# Time-course Study of Translocation of Products of Photosynthesis in Soybean Plants [1, 2]

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## Introduction

In a previous report (7) we have shown that soybean plants, in response to age and culture conditions, selectively translocate varying proportions of $C^{14}$-labeled sucrose, serine-glycine, and malic acid from among the products of photosynthesis in a primary leaf exposed to $C^{14}O_2$ for 10 minutes. Regardless of age or culture conditions or the amount or type of compound exported, total radioactivity in the stem decreased logarithmically with distance from the input node to the root. Moreover, the concentration of radioactivity at the input node did not vary significantly in spite of some relatively large changes in the total export in the 10 minutes interval of these experiments. Further information on these observations drawn from short-term experiments required data which could be obtained only by extending the duration of the experiments.

Logarithmic gradients of translocated radioactivity in stems have been noted by others (1, 9, 10) and attempts have been made to interpret them in relation to velocity or rate of translocation or to concurrent losses from the sieve tubes to the surrounding tissues (1, 2, 3, 4). In all cases but one (9), these gradients have been observed in short-term experiments. Only 2 reports (1, 10) have noted the changes in gradient characteristics that occur when the duration of $C^{14}$-assimilation and translocation is increased, and in these the experiments were limited to periods from 5 to 30 minutes.

Kursanov and Pristupa (5, 6, 8) have recently reported evidence in support of the classical concept of a circulation of carbon compounds from the leaves to the roots and back to the tops with metabolism in the roots playing an important role in the overall process. They have found that bean, pumpkin, and maize translocate mainly sucrose from the leaves to the roots. There it is metabolized to organic acids and amino acids in varying degree, (depending on the status of the mineral nutrition) and these acids are then translocated to the tops in the xylem sap. If such a criculation exists in the soybean, it was reasoned that, with increasing translocation time, the initial logarithmic decrease in $C^{14}$ concentration with distance down the stem could change to an increasing gradient. This alteration might result from the combined effects of the decrease in export from the leaf and the increase in return flow from the root.

The influence of increasing time on the translocation of the products of photosynthesis was, therefore, followed in young soybean plants maintained under defined conditions for intervals of 20 minutes to 70 hours after $C^{14}O_2$ was assimilated for 10 minutes by one of the primary leaves. At each time interval the total amount of $C^{14}$ exported by the leaf, its distribution within the plant and the identity of the labeled compounds were determined. The results of these experiments are interpreted in terms of the significance of the $C^{14}$-concentration gradients in the stem as a measure of the rate of downward translocation and as an indicator for return flow from the root.

## Material and Methods

Plants of *Glycine max* var. Comet were grown in aerated nutrient solution in a growth chamber maintained at 21° and illuminated with water-filtered tungsten lamps providing an intensity of approximately 20,000 lux at the leaf surface for 16 hours per day (7). The plants were used at the 14 day stage, when the primary leaves and first trifoliate leaf were fully expanded and the second trifoliate was just opening (7, fig 1C).

About 30 minutes prior to the experiment and 4 to 6 hours after the start of the daily photoperiod, plants were illuminated with tungsten light filtered through 5 cm of water. The intensity at the surface of the primary leaves as measured with a Weston foot candle meter was 20,000 lux. At zero time 50 $\mu$c of $C^{14}O_2$ (38.5 $\mu$c/mmole) was injected from a hypodermic syringe into a 50 ml polyethylene bag sealed around the petiole of one of the primary leaves. After 10 minutes of photosynthesis the bag was removed and the plant was sectioned immediately or after periods up to 70 hours during which it was maintained under the defined culture conditions. Each plant was divided into the following parts: treated primary leaf including the primary node, the opposite primary leaf, the top, the stem, and the root. The stem between the primary node and the root was subdivided into 4-cm sections (usually 4). Each part of the plant was ground under liquid nitrogen, extracted with hot 80% ethanol and assayed for $C^{14}$.

Aliquots of the ethanol-soluble extracts were sub-

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jected to paper chromatography and autoradiography (7). Radioactive areas located by reference to the autoradiogram, were cut out, mounted on aluminum planchets, and counted under a thin-window Geiger flow detector (D47, Nuclear-Chicago). The sum of the recorded counts was taken as the base for calculating the percentage distribution of $C^{14}$ among the several compounds. The radioactivity in the ethanol-insoluble residues was determined after combustion to $CO_2$, either as a gas in an ionization chamber (Dynacon, Nuclear-Chicago) or after precipitation as barium carbonate in a methane-flow proportional counter.

Although the data presented are from individual experiments, each phase of the work was replicated in whole or in part, at least twice. Questions involved in the reproducibility of results in this type of experiment were considered earlier (7). Analyses from more than 60 plants were used directly or indirectly in this work.

## Results

The total amount of $C^{14}O_2$ assimilated by the primary leaf during 10 minutes of photosynthesis was about one third of the 50 $\mu$c offered. The translocation of this $C^{14}$ out of the treated leaf to the rest of the plant followed a characteristic course as a function of time. The data for periods up to 70 hours after the treatment are shown in table I. The ethanol-soluble and -insoluble fractions were assayed separately but the data have been combined for this table. The assimilated $C^{14}$ moved out of the leaf at a fairly steady rate of 4 to 5% per hour during the first 6 hours, after which the rate decreased abruptly to a very low value. Additional slow export during the first night period raised the total amount of $C^{14}$ translocated to 33%, and this level remained unchanged during the remaining 50 hours.

The overall relation between the ethanol-soluble and -insoluble portions of the translocated $C^{14}$ as a function of time is shown in figure 1. During the first 2 hours most of the exported $C^{14}$ was in the

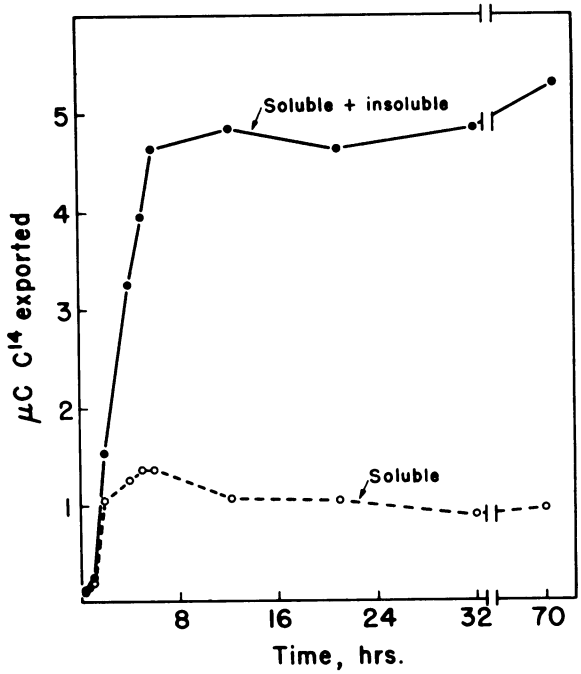


Fig. 1: Curves showing the changes in quantity of photosynthetically assimilated $C^{14}$ exported from soybean leaves as a function of time following 10 minutes of photosynthesis in 50 $\mu$c of $C^{14}O_2$ by a primary leaf. Fractions shown were soluble and insoluble in hot 80% ethanol.

ethanol-soluble fraction but in the period between 2 and 6 hours increasing proportions were recovered in the insoluble fraction. After 6 hours, when there was little or no further increase in total $C^{14}$ exported, there was no significant change in distribution between the soluble and insoluble fractions. The changes with time in distribution of translocated $C^{14}$ between the soluble and insoluble fractions in the root, the top, and the stem are shown in figure 2. Each of the parts followed essentially the same pattern, converting of a major part of the translocated carbon

**Table I**

*Distribution of Assimilated $C^{14}$ between Treated Leaf and Remainder of Plant Following Various Periods of Translocation*

One primary leaf of each plant was offered 50$\mu$c of $C^{14}O_2$ for 10 minutes, followed by various periods in $C^{12}O_2$ as indicated. The plants were in darkness during the intervals: 12 to 20, 36 to 44, and 60 to 68 hours.

| Total time (hr) | 0.16 | 0.33 | 0.5 | 1 | 2 | 4 | 5 | 6 | 12 | 20 | 29 | 36 | 44 | 70 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total $C^{14}$ recovered ($\mu$c) | 16.2 | 23.9 | 17.1 | 13.7 | 15.4 | 15.9 | 20.4 | 18.3 | 18.2 | 14.6 | 15.3 | 12.4 | 11.9 | 16.3 |
| Treated leaf* ($\mu$c) | 16.2 | 23.8 | 17.1 | 13.4 | 13.9 | 12.6 | 16.5 | 13.7 | 13.4 | 10.0 | 10.4 | 8.4 | 7.5 | 11.0 |
| Translocated ($\mu$c) | tr.** | 0.1 | tr. | 0.3 | 1.5 | 3.3 | 3.9 | 4.6 | 4.8 | 4.6 | 4.9 | 4.0 | 4.4 | 5.3 |
| Translocation as % of total | 0 | 0.5 | 0 | 4.6 | 10.2 | 20.8 | 19.2 | 25.1 | 26.4 | 32.8 | 32.0 | 32.3 | 37.0 | 32.5 |

* About 80% of the $C^{14}$ in the treated leaf was soluble in 80% ethanol after the first 2 hours, 50% after 4 hours, 40% after 20 hours, and about 30% after 70 hours remained soluble.

** tr. = trace = $< 0.1$ $\mu$c.

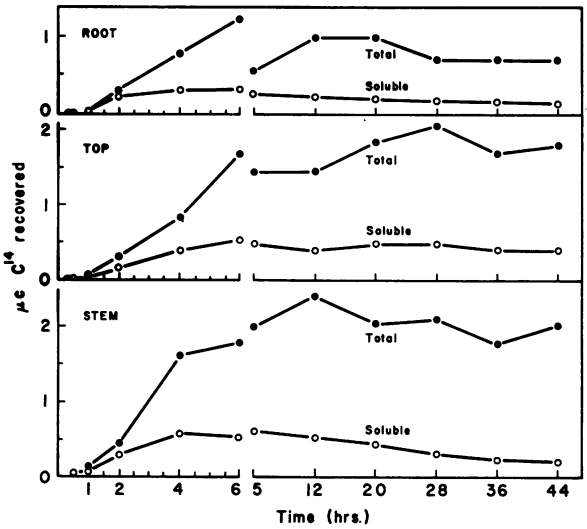


FIG. 2: Distribution of $C^{14}$ exported from a primary leaf of soybean to the root, top and stem, as a function of time following 10 minutes of photosynthesis in $C^{14}O_2$. The data are from 2 experiments in which the times were overlapped.

into insoluble form with equal ease and at about the same rate.

The $C^{14}$ gradient in consecutive pieces of the stem below the primary node was determined after increasing periods of translocation (fig 3) and was found to decrease logarithmically with distance from top to bottom (7). The amount of $C^{14}$ in the stem increased rapidly during the first 2 hours when most of it was in the soluble fraction, and then more slowly up to 6 hours when it was predominantly in the insoluble fraction (fig 2). After 6 hours the total $C^{14}$ in the stem gradually decreased in spite of the fact that a major proportion of it was in insoluble substances. The negative logarithmic gradient was maintained throughout the entire period of 44 hours.

A comparison of the distribution of $C^{14}$ in the products of photosynthesis in the leaf with those in the first 4 cm of stem showed that the early dominance of radioactive serine-glycine and sucrose in the leaf was reflected in the first products to reach the stem, (fig 4). With increasing time, the amounts of serine-glycine and sucrose in the leaf decreased while sucrose in the stem increased rapidly and serine-glycine decreased. The slopes of the curves in figure 4 indicate that serine-glycine was formed more rapidly than sucrose in the leaf and reached the stem ahead of sucrose. Figure 4 also shows that significant amounts of asparagine and malic acid were present in the leaf, and after a delay of 30 minutes, in the stem. It is, however, not clear from the data whether these substances were translocated from the leaf more slowly than sucrose and serine-glycine or were formed in the stem by metabolism of translocated sucrose or serine-glycine. Still smaller amounts of $C^{14}$ were recovered in aspartic and glutamic acids, alanine ,and the hexoses, but the radioactivity in these substances increased only very slowly in both leaf and stem, and was therefore, attributed to secondary metabolic pathways.

In the interval between 2 and 44 hours, the distribution of radioactivity among the compounds in the stem underwent changes, but no new ethanol-soluble

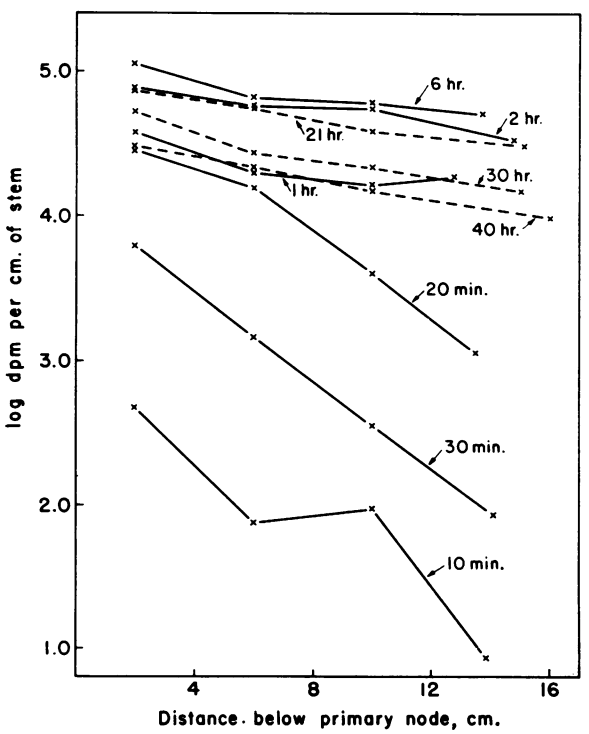


FIG. 3: Changes in the $C^{14}$ gradient in the stem of young soybean plants at various times after 10 minutes of photosynthesis in $C^{14}O_2$ by a primary leaf.

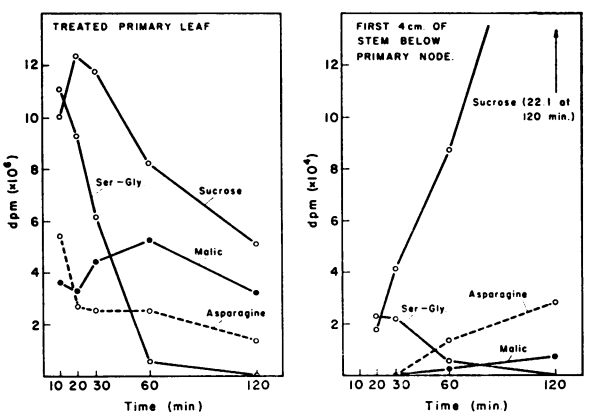


FIG. 4: Changes in the distribution of $C^{14}$ in the ethanol soluble compounds of the treated primary leaf, and the first stem section at various times following 10 minutes of photosynthesis in $C^{14}O_2$ by the leaf. Small amounts of $C^{14}$ (less than $2 \times 10^6$ dpm in the leaf, and less than $2 \times 10^4$ dpm in the stem) were recovered in each of asparatic, and glutamic acids, alanine, and the hexoses.

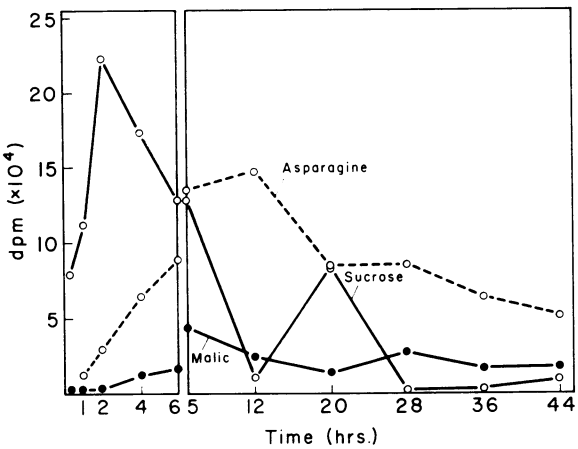


FIG 5: Changes in distribution of $C^{14}$ in the ethanol soluble compounds of the stem at various times after 10 minutes of photosynthesis in $C^{14}O_2$ by the leaf. Curves for aspartic and glutamic acids, and the hexoses paralleled (at a slightly higher level) that shown for malic acid. The plants were in darkness from 12 to 20 and 36 to 44 hours (data from 2 experiments in which the times were overlapped).

compounds became labeled (fig 5). The level of radioactive sucrose varied considerably with time. The peak level in the stem occurred after 2 hours, then decreased to a low level during the remainder of the day, rose again during the first night to a smaller maximum, decreased again to a low level during the following day, and remained low for the rest of the experiment.

After the first 6 hours, a major portion of the $C^{14}$ in the stem was recovered in asparagine. Small amounts, of the same order as that shown on the graph for malic acid were also recovered in aspartic and glutamic acids, alanine, and the hexoses. The radioactivity in all of these substances was highest after about 6 hours, then decreased slowly throughout the remaining time.

## Discussion

The translocation of photosynthetically assimilated $C^{14}$ from the primary leaves of young soybean plants is essentially completed in 6 hours after exposure to $C^{14}O_2$ for 10 minutes. The data for total $C^{14}$ export can be divided into 3 distinct time periods. From 0 to 2 hours there is a rapid increase in both soluble and insoluble forms; from 2 to 6 hours export continues, but a much higher proportion of the $C^{14}$ is recovered in insoluble fractions; and after 6 hours little or no change in overall $C^{14}$ distribution occurs. The increased conversion of the exported $C^{14}$ from a soluble to an insoluble form after 2 hours in all parts of the plant must be an expression of the steady accumulation of $C^{14}$ in insolubles superimposed on a reduction in the output of $C^{14}$-labeled precursor from the leaf. After 6 hours the supply of precursor must be exhausted since there is no further change in distribution. Although approximately 80% of the exported $C^{14}$ was found in the insoluble fraction after 6 hours, no attempt was made to characterize this material beyond recognizing its carbohydrate nature since it presumably could not be part of the moving stream.

The distribution of $C^{14}$ among the ethanol-soluble compounds extracted from the leaf and stem varies considerably with time. The early dominance of serine-glycine in the leaf is reflected in their early appearance in the stem. Sucrose becomes significant in the stem after a delay of about 20 minutes and reaches its maximum after 2 hours. This movement of sucrose characterizes the first period. The second period is marked by the gradual disappearance of labeled sucrose from the stem, concurrent with the build up of other radioactive compounds. During the third period asparagine becomes the dominant radioactive compound in the stem, followed by sucrose and several other compounds, malic, aspartic and glutamic acids, the hexoses, and alanine, which together contribute a considerable amount of radioactivity. All of these substances increase gradually during the first 6 hours, presumably at the expense of sucrose, since the latter is the only compound showing a significant loss of radioactivity. The fact that the same compounds are radioactive in the stem after 44 hours as after 6 hours, suggests that most of these substances are accumulated in stem tissues which are not in direct equilibrium with the translocating channels.

The appearance and accumulation of radioactive asparagine in the stem does not seem to be related to its export from the leaf, since the main loss of asparagine in the leaf occurred 10 minutes before significant quantities appeared in the stem, and the accumulation in the stem continued long after the level in the leaf had stabilized. Other possible sources for the asparagine include biosynthesis in the stem from other radioactive compounds, and biosynthesis in the root from translocated sucrose combined with return flow up the stem. The latter suggestion is based on the circulation concept of Kursanov (5, 6) and is supported by the results showing the early appearance and continuing presence of soluble, $C^{14}$-labeled substances in the root. It is, therefore, possible that some of the radioactive compounds extracted from the stem were components of a return flow of the products of root metabolism. There was not, however, sufficient change in the $C^{14}$ gradient in the stem during the time of these experiments to provide additional evidence for the existence of the return flow.

Throughout all of these changes in quantity and kind of radioactive compound in the stem, the overall gradient of radioactivity maintains a negative logarithmic slope from top to bottom. During the first 2 hours the gradient must describe movement of sucrose out of the leaf but the character of the gradient does not change when most of the radioactivity resides in substances apparently immobilized in the stem. The significance of the logarithmic relation as a measure of the velocity or the rate of translocation in soybean plants is, therefore, doubtful. In

short-term experiments, it is clear that the $C^{14}$ gradient includes products of photosynthesis which are in motion toward the root, but it is probable that the exponential character of the distribution in the stem is determined at all times by the $C^{14}$ withdrawn from the translocation stream and accumulated in the stem.

## Summary

Primary leaves of young soybean plants were exposed to $C^{14}O_2$ under photosynthetic conditions for a period of 10 minutes. The plants were held under defined conditions of day and night for various intervals up to 70 hours.

About 25% of the $C^{14}$ assimilated during the initial 10 minutes moved out of the leaf during the next 6 hours. Additional translocation during the following 18 hours raised the export to its maximum of about 33%. During the first 2 hours, most of the translocated $C^{14}$ was in ethanol-soluble products but between 2 and 6 hours a major part of this was converted to the ethanol-insoluble form in the stem, root, and top of the plant. The gradient of $C^{14}$ concentration per unit length of stem decreased logarithmically from the input node to the base at all time intervals up to 44 hours. The first $C^{14}$-labeled compounds to reach the stem were serine and glycine. These were followed and replaced by the main output of sucrose-$C^{14}$, which continued for about 2 hours. After 2 hours, other compounds began to replace the sucrose in the stem. These included asparagine, malic, aspartic and glutamic acids, alanine, and the hexoses. These compounds were recovered from the stem in varying proportions at all times up to 44 hours. It is suggested that some of these may have originated in the root. It is concluded that most of the $C^{14}$ translocated to the stem from the primary leaf of a young soybean is rapidly withdrawn from the conducting tissue and accumulated in other stem tissues. The logarithmic gradient of $C^{14}$ in the stem arises from this accumulation.

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