. III-1, III-2), but it may be that a high utilization of sugar is necessary before import becomes massive. On the other hand, the relative lack of import into mature leaves may well be due in part (as envisaged by Leonard and others—see Crafts 1951) to an active secretion of sugar into the phloem from the border parenchyma. It is hoped to use radiocarbon to test the possibility that such a secretion is not operative until the leaf is reaching its full size.

V. ACKNOWLEDGMENTS

Financial assistance from Monsanto Chemicals (Australia) Limited and from the Commonwealth Bank (Rural Credits Account) is gratefully acknowledged. We also owe thanks to Dr. R. T. Leslie and Mr. G. W. Rogerson, Statistics Department, University of Melbourne, for Appendix I and for advice on statistics, and to the late Professor E. J. Maskell for his valuable criticisms of a draft of the manuscript.

VI. REFERENCES

EXPLANATION OF PLATES 1-3

PLATE 1

Experiment I-1: application chamber circular in cross section. Note overexposure at site of application of $^{14}CO_2$ (Figs. 2 and 3)

Fig. 1.—Radioautographs of stems: (a) source leaflet lateral; (b) source leaflet terminal.

Fig. 2.—Radioautographs of terminal source leaflet, petiole, apex, and expanding trifoliate leaves. Radioautographs not obtained with lateral leaflets of source leaf or with other mature leaves.

Fig. 3.—Radioautographs of lateral source leaflet, petiole, apex, and expanding trifoliate leaves. Radioautographs not obtained with other lateral or terminal leaflet of source leaf or with other mature leaves.

Fig. 4.—Radioautograph of root system—source leaflet terminal.

Fig. 5.—Radioautograph of root system—source leaflet lateral.

PLATE 2

Figs. 1 and 2.—Radioautographs of segments of soybean leaves: experiment III-1 (Fig. 1), experiment III-2 (Fig. 2). After destarching, each attached leaf was illuminated and $^{14}CO_2$ applied to the central (bright) area. All leaves were then completely masked. They were cut into segments, dried, and radioautographed after the periods (hr) stated. In Figure 2 each segment is approximately the size of the black rectangle.

Fig. 3.—Experiment II-2: radioautographs of source leaflets. Plants $A$ and $C$, apical buds illuminated; plants $B$ and $D$, apical buds masked. The apical bud of plant $B$ was "plated", but did not record a radioautograph.

Fig. 4.—Experiment III-4: radioautographs of source leaflet ($s$). The results show import into mature primary leaves ($p$, $p$), lateral leaflets of source leaf ($l$, $l$), mature trifoliate leaf ($t$). Note that import into the apex ($a$) is much greater than that into the leaves.

PLATE 3

Fig. 1.—Experiment III-6, plant $A$: radioautograph of source leaflet (of which the upper half was masked), the two other leaflets of source leaf, and one of the (stalked) primary leaves. Outlines drawn on negative.

Fig. 2.—Experiment III-6, plant $B$: as in Plate 3, Figure 1, but the source leaflet shows movement of tagged assimilate into the masked upper half and accumulation around chlorotic spots. Both primary leaves are shown to be free of activity, but there is local activity in the lateral leaflets of the source leaf (see text).

Fig. 3.—Experiment III-7: radioautograph of leaf which was completely masked except at the application site, and cut into three segments before air drying. There has been little acropetal movement and no movement across the main vein.

Fig. 4.—Experiment III-8: radioautograph of source leaflet with masked upper half, stem apex removed, showing acropetal movement into masked half, little movement across the main vein.
Plants from experiment II-8 (see Table 4) were compared in a balanced, incomplete block design, two treatments per block, three blocks per replication. In addition, the experiment was replicated three times.

Two apical treatments—apex masked and apex illuminated—were introduced as split plots within the main design. The apical treatment was repeated on duplicate plants within each split plot.

There were thus three independent estimates of variability:

1. Variation between duplicates, for four degrees of freedom in each block, giving a total of 36 degrees of freedom;
2. Within-block variation, after allowing for treatment effects, for 15 degrees of freedom;
3. Between blocks, for 7 degrees of freedom.

Strictly, (1) is appropriate for evaluation of apical treatments, (2) and (3) for leaf treatments, but as differences between (1), (2), and (3) were non-significant, a pooled error variance was used.

The usual analysis for balanced incomplete blocks was applied separately to the two apical treatment series, and subsequently comparisons were made between apical treatments.

As the bulk of the uncontrolled variation was thought to be due to variability between different plants rather than to the random nature of a radioactive count, a logarithmic transformation was applied, corrected zero counts being given an arbitrary value of 0.5.

(a) Numerical Results

The observed errors were as follows:

<table>
<thead>
<tr>
<th>Type of Error</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-block</td>
<td>7</td>
<td>0.114083</td>
</tr>
<tr>
<td>Within-blocks</td>
<td>15</td>
<td>0.119150</td>
</tr>
<tr>
<td>Between duplicates</td>
<td>36</td>
<td>0.291590</td>
</tr>
</tbody>
</table>

These were not significantly different and were pooled to give an error of 0.225569 for 58 degrees of freedom. This was used as error in all tests of significance. A complete analysis of variance is given in Table 6.

(b) Conclusions

1. The effect of masking the apex was not significant.
2. The overall differences between levels of factor \((L)\) were highly significant (at 1 per cent. level). The mean square for \((L_m)\) was not significant, but that for \((L_o)\) was highly significant (at 1 per cent. level). Cutting off the side...
leaves increased the amount of tagged assimilate reaching the apex by about 60 per cent.

(3) Differences between "similar" blocks were significant (at the 5 per cent. level). Thus, some reduction in the error mean square was obtained by carrying out the work in blocks. In view of this, the design adopted in the present experiment seems very suitable for this type of work.

At first sight it seems that conclusion (2) is at variance with the means quoted in Table 4 for the three groups $L_i$, $L_m$, $L_o$. It must be noted, however, that the three-leaf treatments are paired in different blocks. Blocks 1, 2, and 3 are very light in yield; blocks 4, 5, and 6 very heavy; and blocks 7, 8, and 9 quite heavy, and a rough indication of adjusted comparisons is obtained if one doubles the results in blocks 1, 2, 3. The effect is clearly to raise the means for groups $L_i$ and $L_o$, leaving that for group $L_m$ unchanged, and consequently the difference between groups $L_i$ and $L_m$ is reduced while that between groups $L_m$ and $L_o$ is increased. Proper calculation shows that the means 5·7, 14·0, and 16·6 are changed to 4·4, -0·9, and 5·4 (general mean zero), and the conclusion stated above becomes reasonable. The effect is more apparent still when the data have been transformed by taking logarithms, in order to make an analysis of variance valid. Finally, the two orthogonal comparisons between the three-leaf treatments considered most appropriate were $L_i - L_m$, $L_i + L_m - 2L_o$. These correspond to the direct evaluation of masking, and the comparison of plants with and without removal of leaves.

**Table 6**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>D.F.</th>
<th>S.S.</th>
<th>M.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf treatments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masking side leaves</td>
<td>1</td>
<td>0·435380</td>
<td>0·435380</td>
</tr>
<tr>
<td>Cutting off side leaves</td>
<td>1</td>
<td>4·958816</td>
<td>4·958816**</td>
</tr>
<tr>
<td>Blocks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varietal component</td>
<td>2</td>
<td>2·292764</td>
<td>1·146382**</td>
</tr>
<tr>
<td>Remainder</td>
<td>6</td>
<td>3·571101</td>
<td>0·595184*</td>
</tr>
<tr>
<td>Inter-block error</td>
<td>7</td>
<td>0·798583</td>
<td>0·114083</td>
</tr>
<tr>
<td>Apical treatments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masking effect</td>
<td>1</td>
<td>0·311524</td>
<td>0·311524</td>
</tr>
<tr>
<td>Masking effect x leaf treatments</td>
<td>2</td>
<td>0·068601</td>
<td>0·034300</td>
</tr>
<tr>
<td>(eliminating blocks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masking effect x blocks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varietal component</td>
<td>2</td>
<td>0·458610</td>
<td>0·229305</td>
</tr>
<tr>
<td>Remainder</td>
<td>6</td>
<td>0·950654</td>
<td>0·158442</td>
</tr>
<tr>
<td>Inter-block x masking effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>7</td>
<td>0·377982</td>
<td>0·053997</td>
</tr>
<tr>
<td>Within blocks duplicate error</td>
<td>36</td>
<td>10·497288</td>
<td>0·291590</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>24·721244</td>
<td></td>
</tr>
<tr>
<td>Pooled error</td>
<td>58</td>
<td>13·083057</td>
<td>0·225569</td>
</tr>
</tbody>
</table>

*P < 0·05. **P < 0·01.
THAINE, OVENDEN, AND TURNER

TRANSLLOCATION IN THE SOYBEAN

Aust. J. Biol. Sci., Vol. 12, No. 4