Translocation of C¹⁴ Metabolites in the Phloem of the Bean Plant [1]

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Introduction

Relatively little is known of the nutritional inter-relationships of plant parts. Currently, however, considerable effort is being expended in such studies, and satisfactory methods for following specific metabolites from their sight of synthesis to their destination have been developed. Kursanov's (9) recent review includes this area of translocation phenomenon.

The general conclusion to be reached from these studies is that there exists a demonstrable localized sink area for the metabolites produced in a particular source area, and during the course of the movement of the metabolite between them there is little lateral transport and general mixing with substances moving in an adjacent source-sink channel (7,9).

Assimilates from mature leaves are said to move to immature ones of the same orthostichy, but there is little movement between mature leaves (7,9).

It is now generally understood that the assimilate flow from a single leaf may be divided, on reaching the stem, into an upward moving and a downward moving component. When the flow from 2 leaves is considered, if 1 is located above the other, the downward moving component from the upper leaf must pass the upward moving component from the lower leaf. This has been loosely referred to as bidirectional movement since a given internode supports movement in opposite directions. How this occurs was the subject of a former study (1), but the details of the movement are better clarified herein.

Zimmerman, in 1959 (11), rightly stated that bidirectional movement in a sense incompatible with mass flow had not been demonstrated. This would probably require that the movement be bidirectional within the smallest functional conduction unit, i.e., within files of sieve cells.

What is required for a better understanding of the nutritional interrelationships of parts and for a clarification of the mechanics of metabolite movement, is a successful linking of detailed flow patterns with detailed phloem anatomy. Anything less has not proved to be of critical value.

The present report is concerned with the information that has been gained from a direct comparison of high resolution autoradiograms of assimilate flow with the fluoresced phloem tissue through which the flow occurred. This permitted the precise identification of the particular phloem bundles involved in the transport of metabolite between a selected source area and its normal receiving sights.

Methods

The study progressed from (A), a determination of the gross export pattern from leaves in different positions, and, therefore, of different ages, to (B), a determination of the precise phloem channels through which the flow occurred, and then to (C), an identification of 2 of the most important mobile metabolites. Only part B required the development of new methods.

The experimental material consisted of Red Kidney bean plants grown in aerated half-strength Hoagland solution, with micronutrients, under the following environmental conditions: Temperature $23 \pm 1°$, fluorescent light at 1000 to 1200 ft-c on a 12-hour photoperiod, and a relative humidity of $60 \pm 5\%$.

The age of the plants when treated with $C^{14}O_2$ varied with the different experiments, but the usual age was 18 days from seed. At this age the third trifoliate leaf had just matured from the stage of a dependent, importing organ to a self-sufficient, exporting organ. For parts A and B of this study, only plants of this age were used. For part C the export pattern of juvenile leaves was determined as they first started to export.

Transparent leaf chambers with volumes of 860 and 112 cc's were employed to confine $C^{14}O_2$ around entire leaves or single leaflets, respectively. The $C^{14}O_2$ generated from either 1 or 2 mg of $BaC^{14}O_3$ was transferred directly into a leaf chamber as indicated with each experiment. For prolonged experiments the cover of the leaf chamber was removed after the first hour of confinement. No attempt was made to control the humidity within the leaf chamber during the period of leaf confinement; however, it was observed that no condensation occurred on the chamber walls. $C^{14}O_2$ treatment was between the first and third hours after the beginning of the photoperiod.

Gross Autoradiography. For each leaf separately, 0.114 ml of $C^{14}O_2$ containing 0.127 mc of radioactivity was introduced into the 860-ml leaf chamber which had been carefully sealed around the entire leaf. After a 1.5 hour migration period the plants were removed and severed into root, individual leaves, upper and lower stem and stem apex. Division between

upper and lower stem was made at the node of the treated leaf. The parts were dried between blotters at 80° and exposed to no-screen X-ray film for 40 hours.

Fluorescence and Autoradiography of Phloem Channels. To determine which phloem bundles conduct the metabolite up and down the stem, 0.228 ml of $C^{14}O_2$ containing 0.254 mc of radioactivity was introduced into the 112-ml leaf chamber surrounding the terminal leaflet of 1 of the trifoliate leaves. The axillary buds of these plants were removed as they appeared in order to obtain a straighter, more easily handled stem. After a 30-minute migration period the stem between the cotyledonary and the third trifoliate leaf nodes was quickly stripped by making a longitudinal slit along its length and peeling the bark from the wood (2). The bark sections were plunged into isopentane chilled in liquid N_2. They were then transferred quickly, while frozen, to a prechilled freeze-drying chamber at −40° and dehydrated at that temperature. The freeze-drying apparatus was of the gas flow variety (3). When dehydrated (after 24 hours in the chamber), the bark was removed, placed on filter paper, and exposed to an atmosphere at 60 % R.H. for about 45 minutes. This treatment relaxed the dehydrated bark and prevented splitting when it was flattened. Since the bean stem is not normally straight (it angles at each node), the handling of the bark as 1 continuous unit was not feasible. It was cut transversely just above or below a node depending upon the situation of interest. In either case, a small longitudinal slit from the shortest cut end toward the node was necessary to obtain the minimum distortion when flattened.

The relaxed bark segments were placed cambial side down on a chrome-plated metal surface and carefully flattened. Layers of filter paper and a weight were added and the whole placed in an 80° oven to dry. It was found by trial that the treatment at 60 % R.H. did not provide sufficient moisture to permit redistribution of the C^{14}-labelled materials in the tissue. When dry, the radioactivity was determined, and the exposure calculated (10) for the film used (Eastman contrast process panchromatic). When a satisfactory autoradiogram was obtained, a fluorescent photograph of the phloem was made for comparison. This was done as follows: The tissue was placed in an aniline blue solution as used by Currier (5) to visualize callose, and a partial vacuum was established to facilitate its penetration. Several hours after full infiltration, the tissue was removed and mounted in glycerol between glass plates. It was then illuminated by the incident light from 2 transformer-operated, General Electric H100 SP4 lamps with Corning CV.X. R.D.L. (dark blue) filters and photographed through a Wrattan #58 yellow-green filter. The callose associated with the sieve plates complexed with the analine blue and fluoresced under the blue light so that the resultant photograph, consisting of fluoresced sieve plates, provided an ideal way to study the whole phloem system.

With a certain number of plants the bark and wood sections were subjected directly to radioactive analysis in order to determine the amount of metabolite that had been transferred from the bark to the xylem cylinder. Determinations were made only on the 80 % ethyl alcohol v/v extractable fraction.

Identification of Mobile Constituents. For this study 0.114 ml of $C^{14}O_2$ containing 0.127 mc of radioactivity was introduced into the 860-ml leaf chamber surrounding the entire third trifoliate leaf. A small number of studies were made on the primary, and the first and second trifoliate leaves, both as mature and as young leaves just beginning to export. Under the growth conditions listed above, export began when the terminal leaflet was about 8.8 cm long.

Two recording rate meters were used to determine the beginning and the rate of export. The G-M tube of 1 was positioned at the internode below the treated leaf and the other at the young unfolding leaflets at the node above. At various times after export began, plants were harvested and extracts prepared for chromatography. By selecting progressively younger leaves to receive $C^{14}O_2$, thereby increasing the time between labelling and export, the normal labelling time for each of the exported materials could be determined. In certain of these experiments a second labelling with $C^{14}O_2$ was required to satisfactorily label the principal downward-moving component. In each case the $C^{14}O_2$ label was retained around the leaf for not more than 1 hour.

Plant tissues were extracted overnight in 95 % v/v ethyl alcohol which was initially hot but allowed to cool slowly. The extract was removed and evaporated at reduced pressure over anhydrous $CaCl_2$. The dried residue was dissolved in 1 ml 80 % v/v ethyl alcohol, and a fraction of sufficient volume to give suitable radioactivity was spotted on Whatman #1 filter paper for ascending chromatography in ethyl acetate: pyridine: water at 120: 50: 40 relative volumes. A 56-cm ascension required approximately 15.5 hours. Sucrose was identified by comparison with known C^{14} sucrose. Two additional spots of current interest showed R_F values of approximately 0.95, a blue color with phosphomolybdic acid, a pink color with phosphotungstic acid, a yellow-orange color with antimony pentachloride, and fluoresced under ultraviolet light when treated with NaOH. These substances appear to be steroids, but saturated cyclic compounds containing hydroxy groups have not been ruled out by means of chemical tests. Both have a carbonyl group (or groups): one has a hydroxy group, the other not, as revealed by ascension in benzene: chloroform on aluminum oxide-treated paper. No negative tests for sterols have been encountered, but more positive identification awaits the collection and purification of suitable quantities of material.

Gross autoradiograms of the stem and young leaves above the node, and detailed autoradiograms of stripped frozen-dried bark from around the node of the treated leaf, were prepared to check the results obtained with the counters.

Results

Variability both in the amount of metabolite translocated and in the destination of the material was often observed for leaves in the same relative position on plants grown under similar conditions. In an attempt to understand the variability, we have investigated the following as probable causal factors: A) the time of applying the tracer with respect to the beginning of the photoperiod; B) various transpiration rates from leaves other than the treated one; C) the effect of holding the plant in a saturated atmosphere. None of these factors overshadowed the random variation among plants. As a consequence of the variability we studied export patterns in more than 50 plants. The results selected for presentation here are representative of the most characteristic export patterns for plants grown under the conditions listed above.

Gross Export Patterns. All mature leaves exported a fraction of their newly synthesized metabolite to the stem apex and its associated immature leaves, but the proportion of the total exported material that moved toward this destination varied considerably with the position of the leaf. It was greatest for the leaf closest to the apex and decreased progressively down the stem until, for the lowest leaves, the primaries, the upward moving component was but a small fraction of the whole and almost the entire flow was downward. From the leaves positioned between the youngest (exporting) and the oldest, the flow was more equally divided between base and apex (figure 1). This flow pattern, while stated here in very general terms, can be more fully understood from the detailed anatomical study presented below.

During the course of the downward flow of metabolite in the phloem, a significant cross transfer to the xylem occurred (table I). A cross transfer of metabolite from each leaf occurred, but the percentage transfer was least for the lowest leaf and greatest from the highest, or youngest leaf. A cross transfer into the transpiration stream, resulting in delivery of traces of metabolite to the mature leaves, was most

marked for the lowermost leaf, and it decreased upward. As will be shown in the following section, the delivery into the mature leaves was not via the phloem. The amount delivered to a mature leaf was very small and the pathway indirect; that is, it involved a transfer into the transpiration stream.

Movement in Relation to Phloem Channels. The phloem anatomy at the first trifoliate leaf node is
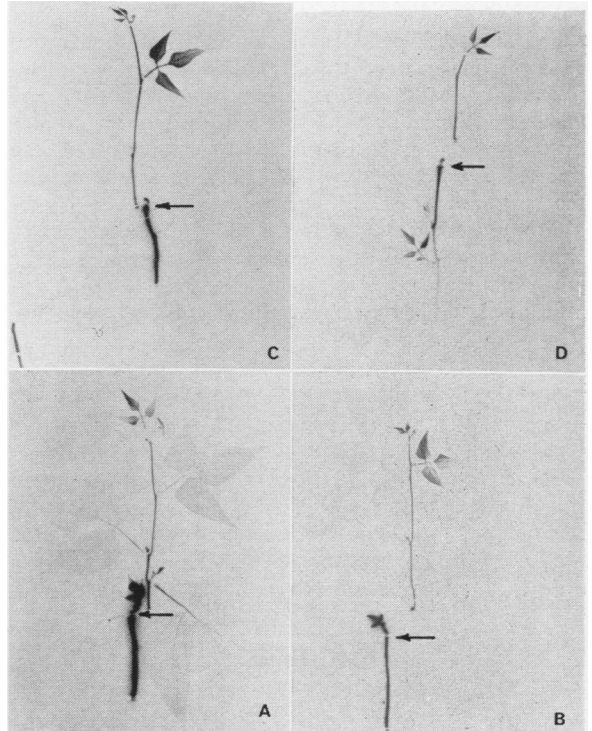

FIG. 1. Gross autoradiograms showing export of metabolite from 1 primary leaf (A) and from the first, second, and third trifoliate leaves (B), (C), and (D), respectively. The treated leaf is not shown, but its position is indicated by an arrow.

Table I. *Transfer of C^{14} Metabolites from Phloem to Xylem*
The metabolites were those soluble in 80 % ethyl alcohol after a labelling period of 30 min from $C^{14}O_2$ introduction.

Stem position	Pri*	Recipient leaf				% Transferred to wood from each recipient leaf			
		1TF**	2TF	3TF		Pri*	1TF	2TF	3TF
Internode		c/s in each stem sect. (total)							
2nd to 3rd	...	5220	2545	> 637.0		...	10	10	> 25
1st to 2nd	229	7850	> 10970	20.6		12	10	> 11	44
Pri to 1st	837	> 27100	2590	15.8		6	> 12	11	49
Cot to Pri	> 10510	20580	604	7.7		> 4	11	7	33
Root to Cot	5420	15920	268	6.0		5	10	8	32

* Only 1 primary leaf treated.
** TF, trifoliate leaf.
> Indicates node of recipient leaf.

shown in figure 2. This nodal tissue was removed and prepared with minimum distortion in order to serve as a guide in the interpretation of the following figures. The autoradiograms representing the export of C^{14}-labelled metabolite from the second trifoliate leaf are shown in figure 3 and the corresponding phloem patterns at each node in figure 4. In opening the cylinder of bark, preparatory to autoradiography, the longitudinal slit was made through the petiole base of the second trifoliate leaf. As a consequence each half of the split petiole base appeared on opposite edges of the flattened bark section, and the branch gaps for the first and third trifoliate leaves were centrally located. The leaf traces that conducted the labelled assimilate into the stem are easily identifiable by the broken stubs of the xylem component of the traces. They are lettered A to E to correspond with the autoradiogram in figure 3. From the autoradiogram (fig 3) it is evident that the metabolite descended in the phloem component of the individual leaf traces to the node below without a significant lateral movement into adjacent bundles.

Consequently, the flow pattern of the descending metabolite was traceable past the first trifoliate leaf node (as shown in fig 3C) and on down to the cotyledonary node (fig 3D). Leaf trace A, the 1 median to the petiole of the second trifoliate leaf, descended past the first trifoliate leaf node without anastomosing. This is characteristic of the second and higher trifoliate leaves. Flow in bundles B, C, D and E became divided through the anastomosing system of bundles at the first trifoliate leaf node, but the division was such that the arms of the flow passed to either side of the leaf traces entering from the first trifoliate leaf. The leaf traces are numbered 1' to 7' in figure 3C. The fluoresced phloem bundles at this node, figure 4C, show the leaf traces similarly numbered so that the path of the descending metabolite can be followed around them.

Two points should be emphasized: First, the labelled metabolite that descended from the second trifoliate leaf did not mix with, or enter, the flow (unlabelled) that descended from the first trifoliate leaf. Second, only the flow in bundle A descended past the
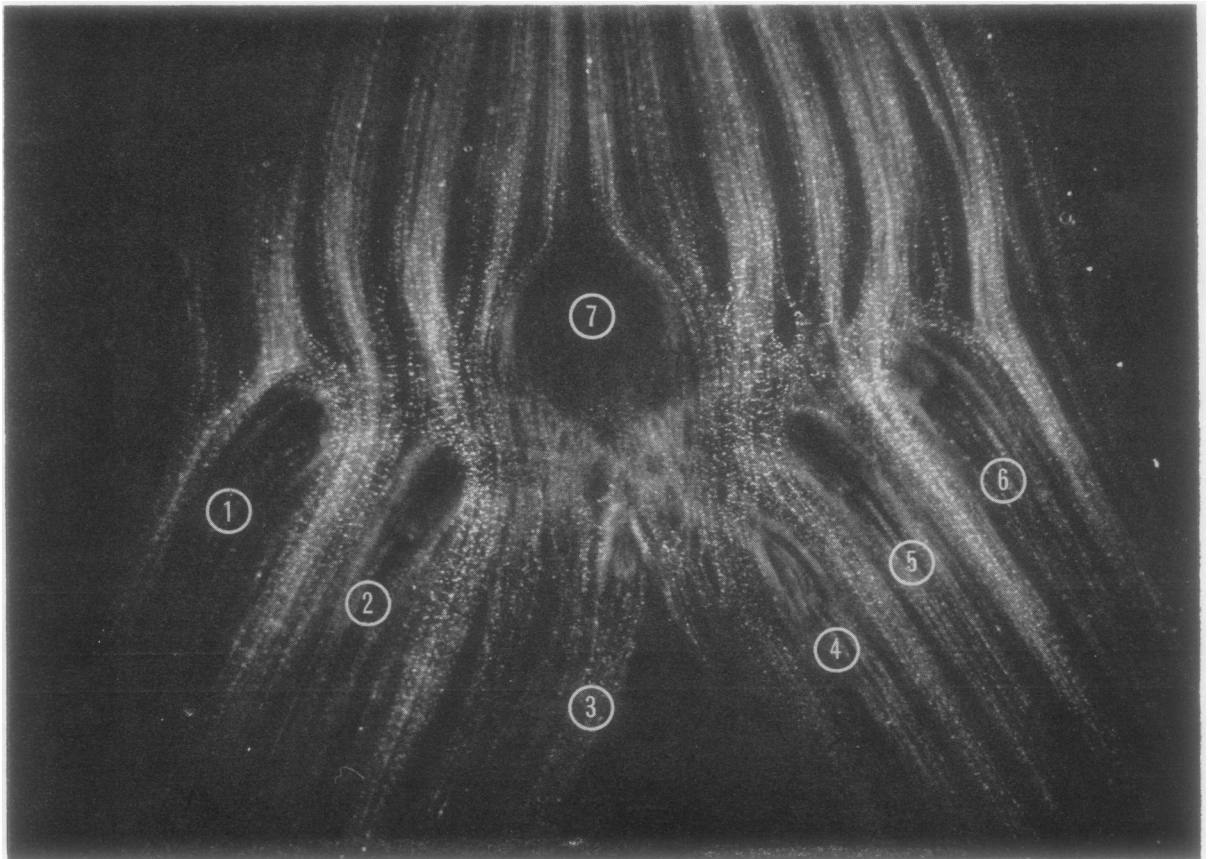

FIG. 2. The fluoresced phloem at the first trifoliate leaf node. Numbers 1 through 6 identify the leaf traces by the broken stubs of the xylem components. The branch gap is identified by number 7. A longitudinal slit along leaf trace number 3 was necessary to flatten the section for photography. In some subsequent sections the slit was made from above into the branch gap.
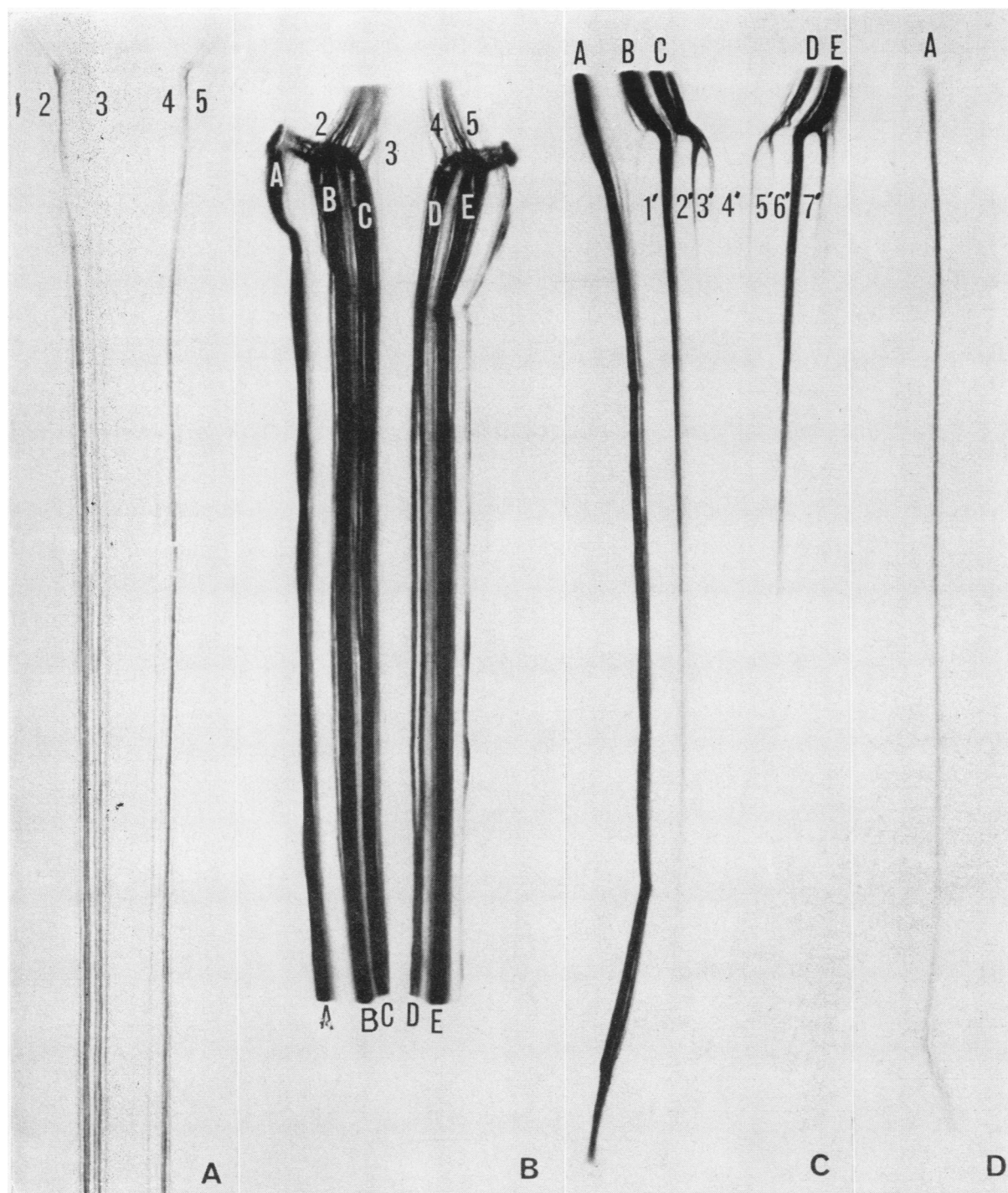
Fig. 3. An autoradiogram representing the movement of metabolite in the phloem. The metabolite entered from the second trifoliate leaf through traces A, B, C, D, and E of figure B. Some metabolite moved directly upward past the third trifoliate leaf whose traces are numbered 1 through 5 in figure A. The remainder moved downward in the leaf traces A through E past the first trifoliate leaf node whose traces are numbered 1′ through 7′ in figure C. Leaf trace A conducted past the cotyledonary node at lower extremity of figure D. Mag. 3.8x.

primary and cotyledonary leaf nodes (cotyledonary leaf node not shown in fig 4).

Upward flow of metabolite directly from the second trifoliate leaf was observed in the autoradiogram of figure 3A.   A major part of the upward flow occurred by a direct transfer of metabolite out of the leaf traces into cauline phloem bundles passing inside of them. A second but very small upward-moving component appeared to consist of a fraction of the metabolite that had descended to the first trifoliate leaf node and
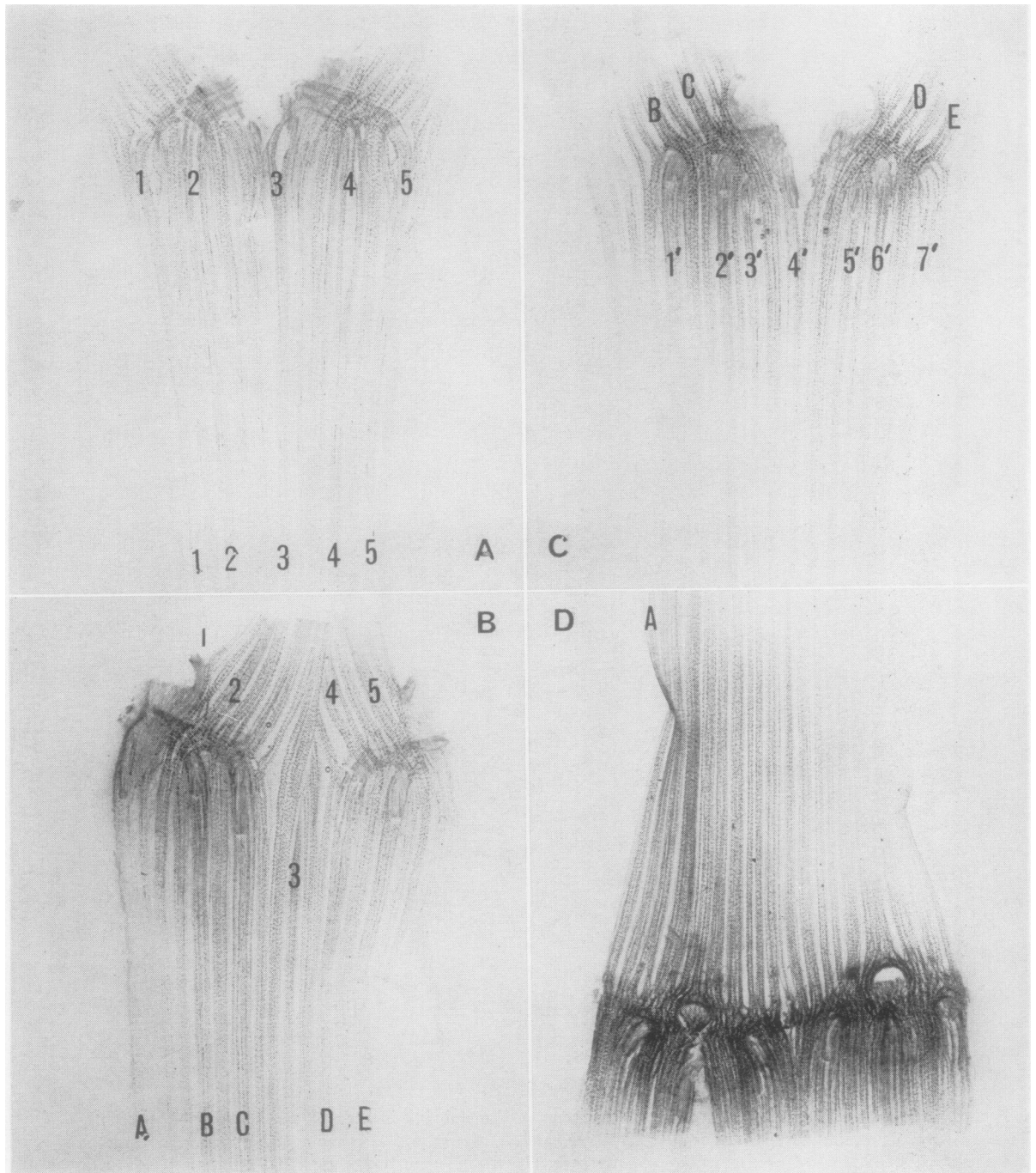

FIG. 4.   The fluoresced phloem at the third, second, and first trifoliate leaf nodes and the primary leaf node are shown in A, B, C and D, respectively. This is the tissue from which the autoradiograms of figure 3 A, B and C was prepared. A, B and C correspond to A, B and upper C in figure 3.   D, the primary leaf node, corresponds to lower C of figure 3.   The cotyledonary node corresponding to lower end of figure 3D is not shown.   The numbering and lettering is consistent with that in figure 3.   The tissue at (1) in figure B is missing.

then reascended in bundles lying adjacent to the leaf traces. This latter pattern of ascent becomes more accentuated from older leaves and can be seen much better in figure 5 which represents export from the first trifoliate leaf. The direct ascent by transfer from leaf traces into ascending cauline bundles was always more pronounced from the youngest leaves. In fact, this is the sole initial mode of export from
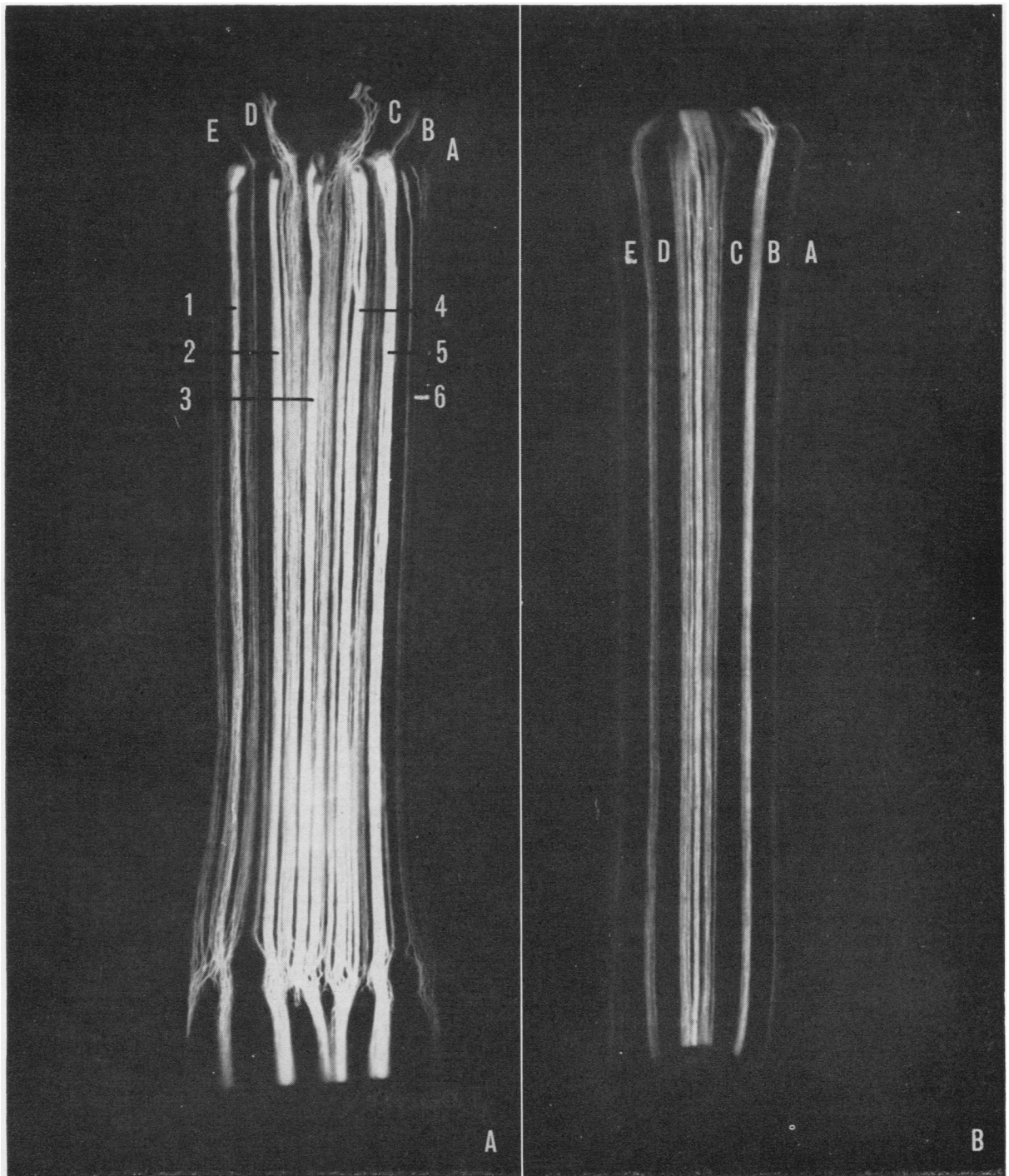

FIG. 5. An autoradiogram (reversed) representing the flow of metabolite (in the phloem) from the first trifoliate leaf. Metabolite entered in leaf traces 1 through 6 at the node in upper figure A. It descended to the primary leaf node at lower figure A. A fraction of the metabolite then ascended in bundles alternating with the leaf traces back past the node of entrance and on up past the second trifoliate leaf node in upper figure B. The descending leaf traces from the second trifoliate leaf are lettered A through E and can be followed to the first trifoliate leaf node.

very young leaves. Figure 6B shows the export pattern for a young third trifoliate leaf.

Ascension of the metabolite originating in the second trifoliate leaf past the third trifoliate leaf node (fig 3A) was in phloem bundles lying adjacent to the descending leaf traces from the third trifoliate leaf. These latter bundles are numbered 1 through 5. There was only a very slight indication, in bundles 2 and 4, of a possible intake of labelled metabolite into the third trifoliate leaf.

The pattern obtained for the export of metabolite from the first trifoliate leaf is shown in figures 5A and B. This figure is a direct projection of the autoradiogram in order to conserve the maximum detail so the labelled metabolite is represented as white on a black background. Downward flow of metabolite is heavy in 5 of the 6 leaf traces and light in the sixth. Downward flow in the leaf traces remains distinct to the primary leaf node. At this anastomosing point part of the flow continued downward and part of it reascended through adjacent phloem bundles back past the node of entrance and past the second trifoliate leaf node, figure 5B. The ascension through the internode above the first trifoliate leaf occurred in phloem bundles lying between the leaf traces descending from the second trifoliate leaf. These latter bundles are lettered A to E. Again it was evident that the descent of unlabelled metabolite from the second trifoliate leaf took place in phloem bundles alternating with those in which ascent of labelled metabolite occurred, i.e., bidirectional movement involved separate distinct phloem bundles. No exceptions to this rule were found in the studies involving the mature leaves on more than a dozen plants. There was no apparent upward movement of metabolite from the first trifoliate leaf by a direct transfer into cauline bundles such as occurred from the second trifoliate leaf and to a still more marked degree from the third.
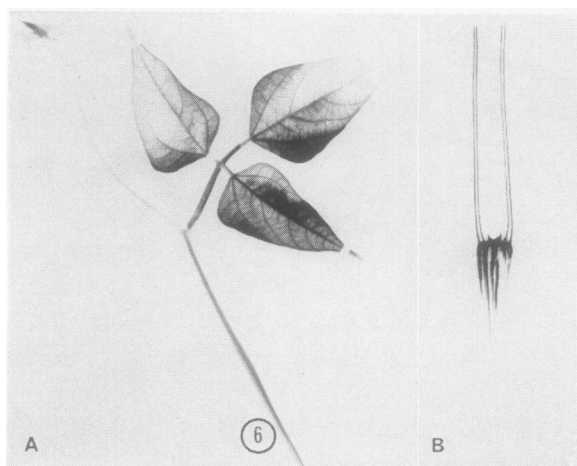

FIG. 6. A, A gross autoradiogram of the fourth trifoliate leaf and stem apex showing metabolite originating in the third trifoliate leaf as it first began to export. B, An autoradiogram of the frozen-dried stripped bark above and below the third trifoliate leaf node. A and B are contiguous sections, upper B joining lower A.

Identification of Mobile Metabolites. Studies of the export of metabolite from the third trifoliate leaf were begun prior to the time it changed from an importing organ and extended well into the period when it sustained a normal export. The destination of the first metabolites exported was almost exclusively upward toward the apex, as shown in figure 6A. This figure, a gross autoradiogram, shows the metabolite distribution in the stem apex. Figure 6B shows the detailed autoradiogram of frozen-dried bark stripped from around the third trifoliate leaf node. The 2 figures are contiguous with each other. There was little downward flow of metabolite; in fact, it extended less than an inch in this direction. The upward-moving component originated from a direct transfer of metabolite from 4 of the 5 leaf traces into the cauline bundles.

The recorder tracings used to determine the time when upward and downward flow of metabolite commenced are shown in figure 7. By selecting leaves of progressively younger age, the actual commencement of flow in either the upward or downward direction was demonstrated. Curve A represents the flow from a newly matured leaf, that is, one from which export was well established and from which labelled metabolite flowed in both directions within 20 minutes after applying the $C^{14}O_2$. In B, a younger leaf, upward flow began within 30 minutes of labelling, downward flow after 1.5 hours. The labelled metabolite was almost exclusively sucrose with a faint trace of steroid. Curve C represents a still younger leaf from which upward export began about 3 hours after labelling. Downward export did not occur within a succeeding 1.5 hours. The mobile metabolite contained sucrose and steroid having a C^{14} ratio of about 12: 1. D represents a young leaf from which upward export began about 2 and one-third hours after labelling. When harvested, after 23 hours, a C^{14} ratio of sucrose: steroid of 1.5: 1 was found. The sucrose label was obviously deficient. Curve E shows a similar experiment in which a second aliquot of $C^{14}O_2$ was added 3 hours after upward export began. At 23.5 hours the sucrose to steroid radioactivity was 5: 1. The sucrose labelling was again deficient. Curve F represents a still younger leaf from which upward flow did not begin until 13 hours after labelling. $C^{14}O_2$ was again added about 6.5 hours after upward flow commenced. Within 20 minutes a well-labelled downward flow was obtained which was permitted to continue for 30 minutes. A sucrose: steroid label estimated at 88: 1 was then found. Typical chromatograms are represented in figure 8.

The following points concerning the foregoing are of interest: First, within 20 minutes from application, a marked labelled sucrose flow was in progress from leaves of sufficient maturity. A strongly labelled flow continued only while the $C^{14}O_2$ was currently available. No significant pool of label was retained from which labelled sucrose was later formed and exported. The steroid required about 2 hours from $C^{14}O_2$ application until it appeared in the stem. Some labelled steroid was exported from a $C^{14}O_2$ label
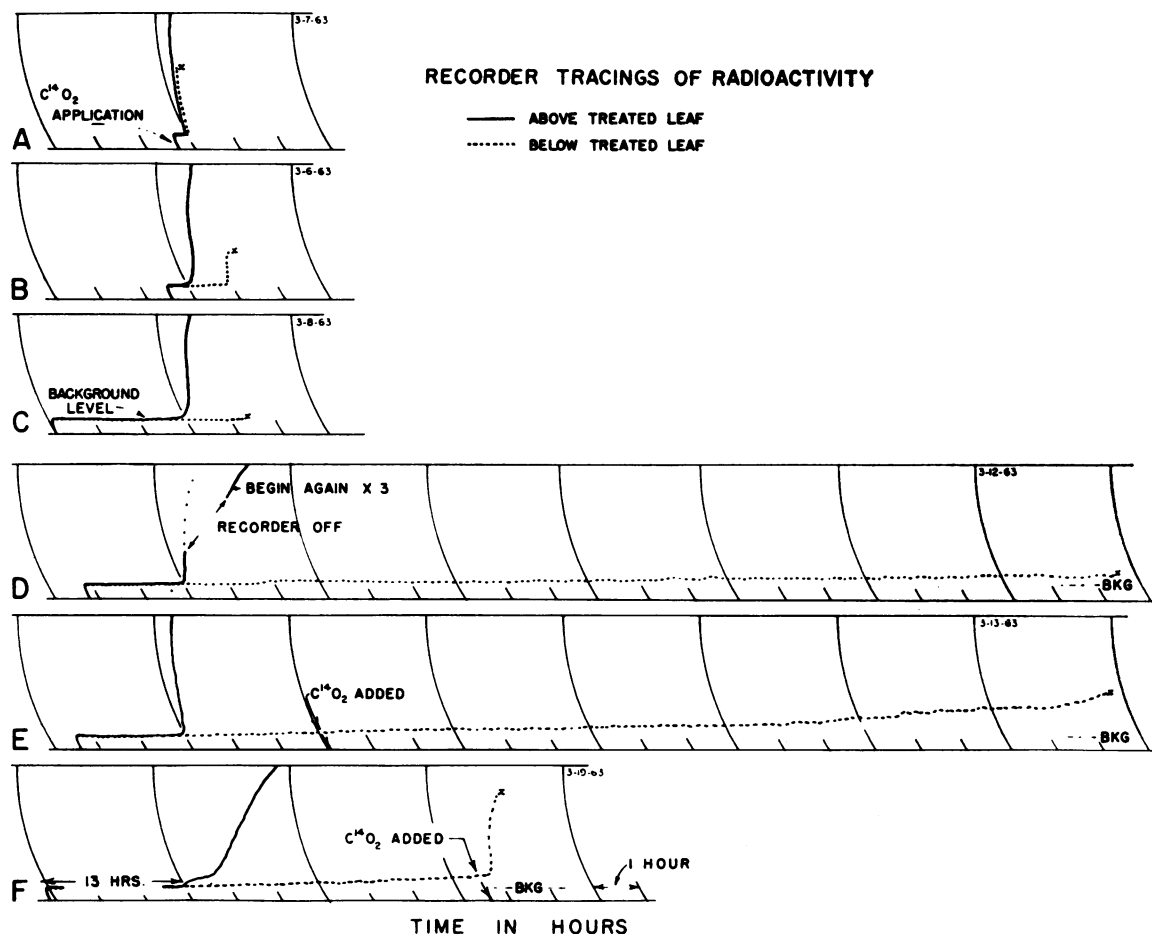
FIG. 7. Recorder tracings showing the appearance of radioactivity in the stem above and below the node of the third trifoliate (treated) leaf. The age of the leaves increased slightly from top to bottom (A through F).

received 13 hours previously, but the amount was small.

Second, upward export preceded downward export by several to many hours. The upward export was relatively rich in steroid, and downward export, through any great distance, did not succeed until the metabolism of the leaf was so altered that a massive supply of sucrose became available for export. Downward export through the leaf traces then became dominant, and the pattern of export developed progressively, as decribed above for leaves of different ages.

Discussion

There are a number of points brought out in the present research that should be correlated with the overall general knowledge of translocation. These deal first with the general export pattern from leaves of different ages, and with the metabolites exported; second, with the detailed pathway that different metabolites follow; and third, with the modes of movement.

The destination of metabolites from a given leaf depends upon its position. Lower leaves export primarily to the root with only a small fraction ascending toward the apex. From the uppermost exporting leaves the metabolite flow is primarily toward the stem apex. Intermediate leaves export in both directions. This is very similar to the export pattern for leaf applied P^{32} (8).

As the metabolites flow through the phloem, little moves tangentially between bundles except, of course, where anastomosing occurs. However, there can be considerable radial loss to the xylem, the amount of which varies with the age of the leaf from which the export occurred. As much as 49 % or as little as 4 % of the metabolite may be found in the xylem cylinder after 30 minutes, the larger being metabolite from a young leaf and the smaller from a mature primary leaf. It is not known which of the metabolites transfer most readily from the phloem, but in both cases where a rapid transfer occurs, metabolite from young leaves is involved. This is in the transfer between phloem bundles, i.e., leaf traces to cauline bundles, and the marked transfer to the xylem as the metabolite descends the stem. The metabolite in both cases is rich in steroid.
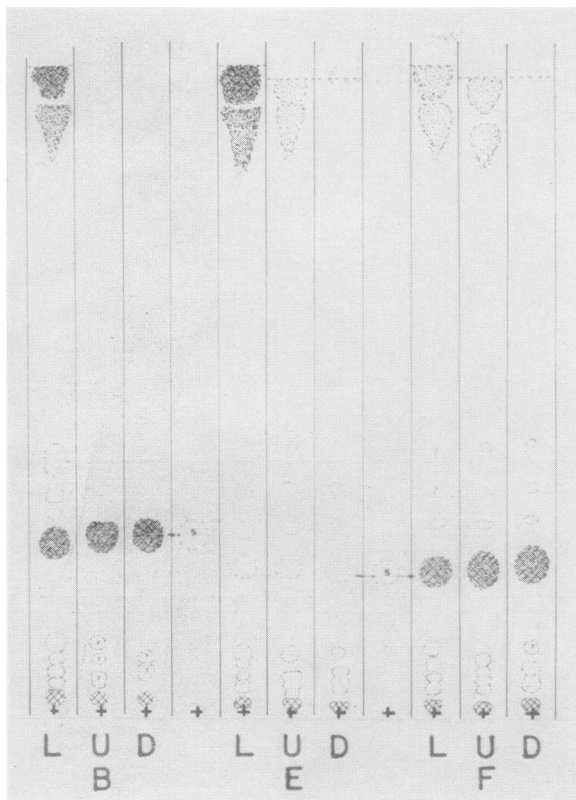
FIG. 8. A compiled free-hand sketch of chromatograms showing the ascension of ethanol-soluble materials from treated leaves (L), upper stems (U), and lower stems (D), for plants B, E, and F of figure 7. Known sucrose is indicated at (S); the 2 steroids are near the front, the other spots are unknowns.

It is highly probable that the metabolite from newly maturing leaves supplies the stem tip and the growing stem with various factors other than the bulk material sucrose and that these are largely consumed within the rapidly growing areas of the stem. It has been observed that very little of the export from young leaves reaches the root, whereas with the roots, the organic nutrient requirement of which consists of sucrose, thiamine and possibly nicotinic acid and pyridoxine, the bulk export is from the more mature leaves where sucrose export predominates. The methods employed in the present survey, if properly extended, should be adequate for the pursual of this subject.

The metabolite that entered the stem from a mature leaf of intermediate position descended in the leaf traces to the node below before a significant movement into adjacent bundles occurred. Here the anastomosing of bundles permitted lateral movement into adjacent channels, some of which conducted metabolite back up the stem. This confirmed earlier evidence for lack of polarity in the phloem (11). The node below a given mature leaf was then, in a sense, the distributing point for the metabolite.

Flow within a given leaf trace frequently involved all files of sieve cells within a particular bundle, but this was not always the case. When the terminal leaflet rather than the whole leaf was labelled, flow in the 2 most laterally located leaf traces was restricted to certain longitudinal files of sieve cells in those bundles. By the time the assimilate had descended through 1 internode, enough lateral movement had occurred to distribute tracer throughout the bundle. Ascent was also sometimes found to begin in certain files of cells within a bundle only to attain a wider distribution with distance of movement. This intercommunication is, presumably, via the sieve plates located on lateral walls (6).

The mixing of metabolites within a single bundle would lead to the conclusion that each phloem bundle acts more or less as a unit, or is capable of so acting. It is probable that certain files of sieve cells may conduct independently of other files so long as the forces associated with supply and demand remain gentle ones, but an unbalancing or an interference could produce cross transfer between files and a reestablishment of the flow in the direction of the net downhill gradient for the bundle as a whole. Canny's (4) calculations have shown that flow from an aphid stylet may involve adjacent files of sieve cells.

In summary, the direction of metabolite movement appeared to be directly related to an intensity factor, represented by concentration differences at source and sink, and inversely to a distance factor between the 2, in other words, to a gradient. Since each of a number of mature leaves served as a source with the major sinks at opposite ends of the plant, the division of the metabolites flowing between each was determined by a complex net gradient within the web of channels available through the anastomosing bundles. Ordinarily this means that the lowermost synthesizing leaves, supplying largely bulk sucrose, export to the roots while the uppermost leaves, when they attain a certain stage of maturity, export a more complete array of metabolites to the apex. The intermediate leaves, each with its progressive metabolic differentiation, supply their current array of metabolites to the intermediate tissues: the cambium, and other differentiating stem tissues, the axillary buds, and the apices (gradients permitting).

The present research clarifies considerably the confusing issue of bidirectional movement and shows its extent and limitations for at least 2 metabolites. It would appear that there is little or no countermovement to the main sucrose flow in well-developed phloem bundles. Thirty cases were examined where the upward-moving component of labelled assimilate flowed past the downward-moving (unlabelled) assimilate from the next higher leaf, or vice versa, and in no case was labelled assimilate found in leaf traces conducting the unlabelled assimilate. Utilizing this criterion, we were unable to confirm our earlier report of bidirectional movement in the same phloem bundle (1). We suggest that in the case of the tissue for figure 2 (1) an overhydration of the frozen-dried bark might have occurred allowing labelled sucrose to move laterally into bundles in which it did not originally occur. It moves readily in such tissue when held at a relative humidity in excess of about

60 %. Figure 1 of that report (1) was amply confirmed by our present results. Figure 2 supporting bidirectional movement in the same phloem bundle is probably at fault.

In young leaves of tobacco there may be a period of simultaneous import and export (7) which raises the question of how it occurred. Judging from the present results, wherein it appeared that there could have been a short period of simultaneous import and export (fig 3A), there was evidence that separate bundles could have been involved: Bundles 2 and 4 importing while 1, 3 and 5 were exporting. This would be rationalized on the basis that certain parts of the leaf were slower to change over to exporting areas than others.

The metabolite that enters the stem from a young leaf, that is, one that had just initiated export, did not descend in the leaf traces but was largely transferred from them directly to cauline bundles where ascent toward the stem apex occurred. This transfer of a metabolite rich in steroid was greatest at the time the initial export began and decreased as the leaf matured, whereas a strong downward flow of metabolite rich in sucrose developed in the leaf traces as the leaf matured. The metabolic pattern within the leaf evidently changed in a way favorable for a direct incorporation of CO_2 into exportable sucrose. The coincidence of an abundance of sucrose in the exported metabolite with the timing of the commencement of a marked downward flow in the leaf traces would suggest the involvement of turgor in the motivating force and, consequently, lends support to a mass flow concept.

In opposition to this, however, there was the direct ascent of metabolite in cauline bundles after a transfer from leaf traces in the instance of the initial export from young leaves. This aspect of translocation is probably better explained by a metabolically activated concept rather than by mass flow.

In agreement with the current trend of the researches on translocation in general (9), the present results lend support to both views of translocation, i.e., solution flow and a metabolically controlled process. The unique feature of the present report is that the 2 aspects of the process have been viewed separately. Each appears in its ascendancy at a different developmental stage, or, similarly, in a different position within the plant. The resolution of these 2 aspects of translocation, though they intergrade with each other, should prove very helpful in designing studies of each. This culminates for us a long search for a means of bringing the various aspects of the translocation process into a more distinct focus with respect to the anatomical and developmental background against which it must be viewed.

Summary

An autoradiographic method was coupled with phloem fluorescence in order to follow the detailed pathway of metabolite movement from bean leaves at 4 positions on the stem.

The lower leaves exported primarily to the root, the upper to the stem apex, and the intermediate ones in both directions. Flow was confined to phloem bundles except for a radial loss toward the xylem. The loss was least for metabolite from older leaves (4 %) and highest for metabolite from younger leaves (49 %).

Metabolite from mature leaves moved downward 1 node where anastomosing of bundles permitted a division into an upward- and a downward-moving component. The upward-moving component was confined to bundles alternating with the downward-moving component from the next higher leaf: Bidirectional movement was in separate phloem bundles.

The metabolite from young leaves just beginning to export left the leaf traces at the node of insertion and was transferred directly into cauline bundles wherein it ascended. A downward flow in the leaf traces developed as the leaf matured.

Three of the 4 major metabolites exported were identified: 2 (temporarily) as steroids and 1 as sucrose. Eleven components were resolvable. Exportable metabolite from young leaves was rich in steroids. Older leaves exported principally sucrose.

Export from older leaves resembled a mass flow: From younger leaves a metabolic (?) transfer was involved, a facet of translocation not easily explained by mass flow.

The fluorescence method should prove valuable for anatomical studies of phloem.

Acknowledgments

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