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The Ohio State University, Ph.D., 1964
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TRANSLOCATION OF PHOTOSYNTHETICALLY LABELED C\textsuperscript{14}
COMPONDS IN BEAN, CUCUMBER, AND WHITE ASH

DISSERTATION
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Terry Mohr Weidner, B.S., M.Sc.

The Ohio State University
1964

Approved by

[Signature]
Adviser
Department of Botany and
Plant Pathology
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ACKNOWLEDGMENTS

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INTRODUCTION

An adequate discussion of organic translocation in plants is possible only if the species of transport molecule is known. Proponents of various theories on the mechanism of this transport have described the movement of an increasingly wide spectrum of naturally occurring transport substances which includes sucrose (8), stachyose (12), mannitol (17), sorbitol (15), and malic acid and serine (11). These compounds have been assigned roles as transport molecules because of their occurrence in sieve-tube exudate or because of their presence as labeled molecules a finite distance from a radioactive source. The aforementioned descriptions are valid only if one assumes that the sieve-tube exudate is a sample of, or is in rapid equilibrium with, the translocation stream and if rapid metabolism of the transport substance does not occur outside the translocation stream.

It is necessary, therefore, to develop methods for determining the transport substance or substances which are independent of the assumptions outlined above. Recently, Geiger (6), using sugar beet as a model plant, was able to determine that the time-course curve showing the relative specific activity of sucrose-$^{14}$C in a mature supply leaf corresponded linearly with the time-course curve showing accumulation of $^{14}$C in a young, expanding sink leaf. In this study, the technique of Geiger is
applied to several other plants which are reported to translocate various compounds. These plants include bean, *Phaseolus vulgaris* L., which is included in a group of legumes that translocate only sucrose (18); cucumber, *Cucurbita sativus* L., which is a member of the Cucurbitaceae, a family in which the translocation of sucrose and stachyose has been reported (16); and white ash, *Fraxinus americana* L., which is reported to translocate mannitol, sucrose, raffinose, and stachyose (17).

A second technique, in which the time-course order of appearance of labeled pools in the sink leaves was determined, was also used. An analysis of sink leaves at various times after the arrival of radioactivity from the supply leaf permits one to estimate, by extrapolation of the data obtained, the percentage of radioactivity in various compounds at zero time. If there is neither biased labeling of the constituent moieties of the transport species nor differential velocities of the translocate species, then the percentage of label in a given compound in the sink leaf at time zero will indicate the quantitative importance of this compound in organic translocation. This approach provides a determination of the transport compounds which is independent of the assumptions necessary for a determination using the time-course relative specific activity data from the supply leaves.

The results obtained from these two approaches provide a means of estimating the mass balance of the $^{14}C$ leaving the
supply leaf and entering the sink leaf in terms of specific carrier compounds. This analysis provides a more refined assessment of the relative translocatability of various photosynthetically labeled compounds.
METHODS AND MATERIALS

A. Experimental Design

In each experiment the photosynthate was pulse-labeled by supplying $\text{C}^{14}\text{O}_2$ in light for a brief period (3.5 to 10 minutes), after which ordinary air replaced the radioactive atmosphere. This technique was used by Burma and Mortimer (2) to study the pathways of short-term carbon assimilation by displacing the $\text{C}^{14}$ in various pools with subsequently supplied $\text{C}^{12}$. This technique can also be used to correlate the relative specific activity in various pools of the supply leaf with the rate of translocation to the sink leaf as suggested by Geiger (6).

At various times after detectable radioactivity appeared in the sink leaf (as measured by a G-M tube positioned against the lamina), the sink leaf was removed and the radioactive compounds determined. Assuming that compounds actually in transit are not metabolized, the translocated molecules should be the only labeled materials in the sink leaf after very short translocation periods. With increasing time, metabolic conversions of the translocate, accumulating in non-transport cells, could produce the other compounds found subsequently in the sink leaf. Figure 1 is a summary of the general analytical procedures used in these experiments.
Fig. 1.—Flow diagram of experimental procedure.
Bean Cucumber White Ash

Seed germination
16 hr. photoperiod
11 to 14 days
16 hr. dark period
1 hr. 3000 ft-c
3.5 to 10 min. labeling
3000 ft-c in 0.1% CO₂, 50% uptake
3000 ft-c in ordinary air 0 to 360 min.
Divide plant into sink and supply
Freeze in liquid nitrogen and/or drop in boiling 80% ethanol
Residue
Oxidize and assay for C₁⁴

Extract
Oxidize and assay for C₁⁴
Chromatograph
Autoradiograph
Scan radioactivity
Planimeter peak area

Supply leaf
Sink leaf
25 ml
25 or 50 ml
B. Experimental Material

All plants used in the experiments were raised in a Sherer-Gillett Model CEL 512-37 controlled environment room. The light intensity six inches above the top of the culture tanks, measured by a Weston Illumination Meter, Model 756, was 1500 foot-candles produced by ten VHO fluorescent and six 100-watt incandescent lamps. The temperature was maintained at 24±1 C during the 16-hour light period and 18±1 C during the dark period. The relative humidity was maintained at 90±5 percent. The composition of the culture solution used is listed in Table 1.

1. Bean

Seeds of *Phaseolus vulgaris* L., Black Valentine, were soaked several hours in aerated tap water and then placed in moist vermiculite. When the seedlings were about eight centimeters tall, they were placed in the light room in culture solution (Table 1). Ten to thirteen days after germination the plants were removed from the light room and trimmed to one primary leaf (the supply leaf, ca. one dm²) and to the terminal leaflet of the first trifoliate leaf (the sink leaf, ca. two cm²).

2. Cucumber

Seeds of *Cucurbita sativus* L., Green Prolific, were soaked two hours in aerated tap water and then placed on moist filter paper in a covered Petri dish. When the roots had elongated about one centimeter, the seedlings were placed on moist cheesecloth suspended over a beaker filled with culture solution in a humid
### TABLE 1

**EXPERIMENTAL PLANT ENVIRONMENT**

**Mineral Solution**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
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<tr>
<td>Ca(NO₃)₂</td>
<td>0.003 M</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.002 M</td>
</tr>
<tr>
<td>KNO₃</td>
<td>0.002 M</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.002 M</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.0005 M</td>
</tr>
<tr>
<td>Fe (as Fe-EDTA)</td>
<td>900 x 10⁻⁷ M</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>800 x 10⁻⁷ M</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>150 x 10⁻⁷ M</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>15 x 10⁻⁷ M</td>
</tr>
<tr>
<td>CuCl₂</td>
<td>8 x 10⁻⁷ M</td>
</tr>
<tr>
<td>MoO₃</td>
<td>7 x 10⁻⁷ M</td>
</tr>
</tbody>
</table>

**Photoperiod:** 16 hours light—8 hours dark

**Relative Humidity:** 90±5 percent

**Light Period Temperature:** 24±1°C

**Dark Period Temperature:** 18±1°C

**Lights:** Twelve VHO fluorescent, six 100-watt incandescent

**Light Intensity:** 1500 ft-c, six inches above the top of the culture tanks

**Mineral Solution Change:** Every seven days
atmosphere. After several days, the seedlings were transferred to the light room and placed in culture solution. Seventeen days after germination, the seedlings were removed from the light room and trimmed to the first leaf (the supply leaf, ca. 0.7 dm²) and the third leaf (the sink leaf, ca. two cm²).

3. White Ash

Seedlings of Fraxinus americana L. were collected on March 26 and April 3, 1964, from under two white ash trees growing near the campus in Columbus, Ohio. The seedlings, with stems 10 to 15 centimeters long, were brought into the laboratory, washed for ten minutes in running tap water, and placed in culture solution in the light room. After 26 to 30 days, the plants were removed and trimmed to a mature leaf (the supply leaf, ca. 0.6 dm²) and to the terminal leaflet of an expanding leaf (the sink leaf, ca. one cm²). Although the leaf type of the supply leaves varied from simple to pentafoilate, it was assumed that there would be only minor metabolic differences between them and that there would be no effect on the molecular species translocated.

C. Pulse-Labeling System

The supply leaf of the plant to be labeled was placed in the dark in a gas-tight Flexiglas chamber between 1500 and 1600 hours the day prior to the experiment. The petiole was sealed in place with Mor-Tite caulking compound or with General Electric RTV-11 silicone rubber. Before and after the labeling period, the chamber was flushed with air from the compressed air lines at
800 cm$^3$ per minute. The system for generating, supplying and monitoring the labeling atmosphere was modified from that described by Geiger (6) and is shown in Figure 2.

The carbon dioxide concentration in the labeling atmosphere was measured continuously with a Liston-Becker Model 15A Infrared Gas Analyzer. A seven-micron filter was placed in the system to protect optical surfaces in the analyzer. The C$^{14}$ concentration in the system was measured with a 250-cm$^3$ flow ion chamber and the Nuclear Chicago, Model 6000, Dynacon electrometer. An insulated mixing chamber, fitted with a plastic vane turned by a magnetic stirrer, occupied approximately half the volume of the system and insured adequate mixing of the C$^{12}$O$_2$ and C$^{14}$O$_2$. The gas was circulated at 800 cm$^3$ per minute by a Sigmamotor peristaltic pump.

The system volume was calculated by releasing a known amount of C$^{14}$O$_2$ into the system and determining the amount of label present in the 250-cm$^3$ flow ion chamber. For example, if one microcurie were released from standardized Na$_2$C$^{14}$O$_3$ into the total system and the 250-cm$^3$ ion chamber contained 0.11 microcuries, then the total system volume would be 1.00 uc/0.11 uc x 250 cm$^3$ or 2273 cm$^3$. The C$^{14}$ content of the Na$_2$C$^{14}$O$_3$ solution, as measured in an ion chamber, had been calculated from the voltage drop across a calibrated resistance with the ion-current factor supplied by the manufacturer. The volume of the total system was calculated to be 2270 cm$^3$, while the volume with the leaf chamber bypassed was 1930 cm$^3$. All readings made with the
Fig. 2.—System for generating, supplying, and monitoring ClO₂.
ion chamber and gas analyzer were made at constant pressure. This procedure is necessary with a closed system since a pressure differential causes a change in the relative distribution of gas in the various parts of the system.

The $^{14}$C was supplied as Ba$^{14}$O$_2$ by Oak Ridge National Laboratories. To facilitate handling, a solution of Na$_2$C$^{14}$O$_3$, approximately one millicurie per milliliter, was prepared. This was accomplished by injecting four milliliters of 85 percent lactic acid through a serum vial stopper into a bottle which contained the BaCO$_3$ (ca. 0.02 gm). One milliliter of NaOH (adjusted to a normality which was at least two times that necessary to absorb all the CO$_2$ produced) for each millicurie produced was placed on the bottom of the specially constructed glass container which held the bottle containing the BaCO$_3$ above this solution. After standing overnight, the solution was calibrated as discussed above, and was found to contain approximately 100 percent of the C$^{14}$ released from the Ba$^{14}$O$_3$.

D. Experimental Procedure

At 0800 the supply leaf of the experimental plant was positioned in the chamber and illuminated one hour prior to the labeling period to allow the development of a steady-state rate of photosynthesis. The light was supplied by a 300-watt flood lamp and filtered through seven centimeters of water. The light intensity at the level of the supply leaf was 3000 foot-candles. The petioles of those supply leaves designated as non-translocating were steamed at the time they were sealed in the leaf chamber.
Preliminary experiments indicated that some labeled compounds were able to pass through a steamed petiole if steaming took place less than an hour before the labeling period.

Radioactive CO$_2$ of a specific activity which would permit the uptake of 50 uc within ten minutes was generated by adding 85 percent lactic acid to a known volume of Na$_2$C$^{14}$O$_3$ solution by means of a hypodermic needle inserted into the generating flask through a serum vial stopper. A magnetic stirrer was used to stir the reaction mixture. The necessary specific activity was determined by measuring rates of photosynthesis on typical leaves of each species and calculating the rate of CO$_2$ removal from the system by using the gas analyzer.

Labeling was begun by admitting C$^{14}$O$_2$ into the leaf chamber from the flow system in which the radioactive gas was circulating. The uptake of C$^{14}$O$_2$ by the supply leaf was monitored by the 250-cm$^3$ flow ion chamber connected to the Dynacon 6000 and chart recorder. A calibration curve, constructed by using the proportion of C$^{14}$O$_2$ in the flow chamber to that in the total system (0.110), was used to determine the amount of C$^{14}$O$_2$ in the system at the beginning of the labeling period. When 50 uc had been removed from the system, as determined on the calibration curve, the labeling was terminated. During the labeling period, the CO$_2$ concentration in the flow system dropped from an initial concentration of about 0.10 percent to a concentration of about 0.05 percent. Preliminary experiments showed that CO$_2$ uptake was almost linear in this range for all plants tested under the environmental conditions described above.
The infrared gas analyzer was calibrated by using tanks of CO₂-enriched compressed air, the calibrated values of which ranged from the upper to the lower ends of the recorder scale. Since the response of the analyzer is not linear, points between the upper and lower concentrations were determined by the following method. Carbon dioxide of a known specific activity was released into the system, and the concentration adjusted to correspond to the highest value on the recorder chart. Increments of the labeled carbon dioxide were then absorbed from the system with Ascarite. The carbon dioxide concentration corresponding to the new gas analyzer chart reading was calculated from the specific activity and the observed C⁴⁰ concentration.

Translocated C⁴⁰ was measured by positioning the sink leaf against the mica window of a D-34 G-M probe having a nominal 200-microsecond resolving time. The counting rate in the sink leaf was recorded on a Texas Instruments Recti-Riter recorder connected to a Tracermatic Ratemeter, Model SC79. The counts per minute recorded by the ratemeter could be equated to microcuries after subsequent assay of the C⁴⁰ in the sink leaf (cf. Section E below). It was necessary to carry out this procedure because geometry varied widely from plant to plant.

Respired C⁴⁰ was measured by connecting an Ascarite (8 to 20 mesh) trap to the effluent air stream in the leaf chamber one minute after the labeling period was terminated. The one-minute delay permitted the flushing of residual C⁴⁰O₂ from the
chamber. Fresh Ascarite was inserted into the air stream at various intervals, the used Ascarite being converted to CO$_2$ and assayed for C$^{14}$ in an ionization chamber.

E. Assay

At the termination of each experiment, the sink leaf blade and that part of the supply leaf inside the chamber were excised from the experimental plant, weighed, and dropped into boiling 80 percent (v/v) ethanol. In some cases the leaves were frozen in liquid nitrogen immediately after excision and stored in a freezer at -25 C prior to weighing and were then dropped into the ethanol. The leaves were extracted with 80 percent ethanol in a Soxhlet for two hours or until all traces of chlorophyll had been removed from the leaves. The extracts were made up to a convenient volume, 25 or 50 ml, with 80 percent ethanol and stored in the freezer. Aliquots were removed and evaporated to dryness at 100 C prior to C$^{14}$ assay. The extracted leaf residue was similarly dried before C$^{14}$ assay.

Radioactivity determinations were made by converting samples to CO$_2$, by using the Nuclear-Chicago GW-1 Glassware System in conjunction with the Dynacon-6000 electrometer. Samples were oxidized to CO$_2$ by using the mixture of sulfuric acid, phosphoric acid, potassium iodate, and potassium dichromate described by Van Slyke, Plazin, and Winniger (14). The micro-curies of C$^{14}$ released into the ion chamber could be calculated from the voltage drop across a calibrated resistance with the ion-
current factor supplied by the manufacturer and corrected by measurements with a standardized ion chamber. The Cl$_4$ in Ascarite was liberated by neutralizing it with acid, collected in an ion chamber, and measured in conjunction with the Dynacon 6000.

Radioactive compounds in the ethanol extracts were separated by using a descending paper chromatography technique. An aliquot of each extract was spotted on a 19-cm by 46-cm sheet of Whatman #1 filter paper in a band 5 mm by 10 cm. Each extract was chromatographed in at least two of three different solvent systems, depending upon which compounds were labeled as determined by preliminary experiments. The solvent systems used included n-butanol:glacial acetic acid:water (3:3:2 by volume) which separated sucrose, raffinose, stachyose, and verbascose; the epiphase of n-butanol:ethanol:water (9:1:10 by volume) which resolved glucose and fructose from sucrose; and 2-butanone:glacial acetic acid:water saturated with boric acid (9:1:1 by volume) which resolved mannitol from glucose. Identification of compounds was based on co-chromatography with authentic samples in two or three different solvent systems. An exception was labeled verbascose, usually present only in small amounts, which was identified using the method of French and Wild (5). This method is based on the fact that the logarithm of the partition function is an additive property of the various structural features of a given oligosaccharide molecule in a family of oligosaccharides.

Labeled compounds on the chromatograms were located by using autoradiographic techniques in which the chromatograms were
placed against Kodak No-Screen X-ray film. After developing, the bands on the film were traced onto the chromatogram, eluted with water, lypholyzed, redissolved in 80 percent ethanol, and then respotted on another chromatogram with authentic samples. Unlabeled compounds were located by using standard spray reagents. Amino acids were detected by using a 0.5 percent solution of ninhydrin in acetone. The sugar spray consisted of 0.5 gm of benzidine dissolved in 10 ml of 40 percent (w/v) trichloroacetic acid, 10 ml of glacial acetic acid, and 80 ml of 95 percent ethanol according to the method of Bacon (1). Organic acids were detected using a 0.04 percent chlorphenol red in 95 percent ethanol solution which was adjusted to pH 7 with NaOH. The organic acids showed up as yellow spots against a dark background when the chromatogram was exposed to ammonia vapors. Mannitol was detected using a combination of two sprays, the first of which was ortho para periodic acid, 114 mg, dissolved in 5 ml of water to which 95 ml of acetone was added. This solution keeps only three hours. The second spray, used after the first is dry, was made by adding 184 mg of benzidine to 0.6 ml glacial acetic acid and 4.4 ml water. Next, a small amount of acetone was added which dissolved the benzidine. Finally, 70 percent acetone was added to a total volume of 100 ml (7). All chromatograms were developed in a forced draft oven for five to ten minutes at 100 C.

A specially constructed chromatogram scanner was used to determine the amount of label in each band. Each chromatogram was directed under a TGC-14 gas-flow carbon counter at 12 inches
per hour by a Mylar belt which was friction driven by the same Texas Instruments Recti-Riter recorder that plotted the output from the ratemeter, a Picker Labmeter I, in the system. The gas flow counter was collimated to a slit two millimeters wide by thin steel strips. After scanning, the area under the curves obtained was determined by using a Keuffel and Esser Compensating Polar Planimeter, Model 4236 M. A 0.01 microcurie band produced an area of $1.44 \text{ cm}^2$ with the ratemeter set at 1000 counts per minute full scale deflection. Scans of the same chromatogram on two different days produced curves with an area that agreed within two percent.

The following protocol was used to determine the micro­curies in each compound. The chromatograms developed in butanol: acetic acid:water were run until the solvent reached the bottom of the paper. All compounds spotted thus remained on the chromatogram. The total area under the curves on a scan of these chromatograms was set equal to 100 with individual curves representing proportionate fractions thereof. Since equal aliquots were spotted on chromatograms run in other solvent systems, the area of individual bands could be compared to bands on the butanol:acetic acid:water chromatograms. For example, if there were 20 microcuries in the ethanol extract, 400 units in the total area on the scan, and 100 units in the curve corresponding to sucrose, the microcuries in the sucrose fraction would be 20 uc times $100 \text{ units}/400 \text{ units}$ or five microcuries.
In a few cases, where the radioactivity in sink leaves was low, 23-mm discs were cut from the chromatograms, where bands had been located by autoradiography, and counted in a Nuclear-Chicago D-47 gas-flow counter. The relative number of counts in each disc was averaged with the relative areas on the chromatograms before plotting on the graphs which follow later.
RESULTS AND DISCUSSION

A. **Comparative Accumulation and Depletion Rates in the Source-Sink System**

All of the experiments were carried out using the pulse-labeling technique in which supply leaves photosynthesized in an atmosphere containing $^{14}\text{C}_2\text{O}_2$ for 3.5 to 10 minutes, followed by photosynthesis in $^{12}\text{C}_2\text{O}_2$ for the remainder of the experiment. Figure 3 shows the time-course of $^{14}\text{C}$ distribution in the soluble and insoluble fractions of the supply leaves of both translocating and non-translocating (petiole steamed) bean plants. The $^{14}\text{C}_2\text{O}_2$ respired was the same, within five percent, for both translocating and non-translocating leaves; thus, only one curve is shown. Data obtained from cucumber and white ash were similar to that reported for bean and accordingly are not presented graphically.

All data presented here have been normalized to 50 uc uptake of $^{14}\text{C}$ to permit comparison of the various experiments. The correction was always less than ten percent for the bean experiments. The corrections ranged to twenty percent for the experiments with cucumber and ash because of the higher specific activities necessary for the desired uptake of $^{14}\text{C}$ during short exposure times.

The time-course of the $^{14}\text{C}$ distribution in the supply leaves can be interpreted as follows. Respired $^{14}\text{C}$ and an
Fig. 3.—Time-course distribution of label in ethanol-soluble and ethanol-insoluble fractions of bean plant. Time course of label lost in respiration.
BEAN

Non-translocating - soluble
Non-translocating - insoluble
Translocating - insoluble
Translocating - soluble

μc in 80% EtOH Soluble and Insoluble Fractions (normalized to 50 μc uptake)

Minutes after C¹⁴O₂ Exposure Begun

Respiration
increase in the $^{14}\text{C}$ content of the insoluble fraction can account for the depletion of label with time in the soluble fraction of the non-translocating leaves. Less than 0.01 percent of the supplied label was detected in any part of the bean plant distal to the streamed petiole. The depletion of $^{14}\text{C}$ in the soluble fraction of the translocating leaves in 300 minutes amounted to 22 uc of which 3 uc can be accounted for by respiration and an additional 1 uc by an increase in the insoluble fraction. The other 18 uc, or 36 percent of the $^{14}\text{C}$ supplied, was presumably translocated. The data in Figure 3 also indicate that 90 percent of the $^{14}\text{C}$ that disappeared from the soluble fraction was dissipated in the first 110 minutes after the pulse labeling began. No analysis was made of the chemical composition of the insoluble fraction. Were this fraction directly involved in translocation, the $^{14}\text{C}$ isotope would have been flushed through by the $^{12}\text{C}$ isotope subsequently supplied and a turnover and depletion of this label in the insolubles would have resulted. Analyses of the soluble fractions are presented later.

The depletion of label in the soluble fraction in translocating cucumber leaves amounted to 17 uc. Respiration accounted for 3.5 uc and the ethanol-insoluble fraction gained 2 uc; the balance of 11.5 uc presumably was translocated. Approximately 80 percent of the $^{14}\text{C}$ that disappeared from the soluble fraction was dissipated in the first 110 minutes after the pulse labeling began.
In ash, a decrease of 15 uc in the soluble fraction occurred in 300 minutes. In this plant, the insoluble fraction decreased an additional 5 uc during the experimental period. Of the total 20 uc depleted, 3 can be accounted for by respiration; the balance presumably was translocated. Only 60 percent of the C\textsuperscript{14} lost from the ethanol-soluble fraction disappeared in the first 110 minutes.

It appears that in bean and cucumber, the ethanol-soluble fraction alone could account for the materials translocated during the light period employed in these experiments. In white ash, however, the C\textsuperscript{14} content of the insoluble fraction also declined, although slow conversion of this fraction to a soluble form could result in the slower decline in the C\textsuperscript{14} content of the soluble fraction noted above. In all cases, 20 percent of the C\textsuperscript{14} supplied was still in the supply leaf in 80 percent ethanol-soluble form 300 minutes after the pulse labeling began. The question arises, therefore, as to whether these compounds remaining are non-translocatable or whether these compounds are in some area (or pool) in the cell from which they are not accessible to the translocation mechanism. Geiger (6), considering this question for sugar beet, came to the conclusion that the depletion of label in sucrose resulted in a decrease in the rate of C\textsuperscript{14} translocation, while other compounds remaining in the supply leaf were not translocatable. Identification of the labeled compounds in the soluble fraction of supply leaves of the three experimental species is presented in the following sections.
l. Bean

Figure 4 shows the results of analyses of the soluble fraction in the supply leaves of the translocating bean plants. The sucrose fraction declined from 22 uc to 3 uc during the 300-minute experimental period, a difference of 19 uc which closely approximates the 18 uc of $^{14}$C translocation in bean discussed above. There was also a decline of serine-$^{14}$C with a concomitant increase in malate-$^{14}$C. The malate-$^{14}$C co-chromatographed with ammonium malate in the butanol:acetic acid:water and the butanol:ethanol:water solvent systems, but was not characterized further. Although not shown on the graph, both glucose and fructose slowly accumulated 1 uc during the 300-minute experimental period. An unidentified band that ran at the front of the butanol:acetic acid:water solvent system accumulated 1 uc within 30 minutes of labeling and remained at this level for the duration of the experiment.

In pulse-labeling experiments, storage compounds and structural components of the cell accumulated $^{14}$C during the $^{12}$C displacement period. These storage compounds apparently undergo little metabolic turnover in the light since the pool size appeared to increase during the course of the experiment. Those compounds in the supply leaf which turn over rapidly, either because of the metabolic utilization or because they are translocated, will show a rapidly decreasing label during the displacement period. Sucrose and serine illustrate this possibility.
Fig. 4.—Time-course distribution of ethanol-soluble compounds in the supply leaf of bean.
Sucrose

Malate

Serine

\( \mu \text{c / Supply Leaf (normalized to 50\% uptake)} \)

Minutes after C^{14}O_{2} Exposure Began

BEAN

20

10

0

0

100

200

300
The compounds labeled earliest may be considered precursors to those which accumulate label during the displacement period, if these early-labeled compounds or their close derivatives are not translocated.

In Figure 5 the average time-course accumulation of translocate in the sink leaf of bean is illustrated. It should be noted that the data from the sink leaf analyses have been graphed with the abcissa representing "minutes after detectable activity in sink." This takes into account the time, usually 13 to 16 minutes, necessary for a detectable quantity of the $^{14}$C\textsuperscript{14} translocate to reach the sink from the supply leaf. It corrects for differences in the length of the translocation path between plants and differences in the velocity of translocation from plant to plant. Although the percentage of the total translocated $^{14}$C delivered to the sink leaf varied from plant to plant, it was assumed that the percentage delivered to the sink leaf in a given plant did not vary significantly during the course of any one experiment. A maximum of 4.3 uc, or about 20 percent of the total $^{14}$C translocated, was delivered to the sink leaf; the remainder to the rest of the plant (the non-monitored sink regions). After 110 minutes the $^{14}$C content of the sink leaf had reached 90 percent of its final equilibrium value. It should be noted that in this same time interval, calculated from the time $^{14}$CO\textsubscript{2} was added, the depletion of label in the soluble fraction of the supply leaf also amounted to 90 percent of its equilibrium value (Fig. 4).
Fig. 5.--Relative accumulation and derived rate of translocation of label in the sink leaf of bean.
Relative Accumulation of \( \text{C}^4 \text{Translocate} \)

Relative Rate of \( \text{C}^4 \text{Translocate} \)

Minutes after Detectable Activity in Sink
The rate of translocation of $^{14}C$ to the sink leaf is also shown in Figure 5. This rate is the first derivative of the accumulation curve. A comparison of this rate curve with the time-course curve in Figure 4 showing the amount of sucrose-$^{14}C$ in the supply leaf indicates a close correlation between the two curves. If sucrose or a close derivative is the translocate species, then the concentration-time curve of sucrose $^{14}C$ in the supply leaf should mirror the rate of $^{14}C$ translocation as indexed by the rate of accumulation of $^{14}C$ at a terminal sink, i.e., a sink from which significant rates of re-export are unlikely. The curve showing accumulation is displaced on the time axis to allow for transit time between supply and sink leaves. It is assumed that the sucrose-pool size and the rate of translocation remain constant during the course of the experiment. If these assumptions are correct, then the amount of sucrose-$^{14}C$ is a measure of the specific activity of sucrose in the supply leaf. Thus, the rate of $^{14}C$ translocation is linearly proportional to the amount of sucrose-$^{14}C$ (the apparent specific activity) in the supply leaf.

It is interesting to note in this connection that the only carbohydrate found in the sieve-tube exudates of leguminous plants is sucrose (18). This correlation provides an independent check on the assumption that the sieve tubes are the dominant channels of organic translocation.
2. Cucumber

In the analyses of cucumber supply-leaf extracts, presented in Figure 6, a wider spectrum of heavily labeled compounds was detected. As in bean, sucrose became most heavily labeled. In addition to the stachyose and raffinose shown on the graph, small amounts of verbascose and another compound, tentatively identified as manninotriose, were consistently labeled. Serine and aspartic acid, though not shown, were also detected in small amounts, usually less than 1 uc. There was a 12 uc decline in sucrose-$^{14}$C during the displacement period, in close agreement with the 11.5 uc translocated from the cucumber supply leaf reported above.

Figure 7 presents the average relative accumulation of label in the sink leaf and the rate of translocation in cucumber. As in bean, the percentage of the total $^{14}$C-translocate which was delivered to the sink leaf varied from plant to plant. The maximum label transported to any sink leaf was 2.0 uc, which was approximately 17 percent of the total translocate. After 110 minutes, the $^{14}$C had accumulated in the sink leaf to within 87 percent of its final value. The cucumber supply-leaf data indicate that approximately 80 percent of the $^{14}$C in the soluble fraction had been translocated in the same interval.

A comparison of the translocation-rate curve (Fig. 7) with the concentration-time curves for sucrose-$^{14}$C and stachyose-$^{14}$C (Fig. 6) reveals that the time of maximum concentration of stachyose-$^{14}$C and the time of maximum rate of translocation of
Fig. 6.—Time-course distribution of ethanol-soluble compounds in the supply leaf of cucumber.
Fig. 7.—Relative accumulation and derived rate of translocation of label in the sink leaf of cucumber.
Relative Accumulation of $^{14}C$ Translocate
Relative Rate of $^{14}C$ Translocation

Minutes after Detectable Activity in Sink

CUMBER

Rate

Accumulation
label to the sink leaf agree almost precisely, correcting for transit time between the two leaves; however, a time lag of some ten minutes is indicated for sucrose $^{14}\text{C}$. Furthermore, after 110 minutes, the rate of $^{14}\text{C}$ translocation and the apparent specific activity of stachyose have both declined an equal amount, namely 90 percent, while the apparent specific activity of sucrose at this time had declined only 70 percent. However, the total amount of label lost from the stachyose pool in the supply leaf was much less than the gross amount translocated, this value corresponding best with the sucrose-$^{14}\text{C}$ pool. It appears that these differences allow one to assign either stachyose, sucrose, or even raffinose a transport role, the latter compound having lost about 4 uc during the displacement period. For instance, the slower decline in sucrose-$^{14}\text{C}$, as compared to the $^{14}\text{C}$-translocation rate, could have resulted from other labeled pools feeding into sucrose during the displacement period. Stachyose might conceivably be the only transport species if stachyose were present only in the sieve tubes. Thus, the instantaneous amount of labeled stachyose in the supply leaves could be less than the amount of $^{14}\text{C}$ translocated while labeled sucrose, a likely precursor, could still decline in an amount equal to the amount of $^{14}\text{C}$ translocated. Stachyose and sucrose have been assigned major transport roles in squash (15) and pumpkin (12). A further discussion of these points is given later.
3. White Ash

In the supply leaves of ash, a still wider spectrum of labeled compounds was detected (Fig. 8), but, again, the major portion was found in carbohydrates. Amino acids contained only trace amounts of radioactivity while labeled malate contained 1.5 to 2.0 uc during the entire displacement period. Sucrose again became most heavily labeled, declining about 12 uc during the displacement period. As indicated above, the translocation of label in ash was calculated to be 17 uc. Labeled stachyose and raffinose were present in small amounts while only traces of labeled verbascose were found. A compound, tentatively identified as manninotriose, was present in surprisingly large quantities. The total decline in label in stachyose, raffinose, sucrose, and manninotriose was approximately equal to the 17 uc translocated. Mannitol, which contained up to five percent of the C\textsuperscript{14} supplied, appears to be a storage compound with very little turnover occurring during the displacement period.

Figure 9 illustrates the average relative accumulation of C\textsuperscript{14} in the sink leaves of ash and the derived rate of translocation. The maximum translocation of C\textsuperscript{14} to the sink leaf was 2.3 uc; the percentage of the total translocate going to the sink leaf was variable as before. This represents about 14 percent of the total C\textsuperscript{14} translocated. After 110 minutes, 65 percent of the final value of C\textsuperscript{14} in the sink leaf had accumulated in this region. This is in close agreement with the 60 percent of the C\textsuperscript{14}-ethanol-soluble fraction dissipated in the same interval. The slower rate
Fig. 8.—Time-course distribution of ethanol-soluble compounds in the supply leaf of white ash.
WHITE ASH

Sucrose
Mannitriose
Mannitol
Stachyose
Raffinose

Minutes after CO2 Exposure Began
Fig. 9.—Relative accumulation and derived rate of translocation of label in the sink leaf of white ash.
of accumulation in the sink leaf may indicate a lower velocity of translocation in ash, a larger pool size of the translocate or its precursor, a slower rate of synthesis of the translocate, or perhaps a greater exchange with unlabeled molecules along the translocation path. The latter possibility has also been explored by Hill (9), who found that in willow, stem tissue may contain large amounts of unlabeled material which can exchange with labeled translocate.

The time of maximum concentration of manninotriose-C\textsuperscript{14} (Fig. 8) correlates closely with the time of maximum rate of delivery of C\textsuperscript{14} to the sink leaf (Fig. 9). After 110 minutes, both labeled manninotriose and the rate of translocation declined 55 percent while labeled sucrose had already declined 75 percent. However, the reservations concerning the cucumber supply-leaf data again apply here. Correlation between the rate of accumulation of C\textsuperscript{14} in the sink and the relative specific activity of a compound in the supply leaf cannot differentiate between transport compounds and their precursors. Using the sieve-tube exudate technique, Zimmermann (17) reports the translocation of stachyose, raffinose, sucrose, and mannitol in white ash. On the basis of the supply leaf analyses reported above, it appears that mannitol is primarily a storage compound and is not a major translocate species under the conditions of these experiments.
B. Order of Appearance of Labeled Pools in Sink Leaves

It is evident that the approach used above failed to answer the question as to the relative translocatability of sucrose, raffinose, manninotriose, and stachyose because it failed to differentiate between transport molecules and their possible precursors. An effort was made, therefore, to determine the order of appearance of transport species and other labeled compounds in the sink leaves.

As molecules in the transport stream are released into the metabolically active sink area, they are changed into cellular components. Analyses of labeled compounds in the sink area may be anticipated to show many radioactive compounds, most of which probably will have been derived from labeled transport molecules. It is essential, therefore, to analyze the sink area immediately upon arrival of detectable radioactivity. However, when the first labeled transport molecules arrived at the sink, as monitored by a G-M tube positioned against the sink leaf, there were too few of them for an adequate analysis. This is to be expected because of the nearly linear relationship which often prevails between the logarithm of the radioactivity at any place in the stem and the distance from the region of application. Horwitz (10) has proposed mathematical models based on these findings. Thus, it was necessary to analyze the sink leaves at various times after detectable activity had reached the sink leaf and to extrapolate the curves obtained from these data to zero time. Those compounds
in the sink leaf which were labeled at time zero would then be considered transport species.

The data obtained from the analyses of bean sink leaves are shown in Figure 10. It should be noted that the sink-leaf data are plotted as a function of the percentage activity in the ethanol-soluble material. The percentage of activity in the ethanol-insoluble fraction increased from 5 percent after 12 minutes to 59 percent after 284 minutes. This would be expected in an expanding leaf as the soluble translocate is utilized in growth. The only two distinct spots detected on chromatogram scans or autoradiograms in the shortest time interval (12 minutes) were serine and sucrose. The curve for sucrose appears to extrapolate to 100 percent at zero time. This can most easily be explained by assuming that all or nearly all of the labeled material was translocated as sucrose and later converted to other labeled compounds, serine being the first one detectable in large amounts. This is in agreement with the inference made earlier citing supply-leaf analyses and translocation-rate data which also indicated sucrose to be the translocate. It is possible to assume that the later appearance of serine reflects its later arrival in the sink area. This point will be discussed later.

The sink leaf data for cucumber are shown in Figure 11 and the data for white ash in Figure 12. The similarity between the two plants is immediately obvious. Again, the insoluble fraction accounted for only 5 percent of the total radioactivity after 12 minutes. This increased to 51 percent for cucumber after
Fig. 10.—Time-course distribution of ethanol-soluble compounds in the sink leaf of bean.
Minutes after Detectable Activity in Sink

BEAN

% Activity in 80% Ethanol Solubles

Sucrose

Serine
Fig. 11.—Time-course distribution of ethanol-soluble compounds in the sink leaf of cucumber.
CUCUMBER

% Activity in 80% Ethanol Solubles

Minutes after Detectable Activity in Sink

Sucrose
Raffinose
Stachyose
Fig. 12.—Time-course distribution of ethanol-soluble compounds in the sink leaf of white ash.
282 minutes and to 39 percent for ash after 240 minutes. In both these plants, it appears that the stachyose curve extrapolates to 100 percent at zero time, the data for ash supporting this view with greater certainty than do the cucumber data. The sucrose curve appears to extrapolate to 0 percent in both plants. Although there was only one plant analyzed for each time interval, the cucumber sink leaf data for 44 and 46 minutes (Fig. 11) give some indication of the reproducibility of the experiments.

Continuing the reasoning suggested above, it appears that stachyose is the major, or perhaps exclusive, translocate species in cucumber and white ash, for it is the only labeled molecule found in the sink leaf at time zero by extrapolation. However, the previously reported supply leaf data for cucumber and white ash indicate that several other kinds of molecules may be transport species. The compound, tentatively identified as manninotriose, was detected in trace amounts, less than one percent, on all chromatograms of the ash sink-leaf extracts. Labeled mannitol first appeared in the sink leaf of ash only after 20 minutes and then in amounts detectable only by long term autoradiography. This is consistent with the earlier inference that mannitol is a storage compound in ash, undergoing very little turnover in the light. Labeled verbascose was detected in both ash and cucumber sink leaves in small, variable amounts. In both plants, the spectrum of compounds became increasingly diverse as the translocation period increased, as was to be expected.
There are several possible interpretations of these data. First, the one described above in which that compound representing 100 percent of the radioactivity at zero time is the translocate species with other compounds being derivatives of this molecule. If two or more kinds of molecules are translocated, however, their sum should equal 100 percent at time zero with their respective percentages giving some information as to the relative portion of the total carbon load carried respectively by these molecular species, or to their relative rate of release into the transport stream. This requires, in ash and cucumber, that galactose be released in the formation of raffinose and sucrose in the sink areas. In both species, however, only trace amounts of tentatively identified galactose were found. It may be that galactose is rapidly metabolized or is seldom found as free galactose in these growing areas, that is, it exists only as a constituent moiety of an enzyme complex.

A second possibility is that stachyose (sucrose in bean) is merely the first labeled compound to reach the sink area, labeled raffinose and sucrose (serine in bean) arriving several minutes later. This might occur if stachyose were unevenly labeled. For example, if the galactose moiety of stachyose were labeled early in photosynthesis and the sucrose pool in the supply leaf were not heavily labeled until several minutes later, then sucrose and stachyose released into the transport stream at the same time would exhibit a bias in the labeling toward stachyose. Labeled stachyose would, therefore, arrive at the sink ahead of
labeled sucrose, even though sucrose might account for a large or even larger percentage of the carbon transported. The data in Figure 6 show that the $^{14}C$ label moves through stachyose rather rapidly, although there does appear to be a rather large pool of sucrose in short time intervals. A slower velocity of transport could also account for the later arrival of sucrose at the cucumber and white ash sink leaves.

The data presented above indicate a transport role for stachyose in cucumber and white ash, but do not eliminate the possibility of sucrose and raffinose translocation in these plants. Although Zimmermann (17) has reported mannitol to be a transport species in white ash, the data reported above indicate that it was not an important transport species in these experiments. What is the reason for this discrepancy? It seems likely that the sieve-tube exudate, as tapped by cuts and aphid stylets is not really the translocation stream, but is only in equilibrium with it. The actual stream may reside in the strands observed by Thaine (13). Canny (3) has elaborated on this hypothesis. Thus, the compounds in the exudate, which may come from the interstrand solution, may really be an easily available organic reservoir for the real stream, and the specific components of this reservoir may, in fact, be entirely different from the translocation compounds. Much of the loss of radioactivity by the translocation stream, as proposed in the Horwitz model system (10), could be accounted for if the sieve-tube inter-strand solution represents a much larger carbon pool than that of the
stream passing through it. It should be noted that Esau, Engleman, and Bisalputra (4) regard the transcellular strands as optical artifacts.

The sink leaf data also demonstrate that samples of the translocation stream must be obtained as soon as possible after the first label reaches the sampling area if the transport species are to be identified. Rapid metabolic conversion of the transport molecules occurs, obscuring the real picture in a short time. Therefore, the finding of a labeled molecule a finite distance from the radioactive supply area does not imply translocation of this molecule. In fact, finding a molecule labeled some time after the radioactivity has reached an area may more likely imply that it is not translocated, but is formed from the translocate species.

Resolution of the sink-leaf data awaits further study into the kinetics of translocation. The data presented in Table 2 allow some speculation as to whether stachyose is, in fact, the major transport species in cucumber and white ash. For example, if the average translocation velocity in cucumber is $30 \text{ cm hr}^{-1}$, and the average distance transgressed by a given stachyose molecule in a sieve tube in the sink leaf is one cm, and all or nearly all the stachyose in the sink leaf is in the sieve-tube system, then the turnover of stachyose in the sieve tubes would be 30 times per hour. Since the data in Table 2 allow one to approximate the stachyose-$\text{C}^{14}$ concentration at any time during the translocation period, it is possible to add the amount of $\text{C}^{14}$ delivered
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<th>$%\text{C}^{14}$ in EtOH Solubles</th>
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to the sink leaf in two-minute increments during the entire translocation period. This addition results in 1.6 uc delivered to the sink leaf in five hours which is approximately equal to the actual amount delivered. While the parameters included in the above calculations are not known with certainty, the measured length of the sink leaf was about one cm and the radioactivity reached the sink leaf in 15 minutes, having traveled about seven cm. Similar calculations for white ash, in which the first detectable activity arrived in the sink leaf after 18 minutes, having traveled a distance of 5.5 cm from the supply leaf, resulted in 2.5 uc being delivered to the sink leaf in 240 minutes. The maximum C\textsuperscript{14} delivered to a white ash sink leaf was 2.3 uc in the same time interval.

If most or all the stachyose-C\textsuperscript{14} is in the translocation stream and if the volume of the translocation stream is approximately equal per unit fresh weight in both sink and supply leaves, then the stachyose-C\textsuperscript{14} concentration should be the same in both leaves providing that corrections are made for transit time between the two leaves and for the percentage of the total translocate going to the sink leaf. In cucumber, the supply leaf fresh weight was about 20 times that of the sink leaf and 20 percent of the C\textsuperscript{14} translocated moved to the sink leaf. Therefore, after displacing the data for the sink leaf along the time axis to allow for transit time, the supply leaf should contain, at any given time, about 100 times as much stachyose-C\textsuperscript{14} as the sink leaf. That this is the case can be seen by comparing the
stachyose-$\text{C}^{14}$ in the supply leaf (Fig. 6) with the stachyose-$\text{C}^{14}$ in the sink leaf (Table 2), the latter being multiplied by 100. In white ash, in which the supply leaf weighed about 15 times more than the sink leaf and 15 percent of the $\text{C}^{14}$ translocated moved to the sink leaf, the stachyose-$\text{C}^{14}$ concentration in the supply leaf should again be about 100 times that in the sink leaf. A comparison of the data in Figure 8 with the data listed in Table 2 reveals that the supply leaf contains only about 30 times more stachyose-$\text{C}^{14}$ than the sink leaf. The discrepancy could easily be caused by an error in estimation of one of the parameters involved in the calculations above (in particular, the sieve-tube volume per unit fresh weight may be distinctly different for the supply and sink leaves, respectively). The calculations cited above indicate the possibility, nonetheless, of stachyose-$\text{C}^{14}$ being only in the translocation stream.

The correspondence between the amount of label in a given compound in the supply leaf and the rate of translocation shows only that the compound either is the transport species or that it is a precursor of that compound. More definitive data could be obtained by sampling the sink leaves at shorter time intervals. However, the logarithmic distribution of radioactivity requires that both the specific activity and the total radioactivity of the compounds in the supply leaf be increased greatly if useful data are to be obtained at intervals shorter than seven or eight minutes. Determination of the specific activity of the transport compounds and their labeling pattern would also facilitate interpretation of these results.
SUMMARY

Translocation of photosynthetically labeled C^{14} compounds was studied by using plants pruned to a mature supply leaf and an expanding sink leaf. A pulse-labeling technique was used in which 50 uc of C^{14}O_2 were incorporated into the supply leaf in 3.5 to 10 minutes. A closed flow system that monitored the uptake of label with a gas flow ionization chamber was used. The labeling period was followed by an experimental period lasting up to five hours in ordinary air during which the translocation of labeled compounds was monitored by a G-M detector positioned against the sink leaf.

At various time intervals after the pulse-labeling, both the sink and supply leaves were removed from the plant for assay. Leaves were extracted and aliquots of the extracts and the extracted residue were assayed for C^{14} by oxidation to CO_2 and measurement in an ionization chamber. Further characterization of the extracts was achieved by paper chromatography, autoradiography, and by scanning the chromatograms for radioactivity. The rate of translocation of C^{14} to the sink leaf was derived by plotting the slope of the curve showing the time course of accumulation of C^{14} (converted to uc C^{14}) in the sink leaf.

A comparison of the curves illustrating the rate of translocation of C^{14} to the sink leaf with the concentration-time curves
resulting from analyses of the supply-leaf extracts was made. In bean, where most or all of the $^{14}C$ was translocated as sucrose-$^{14}C$, there was a close correlation between the rate curve and the sucrose-$^{14}C$ time-course curve. In addition, the depletion of label in sucrose in the supply leaf was approximately equal to the amount of $^{14}C$ translocated. In those species in which sucrose apparently is not the major translocate, the rate curve correlated best with the stachyose-$^{14}C$ time-course curve (in cucumber) and the manlinotriose-$^{14}C$ (tentatively identified) time-course curve (in white ash). In cucumber, as in bean, the depletion of label in sucrose was approximately equal to the $^{14}C$ translocated, but, in white ash, the depletion of label in the total carbohydrate fraction was more nearly equal to the amount of translocated radioactivity. Therefore, it appears that a correlation between the time-course distribution of label in a compound in the supply leaf and the time-course rate of $^{14}C$ translocation cannot differentiate between a translocate species and its precursor. Furthermore, the correspondence between the depletion of label in a given compound and the amount of $^{14}C$ translocated again indicates only that the compound is, or is easily converted to, the translocate species.

The data from the analyses of sink leaves of bean further substantiate the conclusion that sucrose is the major or only organic transport species, since extrapolation to time zero indicates that sucrose would account for 100 percent of the radioactivity in the sink leaves. In cucumber and white ash,
extrapolation to time zero indicates that stachyose could account for 100 percent of the radioactivity. Curves for sucrose-$C^{14}$ and raffinose-$C^{14}$ apparently extrapolate to zero percent for these compounds at zero time. This treatment of the data suggests, therefore, that stachyose is the major, or perhaps exclusive, translocate species in cucumber and white ash. In addition, it can be shown for both cucumber and ash that by assuming a reasonable turnover rate for a given cohort of stachyose molecules in the sieve tubes, the instantaneous concentration of stachyose-$C^{14}$ in the supply leaf can account for all the label translocated and that the instantaneous concentration of stachyose-$C^{14}$ in the sink leaf could account for all the $C^{14}$ imported. Alternative explanations are not excluded, however, such as a differential velocity of various translocate species or differences in arrival time of various labeled compounds at the sink leaf because of biased labeling of transport species in the supply leaves. At least one compound, mannitol, previously assigned a transport role in white ash, is apparently a storage compound, very little turnover having taken place in the supply leaf in these experiments. Thus, with the reservations noted above, these data support the contention that a wide variety of labeled compounds found in sieve-tube exudate may be derivatives of the translocate compounds, and may be relatively immobile within the sieve tubes, although recoverable in the sieve-tube exudate. It would follow from this view that the sieve-tube exudate is not an authentic sample of the translocation stream.
LITERATURE CITED


I, Terry Mohr Weidner, was born in Allentown, Pennsylvania, May 31, 1937. I attended the public schools in Emmaus, Pennsylvania, from grades one through twelve. West Chester State Teachers College granted me the B.S. degree in 1959 upon my completion of the undergraduate training. From 1959 to 1962 I was the recipient of a Kettering Foundation Fellowship. During my tenure as a National Science Foundation Cooperative Fellow from 1962 to 1964, I gained college teaching experience through participation in the general botany program. I served as an Instructor in the Radiation Biology Summer Institute in 1964. I have accepted an appointment as Assistant Professor in the Botany Department at Eastern Illinois University.