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Translocation of Photosynthetically Assimilated C14 in Straight-Necked Squash [1,2,3]

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Introduction

Studies of translocation in higher plants derived from sugar analyses of sap (10, 26, 27, 28), aphid stylet exudates (24, 28) and by the displacement of C14 from mature leaves (1, 6, 7, 11, 12, 20, 23), have produced the generally accepted view that sucrose is the main form of transported carbon in phloem tissue (19). These studies however have been confined to a very limited number of plant species. Recent investigations have now shown that sucrose is not the only sugar involved in translocation. Zimmermann (27) has found raffinose, stachyose and mannitol in the sap exudates of a number of trees and other plant species and Starck and Gorham (unpublished data) have found by C14 displacement verbascose and stachyose as the major compounds in the translocation stream of *Mentha* and *Saintpaulia*. Pristupa (15) has also found translocation of verbascose and stachyose in the pumpkin plant. Evidence for the

translocation of sorbitol in the apple has recently been published (25) and amino and organic acid movement from leaves has been reported by Nelson et al. (13) in soybean under certain physiological conditions, and in the rhubarb by Kursanov et al. (9). Evidence for the movement of many other compounds in the phloem may be found as more plant species are investigated. These new discoveries must modify the concept of the unique position of sucrose in trans-location and before ascribing any mechanism to the process it will be necessary to know the full range of compounds transported by the phloem.

A preliminary investigation of the young squash plant had shown that it translocated both stachyose and sucrose. Furthermore freeze-dried squash tissues, in contrast to the tissues of other plant species investi-gated (5, 14), were readily infiltrated and prepared for tissue autoradiography. A detailed study of translocation was therefore carried out in young squash plants using the methods of C14 displacement under controlled conditions and tissue-autoradiog-raphy. The products of short-term $C^{14}O_2$ assimila-tion were determined and their translocation within the plant was measured as a function of time and stage of leaf development.
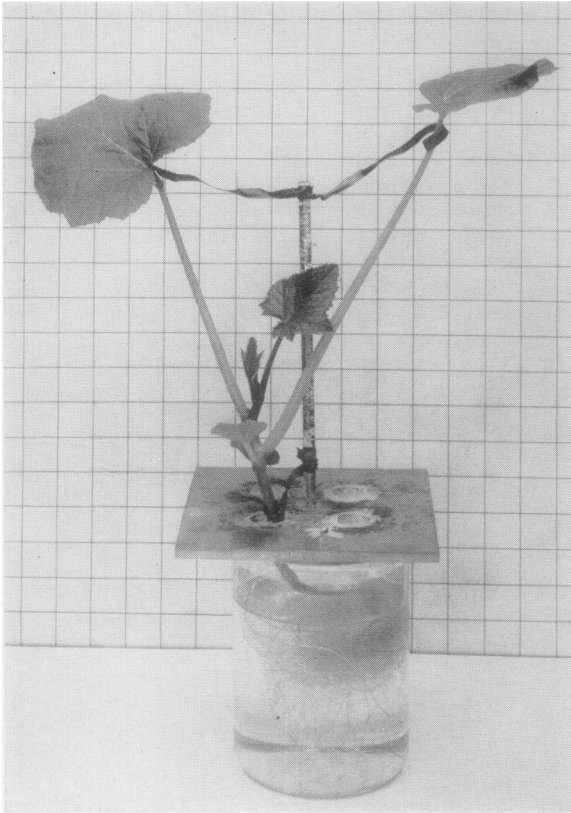
FIG. 1. Early prolific straight-necked squash grown as described for 13 days. Mature primary leaf on the right. One inch squared grid behind plant.

Materials and Methods

Plant Materials. Seeds of the early prolific straight-necked squash, *Cucurbita melopepo torticollis* Bailey, were germinated in vermiculite in an incubator at 25° in the light. At 5 days a vigorously growing shoot and root had formed and the seedlings were transferred to one liter polythene beakers and grown for 7 to 8 more days in aerated modified Hoagland solution (0.005M KNO_3, 0.005M $MgSO_4 \cdot 7H_2O$, 0.002M $Ca(NO_3)_2 \cdot 4H_2O$ and 0.001M KH_2PO_4 with 7.2 mg $Fe(NH_4)_2SO_4 \cdot 6H_2O$, 15 μl Versenol and 1.0 ml Arnon's A-5 minor element solution per liter, pH 3.8) at 25 $\pm$ 1° under a 16-hour day with 20,000 lux provided by water-filtered tungsten lamps. Experimental plants (fig 1) were usually selected 2 hours after commencement of the light period on the twelfth or thirteenth day following seed planting. The design of a few experiments required plants grown for longer periods up to the emergence of the eighth and ninth leaves.

Leaf Chamber. A lucite chamber of about 500 ml volume (fig 2) was assembled to accommodate a mature squash leaf blade under conditions of controlled temperature, humidity and light intensity. From a temperature-controlled reservoir a flow of 80 %

ethanol was maintained under an aluminum plate sealed across the bottom of the chamber providing a rapid heat exchange between the air flowing around the leaf and the circulating fluid. By adjusting the temperature of the reservoir and of the glycol in the glass dish above the chamber a range of leaf blade temperatures from 0 to 35° could be maintained under an illumination of 20,000 lux (provided by two 1000-w reflector-type tungsten lamps) with a filtered air flow across the leaf blade surface of between one and 5 liters per minute. In the experiments reported here
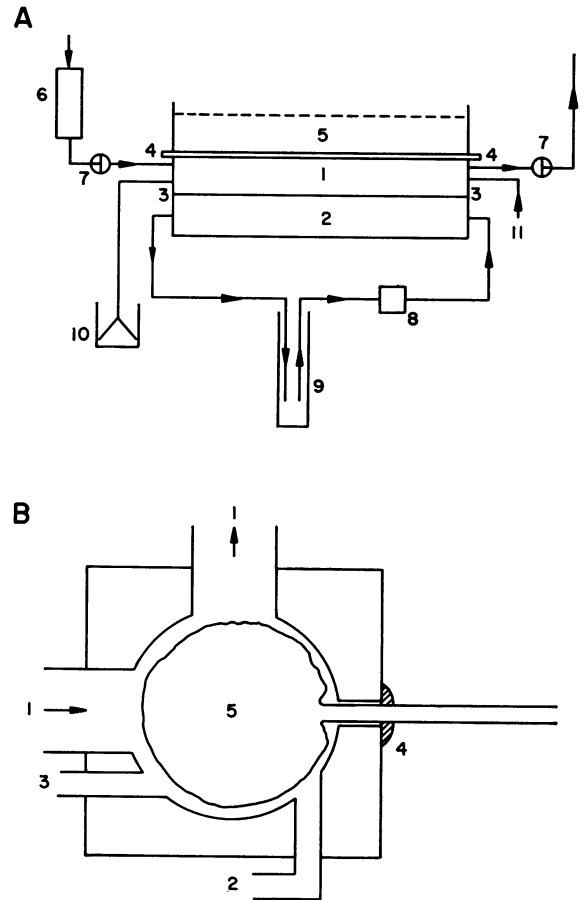

FIG. 2. Schematic views of the thermoregulated leaf chamber. A) Side view. The lucite chamber (1) encloses the leaf blade separated from the circulating ethanol in (2) by an aluminum plate (3-3). A grease-sealed glass plate (4-4) completes the assembly and is surmounted by a dish of glycol (5). Filtered air flows through the gauge (6) into and out of (1) via the large-bore 2-way aluminum taps (7). A pump (8) maintains the circulation of ethanol from the temperature-controlled reservoir (9). A mercury column (10) allows for volume expansion when CO_2 is pumped in at the feeding port (11). B) Top side view, showing diameter of the air leads (1), the CO_2 feeding port (2), the outlet to Hg column (3), the modeling-clay seal around the petiole (4) and the distribution of the leaf blade (5) across the chamber.

the leaf blade was maintained at 25° with an air flow of five liters per minute. Temperature was measured by inserting fine copper/constantan thermocouples into leaf veins. The remainder of the plant also received water-filtered illumination of 20,000 lux.

Assimilation of $C^{14}O_2$. The mature primary leaf blade, unless otherwise stated, was sealed into the chamber with modeling clay and equilibrated for 30 minutes under experimental conditions. After equilibration, 60 μc of $C^{14}O_2$ (specific activity 39 mc/mmole generated in a 50 ml hypodermic syringe) was introduced into the leaf chamber through the feeding port (fig 2) by pumping the syringe back and forth for 15 seconds. The pressure within the chamber during pumping was maintained close to atmospheric by the mercury column in the side tube. The initial total CO_2 concentration in the chamber was approximately 0.04 % by volume. Immediately following withdrawal of the syringe the feeding port was closed, the aluminum taps were reopened and remaining $C^{14}O_2$ swept out of the chamber by the inrush of the renewed air flow. The leaf was then maintained under the previous conditions for periods ranging from 10 seconds to 14 hours.

C^{14} Analyses. Each plant, unless otherwise stated, was rapidly dissected into 13 parts as follows: treated blade, 6 alternate 2-cm segments from the petiole attached to the treated blade, stem, hypocotyl, roots, second leaf, third leaf and the remaining shoot. Each part was separately frozen in liquid nitrogen, ground to a fine powder and extracted twice with hot 80 % ethanol. Both the soluble and insoluble fractions were retained.

Aliquots of the soluble fraction were counted in a windowless methane flow proportional counter of approximately 50 % efficiency. The distribution of radioactivity was determined by 2-dimensional chromatography with butyl acetate : acetic acid : water (3: 3: 1), and pyridine : 28 % ammonia, sp.g 0.90 : isobutyl alcohol (4: 2: 1). The developed chromatograms were exposed to Kodak No-Screen X-ray films. The labeled compounds were cut out from the chromatograms and their surface activities were determined in the windowless proportional counter. The results were expressed as percentages of the total activity in the ethanol-soluble fraction. The compounds containing the bulk of the radioactivity on the chromatograms were identified using the standard procedures of elution and cochromatography in different solvent systems. The products of dilute HCl and invertase hydrolyses were chromatographed for further identification of stachyose, raffinose and sucrose as outlined by French (4). The sugars were identified against known standards. Sucrose and raffinose were obtained commercially and pure crystalline stachyose isolated from *Stachys tuberifera* (17) was kindly supplied by Dr. R. S. Shallenberger.

Preliminary studies of translocated C^{14} compounds showed some incorporation of radioactivity into the ethanol-insoluble residues throughout the plant and

an estimate of this C^{14} deposition was necessary. The dried, finely powdered ethanol-insoluble residues were transferred to tared beakers as a suspension in benzene, redried and reweighed. Approximately 4 mg quantities of the powder were weighed onto an aluminum planchet and spread uniformly across an area of 7.5 cm² in a slurrey of benzene, carefully dried, and counted with an end-window GM tube which was set at 2.0 mm above the level of the powder. Activities were corrected for GM tube efficiency and for self-absorption. Tube efficiency was calculated using a standard C^{14}-plastic disc while self-absorption by the dried residues was determined by oxidizing 4 mg test samples in a Van Slyke procedure and measuring the radioactivity of the total released CO_2 in a Nuclear-Chicago Dynacon chamber. While the method for estimating the residual activity compromised accuracy for speed of determination, this was justified on the basis of the minor importance attached to this non-mobile fraction.

The results of the C^{14} analyses represent average values computed from 4 to 5 replicate experiments, with the exception of those results from radiochromatogram distributions which were obtained from single determinations.

Tissue-Autoradiography. Slices of squash tissue 3 to 4 mm thick were rapidly cut and immersed in isopentane cooled by liquid N_2 at about − 160°. The frozen slices were transferred in small beakers to a vacuum desiccator, previously cooled to − 40°, and evacuated over P_2O_5 to a pressure of about 5 mm Hg. The desiccator was stored at − 40° for at least 7 days by which time dehydration was complete. The dried tissues were readily infiltrated with paraffin wax in a vacuum oven overnight at 80°. They were then sectioned 5 to 10 microns thick, mounted on Kodak NTB autoradiographic plates with a 10 micron emulsion layer and left in sealed boxes at a uniform temperature for an experimentally predetermined exposure time (14). The paraffin wax was removed from the sections by carefully dipping the plates into xylene. Development was carried out as previously described by Perkins et al. (14). Great care was exercised throughout not to disturb the position of the sections on the emulsion. Well-preserved sections superimposed upon their developed images were mounted in a medium having a refractive index of 1.640 and photographed.

Results

Assimilation of $C^{14}O_2$ by the Mature Primary Leaf Blade. All primary leaves that had attained their maximum size produced similar patterns of C^{14} assimilation and export. It was impossible however to achieve a constant quantitative assimilation of $C^{14}O_2$ between replicate experiments. Of the 60 μc of $C^{14}O_2$ presented to a leaf for 15 seconds from 6 to 12 μc were assimilated. This variability was probably due to an inaccuracy of timing the brief exposure period coupled with the varying efficiency of the re-

newed air flow to sweep out remaining $C^{14}O_2$ from the chamber. Between the highest and lowest tracer uptakes the only detectable difference was the increased specific activities of labeled fractions and compounds at the higher levels of uptake.

Calculations involving total amounts of C^{14} translocated were expressed as a percentage of the total amount originally assimilated since the loss of C^{14} as respired CO_2 during the first 3 hours was small. An assay of the respired CO_2 from the entire plant indicated losses of less than 4 % of the total C^{14} within the first hour after $C^{14}O_2$ assimilation and a further 2 to 3 per cent loss during the next 2 hours.

The compounds that incorporated the major proportion of the C^{14} following a 15-second period of $C^{14}O_2$ assimilation are shown in figure 3A, B. Within one minute a high C^{14} incorporation occurred in the ethanol-insoluble residue and among the phosphorylated sugar derivatives. In the next few minutes the C^{14} from these compounds appeared in su-
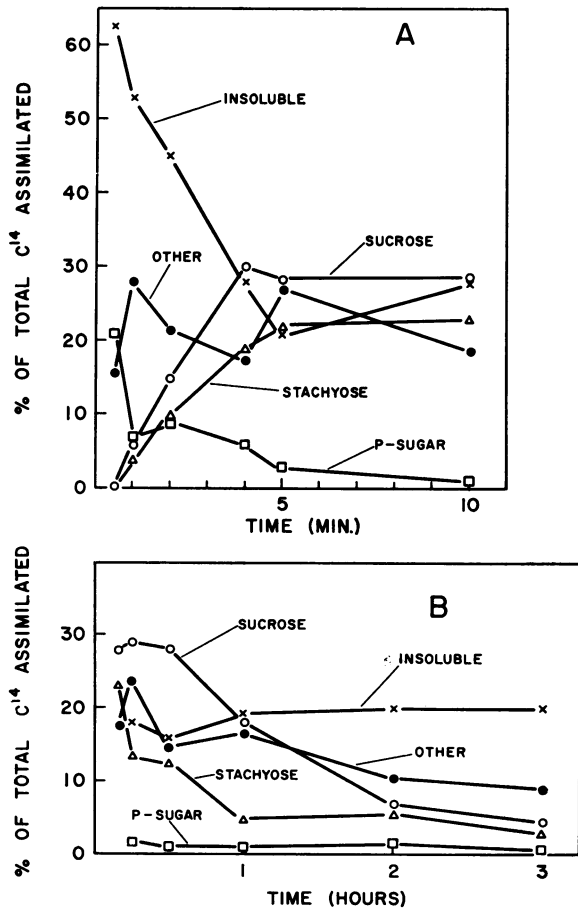

Fig. 3. Distribution of C^{14} in the primary leaf, A) within 10 minutes and B) within 3 hours of assimilation. The activities in amino and organic acids have been combined and expressed as Other. The phosphorylated sugars, P-Sugar, were not individually resolved on the radiochromatograms.
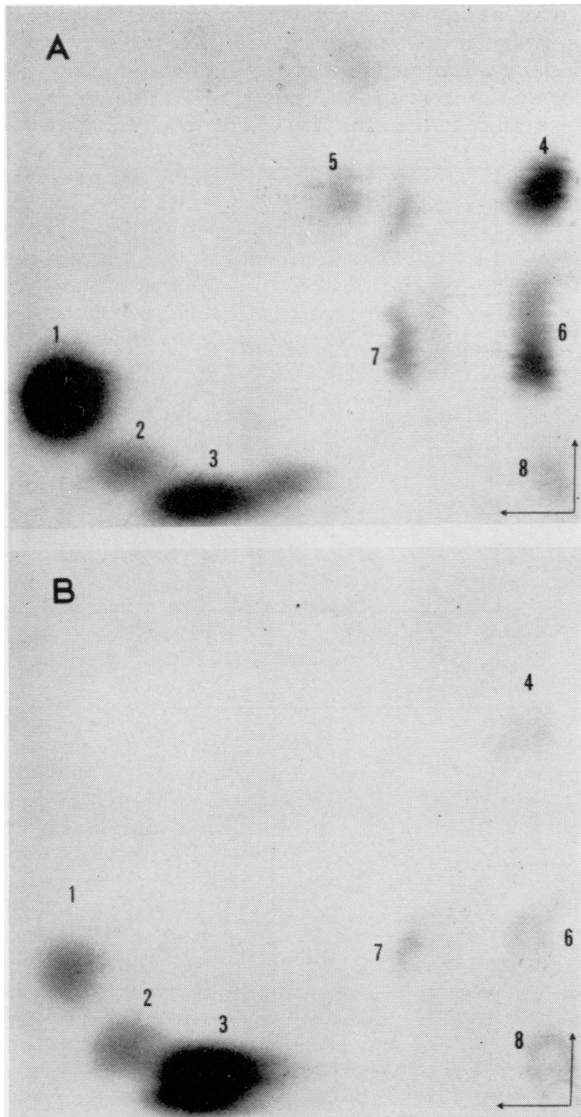
crose, stachyose, raffinose, and in smaller amounts in a wide range of other ethanol-soluble substances. Maximum incorporation of C^{14} into sucrose, stachyose and the remaining ethanol-soluble fraction occurred between 5 and 10 minutes following $C^{14}O_2$ assimilation. After 5 minutes there was little further change in the activity of the ethanol-insoluble residue for at least 2 to 3 hours.

To simplify presentation throughout this work the changes in C^{14} content of both stachyose and raffinose have been combined. The C^{14} content of raffinose was at all times a constant but small percentage of that in stachyose. No immediate relation appeared to exist between the labeled sucrose and stachyose molecules that were simultaneously synthesized during the first 5 minutes. Acid- and enzyme-hydrolysates of the 2 sugars, purified by ion-exchange resins and paper chromatography showed sucrose to be composed of approximately equally labeled fructose and glucose. Stachyose was labeled predominantly in the galactose residues with only traces of activity in the fructose-glucose moiety. There was no evidence for free labeled galactose existing in leaf tissues during the period of C^{14} incorporation into stachyose.

Figure 4A shows a typical radiochromatogram of the ethanol-soluble fraction of the leaf blade 15 minutes after $C^{14}O_2$ assimilation.

Export of C^{14} from the Primary Leaf Blade. Exported soluble C^{14}-compounds from the mature leaf blade were detected in the petiole at one to 2 minutes after $C^{14}O_2$ assimilation. The initial rate of export was rapid (fig 5) and coincided with the disappearance of C^{14}-labeled stachyose in the blade during the first 30 to 45 minutes (fig 3B). The depletion of sucrose-C^{14} commenced after an initial lag period lasting about 30 minutes (fig 3B). Approximately half of the total C^{14} assimilated by the blade had been exported in the 3 hours following assimilation and there was no further net decrease of C^{14} in the leaf blade after this time (fig 5). Figure 5 also shows how the C^{14} was distributed to the parts of the plant.

Movement of C^{14} through the Primary Petiole. During and immediately following $C^{14}O_2$ assimilation by the blade small amounts of C^{14}, below the limits of detectability, undoubtedly passed through the petiole. At 5 minutes a C^{14} gradient was measured down the length of the petiole, (fig 6). The minimum velocity of this first detectable translocate in the petiole was at least 290 cm/hour. The amount of C^{14} in the petiole rapidly increased (fig 6) and within 15 to 20 minutes the downward gradient first observed was replaced by a uniform distribution of activity. With increasing time a marked reversal in gradient occurred which reached its steepest slope at 45 minutes. This slope was more or less constantly maintained for a further 2 to 14 hours even though apparent translocation had ceased. Figure 5 shows that maximum activity in the petiole was attained 10 to 20 minutes after assimilation and remained constant for several hours at 6 to 8 % of the total C^{14} assimilated. The ethanol-insoluble fraction contained a negligible amount of

activity was obtained. Measurements of cross-sectional area of phloem and surrounding tissues showed that these also increased proportionately with diameter and dry weight down the petiole.

Tissue-autoradiographs of transverse sections of petiole taken 30 minutes after C^{14} assimilation by the blade show the main pathway of C^{14} transport associated with the phloem (fig 7A, B, C). Labeling of

FIG. 4. Radiochromatograms of ethanol-soluble extracts. A) from a primary leaf blade and B) from a 2-cm segment of the primary leaf petiole 12 cm from the blade, 15 minutes following $C^{14}O_2$ assimilation by the leaf blade. 1) Sucrose; 2) Raffinose; 3) Stachyose; 4) Malate; 5) Glycerate; 6) Aspartate/Glutamate; 7) Serine; 8) Phosphorylated sugars.

C^{14} during the first hour but by 3 hours it had accumulated about 40 % of the total C^{14} in any one petiole segment.

The establishment of the stationary upward gradient of C^{14} very closely paralleled the increase in diameter, dry and wet weight of the petiole from top to base. When the C^{14} distribution along the petiole was expressed on the basis of activity per unit dry (or wet) weight per 2 cm segment vs. distance from the leaf blade, a more or less uniform distribution of
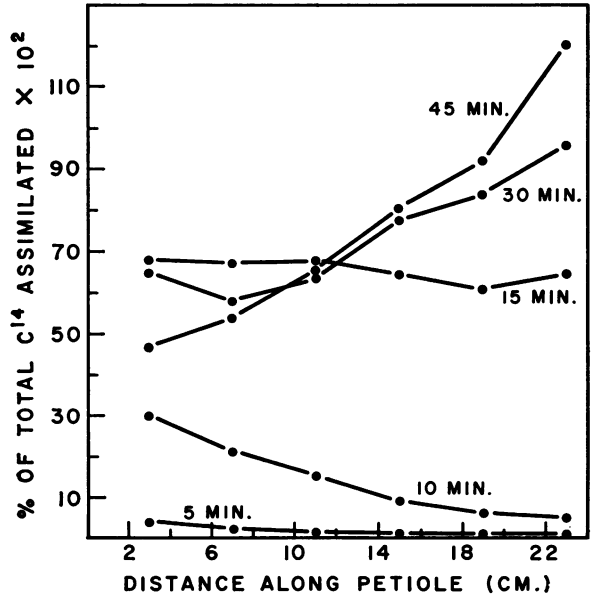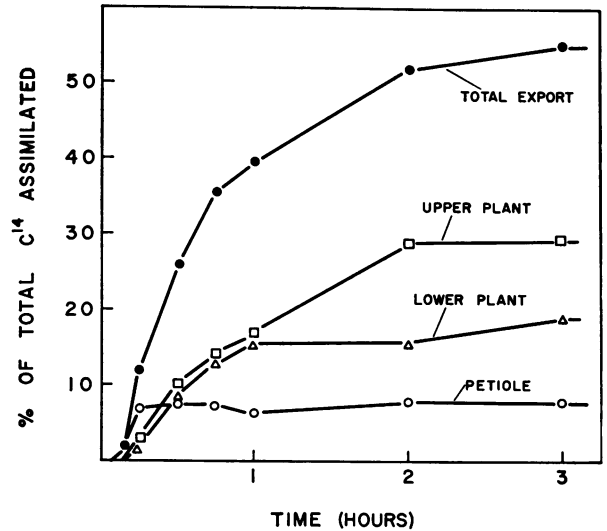

FIG. 5 (*upper*). The distribution of translocated C^{14} from the primary leaf blade to all parts of the squash plant. Data includes activity in both the ethanol-soluble and insoluble fractions. Upper and Lower designate above and below the primary leaf node respectively; Petiole refers to that of the labeled primary leaf.

FIG. 6 (*lower*). Distribution of translocated C^{14} down the length of the petiole from the leaf blade. Each point plotted represents the total activity in a 2-cm segment.
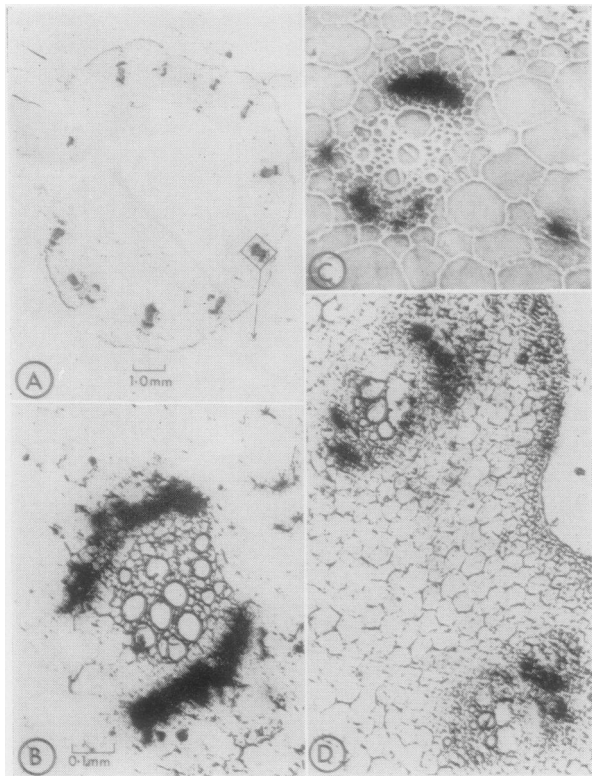
both the internal and external phloem in the bicollateral bundles appeared to be equally heavy by this time. Discrete patches of C^{14} can still be seen in the internal phloem (fig 7B) showing that certain cells (sieve elements?) initially contained considerably more activity than the surrounding cells of the phloem tissue. During 30 minutes of translocation from the leaf blade there was little radial spread of C^{14} in the petiole. The slight contamination observed in the xylem and surrounding parenchyma cells may have been due to a prefreezing artifact caused by the rapid uptake of labeled exudate from the cut phloem by tissues such as the xylem which were in a state of hydrostatic tension prior to the cutting of the slice. The commissural sieve tubes of the petiole forming a lateral bridge between the vertical phloem also translocate C^{14}-containing compounds (fig 7C).

At the basal end of the petiole, 5 minutes after $C^{14}O_2$ assimilation by the blade, labeled stachyose, sucrose, serine/glycine, aspartate/glutamate and malate were detected on radiochromatograms. With increased time the change in distribution of C^{14} among the compounds of the petiole is shown in figure 8. A

typical radiochromatogram at 15 minutes following assimilation is shown in figure 4B. Figures 3B and 8 clearly demonstrate that the disappearance of stachyose-C^{14} from the leaf was closely followed by its appearance in the stachyose of the petiole.
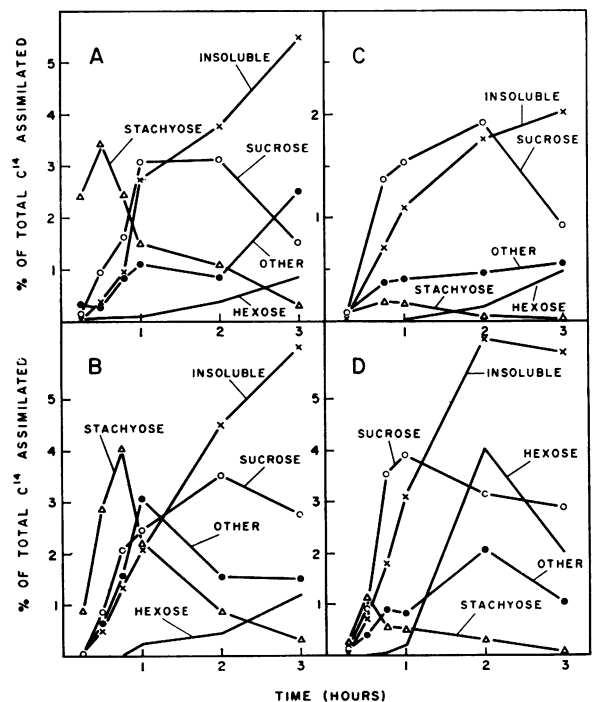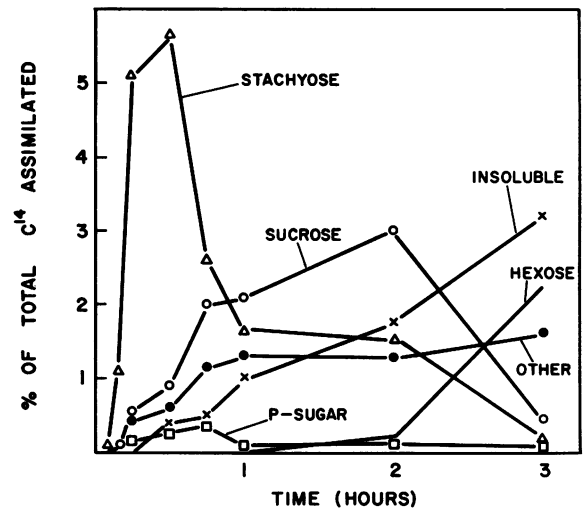
Fig. 8 (upper). Distribution of translocated C^{14} in the primary leaf petiole. Radioactivities in amino and organic acids have been combined as Other and in the chromatographically unresolved phosphorylated sugars as P-Sugar.

Fig. 9 (lower). Distribution of translocated C^{14} in parts of the squash plant: A) The Stem and hypocotyl; B) the root; C) the shoot; D) immature third leaf. The total radioactivity in organic and amino acids and the phosphorylated sugars are included in Other. Note the change of scale along the ordinate in C.

Movement of C^{14} through the Stem and Hypocotyl. Over a 3-hour period the stem and hypocotyl did not accumulate more than 10 % of the total C^{14} assimilated by the leaf blade. The distribution of C^{14} with time among the compounds of stem and hypocotyl (fig 9A) was similar to that of the petiole. Activity was detected simultaneously in both stem and hypocotyl 5 to 10 minutes after C^{14}O$_2$ assimilation (fig 5) indicating an immediate bifurcation of the flow of C^{14}-compounds from the petiole upon reaching the node.

Movement of C^{14} into the Root. Import of C^{14}-compounds into the root was detected 10 minutes after C^{14}O$_2$ assimilation by the primary leaf. A maximum import into the lower plant of about 16 % of the total C^{14} assimilated occurred within one hour (fig 5). The C^{14} content remained at this level during the next 12 to 13 hours in light. There was therefore no net loss of C^{14} from the roots during this period due to the recirculation of soluble carbon compounds in the transpiration stream, as suggested by Kursanov and Pristupa (7, 16).

The distribution of C^{14} with time amongst compounds of the root (fig 9B) was similar to that in the petiole, stem and hypocotyl. Ninhydrin analyses showed that the roots contained appreciable amounts of glutamate and aspartate and other amino acids, but these did not become heavily labeled during the first 3 hours following C^{14}O$_2$ assimilation by the leaves.

Movement of C^{14} into the Developing Shoot and Leaves. Movement into the upper plant continued for at least 3 hours by which time a maximum of about 30 % of the total C^{14} assimilated by the primary leaf had moved into this region (fig 5).

Although the differentiating shoot was of comparatively small size, its accumulation of C^{14} on a weight basis surpassed all other importing regions of the plant. Dry weight and total activity analyses of the shoot gave rates of C^{14} accumulation per unit mass that were 4 times the maximum rate of an enlarging young leaf. The distribution of C^{14} among the ethanol-soluble and insoluble compounds of the shoot is shown in figure 9C. The most interesting feature observed was the contrast between the rates of stachyose-C^{14} metabolism in the stem and the shoot (fig 9A and C). During the first 30 minutes stachyose-C^{14} reaching the shoot from the stem was almost completely converted to sucrose-C^{14} and other compounds.

Movement of C^{14}-compounds into leaves depends on their stage of development. This is clearly demonstrated for the second leaf in figure 10A where the import/export relation to leaf development is shown. Increased import capacity for translocated compounds in the young leaf closely coincided with increased dry mass as the leaf and petiole expanded. Maximum total importation was reached at a petiole length of about 8 cm. At this stage the young leaf imported in one hour as much as half of the total C^{14} exported by the mature primary leaf. With further increase in size acceptance of translocated C^{14}-compounds rapidly

declined to zero, and the ability of the leaf to export its own photoassimilated carbon compounds was developed. Figure 10A indicates that a small simultaneous bidirectional flow occurred in the petiole at about the time that the leaf had attained half its maximum size. This period of simultaneous 2-way movement of C^{14} in the petiole was maintained for approximately 24 to 36 hours.

The distribution of C^{14} among the compounds imported by a young leaf with a petiole 8 cm in length, is shown in figure 9D. As in the shoot the disappearance of stachyose-C^{14} arriving from the stem (fig 9A) was closely correlated with its appearance in sucrose and other compounds.

A typical tissue-autoradiograph of a transverse section of an immature third leaf petiole 3 cm long, is shown in figure 7D. The tissue was analyzed 30 minutes after the primary leaf had assimilated C^{14}O$_2$. Imported C^{14} was mainly associated with the phloem but the movement of labeled compounds into the sur-
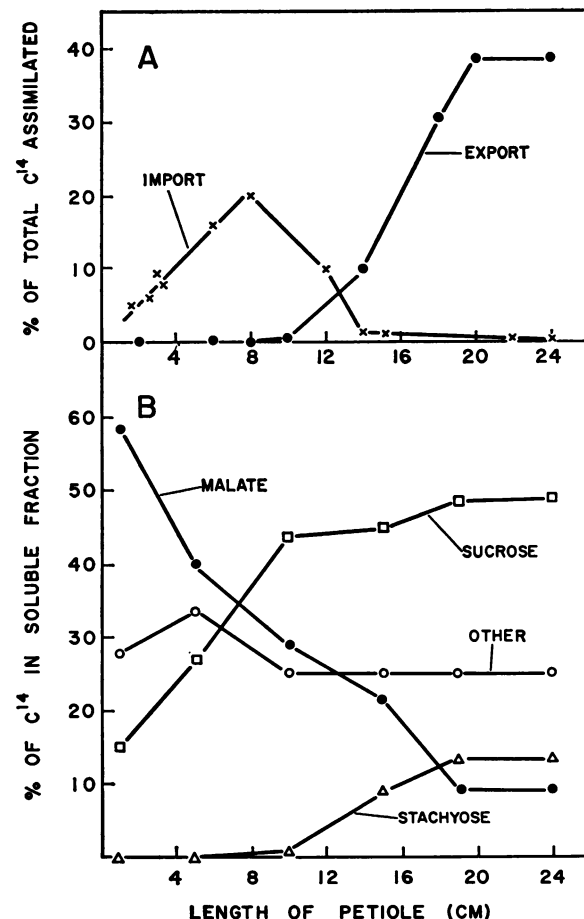

FIG. 10. A) The C^{14} import and export variation with leaf development and B) changes in the percentage distribution of assimilated C^{14} within the ethanol-soluble fraction of a young leaf at different stages of development. Analyses carried out one hour after C^{14}O$_2$ assimilation. Stage of leaf development is arbitrarily expressed as total length of petiole.

rounding tissues could be discerned. The rapid radial movement of C^{14} from the phloem into the surrounding tissues is a characteristic feature of leaves that are rapidly expanding.

Cotyledons. These organs actively assimilated $C^{14}O_2$ until at least the third leaf had matured, at which point they began to yellow and quickly withered. There was no translocation of C^{14} into the cotyledons from other regions of assimilation.

Developing Leaves. As the developing leaf blade increased in size pronounced quantitative changes occurred among the ethanol-soluble compounds analyzed one hour following 15 seconds $C^{14}O_2$ assimilation. This is shown for the second leaf in figure 10B where the length of the petiole was taken as a convenient index of growth. The maximum lengths attained by petioles of this plant under the culture conditions employed were 24 to 26 cm. It is of interest to note that at about half maximum petiole length labeled stachyose and raffinose appeared on radiochromatograms coincident with the commencement of translocation (fig 10A, B).

Mature Leaves. The second, third, and fourth leaves behaved similarly to the primary leaf in both assimilation of C^{14} and in its translocation to other parts of the plant. As leaves aged and yellowed their capacity for assimilating and exporting C^{14} declined. What little was exported went primarily to the actively growing regions of the plant with the shoot exerting the greatest pull. An aged and yellow primary leaf was still capable of contributing to the translocates being imported by the eighth and ninth growing leaves of a plant, but was incapable of supplying any detectable C^{14} to the hypocotyl and root system.

Movement of C^{14} in the Transpiration Stream. In experiments of up to 14 hours following $C^{14}O_2$ assimilation, no significant return flow of C^{14} from the root system via the xylem tissue was found. Mature leaves, although transpiring freely, did not receive any detectable C^{14} from the transpiration stream. Furthermore the absence of any significant concentration of C^{14} from the xylem has been confirmed by tissue autoradiographs of the primary petiole.

Discussion

The results have clearly established that sugars translocated in the phloem of the squash are derived only from mature leaves. The tobacco and soybean have been found to behave similarly (6, 22).

In the young leaf blades of the squash the metabolic rate of photoassimilated carbon is geared to its complete consumption in situ. In these young organs, however, demand exceeds local supply and a rapidly expanding leaf draws heavily upon carbon translocates obtained from mature leaves. As the leaf approaches maturity there is a gradual change in metabolic pattern leading to a greater sucrose surplus and to the synthesis of stachyose. Coinciding with the presence of stachyose in the blade export slowly develops producing a reversal of the direction of trans-

location in the phloem. It is possible that some phloem channels may lag behind others in carrying out this reversal, thereby accounting for the simultaneous bidirectional movement observed over a period of 24 to 36 hours in the leaf petiole (fig 10A). An understanding of this phenomenon may supply an important clue to the mechanism of phloem translocation.

In the mature leaf, assimilated C^{14} is immediately bound in an ethanol-insoluble fraction followed by its rapid incorporation into sucrose, stachyose, raffinose and a variety of other ethanol-soluble compounds. Free phosphoglyceric acid is not among the first products of C^{14} assimilation. Diffusion of translocates from the sites of production across the mesophyll tissue and into the phloem channels must be rapid because ethanol-soluble C^{14} compounds are detectable in the petiole below the leaf blade in one to 2 minutes following $C^{14}O_2$ assimilation. However, the possibility that this initial movement represents gaseous diffusion of $C^{14}O_2$ accompanied by fixation cannot be excluded.

A 15-second exposure of the mature blade to $C^{14}O_2$ produces an export of C^{14} compounds for at least 3 hours. Presumably this results from both a temporary storage and a continuous recycling within the blade of C^{14} even to the extent of a reassimilation of $C^{14}O_2$ liberated through the respiratory activities of the blade tissues. Stachyose and sucrose are the compounds in which most of the C^{14} is exported from the leaf blade. The rapid loss of C^{14} from the blade during the first 20 to 30 minutes is primarily a decline in stachyose-C^{14} which can be traced in the petiole, stem, hypocotyl and root as it moves through the plant. During this time stachyose lost from leaf blade can be almost accounted for by that recovered elsewhere in the plant, indicating little metabolism of stachyose during this period in the conducting channels. Sucrose-C^{14} appears to behave differently. It is held by the blade tissue for 30 minutes during which time it is probable that only a small quantity is exported. Following this lag period the steady translocation of sucrose-C^{14} begins and continues for a further hour. It is transported along the same routes as stachyose replacing the latter as the major form of exported C^{14}. Further proof for sucrose translocation is shown by the fact that sucrose-C^{14} recovered outside the blade cannot all have been derived from the hydrolysis of exported stachyose, as the total amount of stachyose-C^{14} lost from the blade amounts to less than half of the total C^{14} exported in 3 hours. From the observed disproportionate changes in radioactivity between sucrose and stachyose (figs 3A & B) it can be concluded that stachyose formation in the blade is not derived from recently synthesized sucrose. The C^{14} distribution within stachyose and sucrose confirmed this conclusion.

Evidence for the movement of labeled organic and amino acids was negligible. A small, continuous loss of these compounds in the leaf blade was measured during 3 hours of translocation and equivalent amounts were recovered elsewhere in the plant, but

there is no direct evidence to prove that these compounds were exported and not secondarily derived from the localized metabolism of either deposited stachyose or sucrose. Evidence for the translocation of organic and amino acids has, however, been obtained in soybeans and rhubarb (9, 13).

The flow of C¹⁴ through the petiole is composed of a longitudinal movement through the phloem and an accompanying deposition either in or near to the channels of conduction. The C¹⁴ retained must remain in the petiole for an appreciable length of time accounting both for the retention of C¹⁴ long after its translocation from the leaf has ceased and for the upward gradient of figure 6, where retention of C¹⁴ was proportional to tissue mass.

All growing regions of the squash incapable of sustaining their own carbon requirements make a demand upon the translocation flow from mature leaves. This is now a well established feature of the higher plants (6, 19, 22). In the squash the demand is transmitted to at least the nodal region of the mature leaf. Here the incoming sugar stream from the leaf blade is distributed simultaneously into an upward and a downward movement with the upper region of the plant having the greatest requirement. Sucrose-C¹⁴ dominates the labeled compounds in young leaves and the shoot within 15 to 30 minutes contrasting with the patterns obtained in the root and conducting organs. With the negligible loss of sucrose-C¹⁴ from the primary leaf blade during this period such exported sucrose-C¹⁴ must be derived from the rapid metabolism of stachyose in these young growing tissues.

There is no evidence for a recirculation of recently assimilated C¹⁴ in organic and amino acids from the roots to the upper plant via the transpiration stream, as proposed by Kursanov (7).

A diagrammatic reconstruction of translocation in the phloem of the squash as judged by C¹⁴ displacement is shown in figure 11, where different rates of movement are indicated by arrows of different thickness. It is important to note that the simultaneous bidirectional flow while only weakly and temporarily established in the third leaf is strongly and permanently established in the stem between the primary and second leaf nodes. The evidence for circular or lateral distribution between the main vascular bundles is derived from the fact that any mature leaf actively exporting C¹⁴-compounds can supply any other importing young leaf irrespective of its phyllotaxy. This appears contrary to evidence from other plant species (8) where patterns of distribution are found closely associated with the vascular connections between plant organs. Part of this lateral movement has now been shown to occur in the commissural sieve tubes which are a characteristic feature of the Cucurbitaceae (2). These phloem interconnections have been known for many years but until now there has been no direct evidence for their function. Most of the lateral flow, however, must take place in the nodal regions where the petiole traces join a complex of phloem tubes oriented circularly around the node with

connections to all the vertical phloem files. No evidence could be found for separate functions of the internal and external phloem in the bicollateral bundles.

Translocation of assimilated carbon in the squash plant is therefore a distinctly channeled multidirectional movement associated with the phloem. This movement appears to be determined by the demands of growth and is switched readily from leaf to leaf as each matures and ceases to require carbon assimilates synthesized elsewhere. The real cause and control of these movements however remain obscure. In spite of abundant speculation in the literature (3, 18, 21, 28), there is negligible experimental evidence to support any one of the proposed theories of either passive or active translocation to the exclusion of all others.

Summary

The formation and movement of C¹⁴-translocates derived from a 15 second period of C¹⁴O₂ photoassimilation by leaves of *Cucurbita melopepo torticollis* Bailey have been studied for periods up to 3 hours.

C¹⁴-translocates from mature leaf blades were detected in the petiole within 5 minutes of assimilation, moving at a minimum velocity of 290 cm/hour. The rate of C¹⁴-export from the blade reached a maximum within 10 to 15 minutes, slightly diminished during the following 30 minutes and declined more rapidly thereafter. By 3 hours, when 55 % of the total assimilated C¹⁴ had been exported, there was no further net output.

Stachyose with a trace of raffinose and sucrose were the principal compounds translocated to all parts of the plant except other mature leaves. There was insufficient evidence to decide whether other labeled
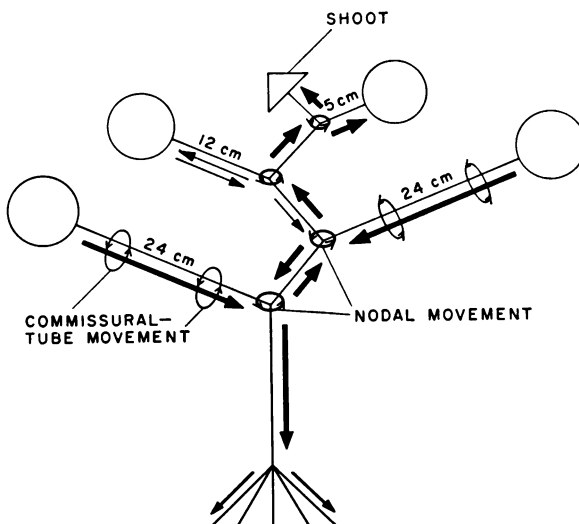

FIG. 11. A diagrammatic pattern of the movement of C¹⁴ throughout a 15-day old plant with mature primary and second leaves. The lengths of the petioles are indicated and the quantities of C¹⁴ moving in any one direction are shown by approximately thickened arrows.

compounds were exported. Stachyose-C^{14} was almost completely exported from the blade within 45 minutes. In the roots and stem it was relatively slowly metabolized but in young leaves and the shoot it was rapidly metabolized to sucrose and other compounds. Sucrose-C^{14} was only partially exported following a lag period of about 30 minutes.

About 14 % of the total translocated-C^{14} was retained by the petiole of the treated leaf. The remaining 86 % was distributed from the nodal region simultaneously toward the upper and lower plant, the orientation of the vascular system facilitating movement of C^{14}-translocates to all immature organs. Immature leaves and the shoot were the regions most actively importing C^{14}-translocates.

Depending on its stage of development an immature leaf photosynthesizing sucrose could import up to 50 % of the total translocate from a mature leaf. There was no export of C^{14} from such an immature leaf and no detectable synthesis of stachyose-C^{14}. As the leaf approached maturity import declined, stachyose synthesis was detected and C^{14} export began, resulting in a simultaneous bidirectional movement of C^{14} in the petiole for 24 to 36 hours. With further leaf growth import ceased.

Partly yellowed, senescent leaves were still able to synthesize stachyose and sucrose and contribute them to the translocation stream.

In mature petioles and stems C^{14}-translocation occurred in the phloem of the bicollateral bundles and in the commissural sieve tubes accompanied by a slow radial movement to surrounding tissues. By contrast, the import of C^{14} in the phloem of immature petioles was accompanied by rapid radial movement of C^{14} into the surrounding tissues.

No return of C^{14}-compounds from the roots to the upper plant in the transpiration stream was detected.

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