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Bidirectional Movement of Labeled Tracers in Soybean Seedlings

A. S. Crafts



Studies on transport mechanism in plants, using soybean seedlings and C¹⁴-labeled tracers, show that: 2,4-D* movement is largely restricted to phloem; monuron* movement takes place in xylem and cell walls; amino triazole* moves in phloem, xylem and cell walls; and maleic hydrazide* moves like amino triazole* and may leak from phloem to xylem and thus, circulate in the plant. Applied to epicotyl, 2,4-D* moves into and along the phloem, monuron* into and along the xylem, and amino triazole* and maleic hydrazide* into and along both phloem and xylem. These distribution patterns show selectivity of solute translocation, and support the concept of mass flow as the mechanism responsible for rapid, long-distance transport of foods in the plant.

THE AUTHOR:

A. S. Crafts is Professor Emeritus of Botany and Botanist Emeritus in the Experiment Station, Davis.

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INTRODUCTION

PREVIOUS PUBLICATIONS have reported that, when applied to leaves, monuron displays only apoplastic movement; that 2.4-D shows restricted symplastic movement; that amino triazole undergoes free symplastic transport; and that maleic hydrazide (MH), following free symplastic movement, may migrate to the xylem and move in the transpiration stream (Crafts, 1959; Crafts and Yamaguchi, 1964).² Evidence has been presented also that a phloem mobile tracer applied to a lower leaf will be transported principally to roots; that if applied to a median leaf, it will move both downward to roots and upward to growing leaves and stem; and that if applied to the uppermost, expanded leaf, it may move only acropetally to the shoot tip (Crafts and Yamaguchi, 1964; Biddulph and Cory, 1965). Finally, if applied to a young, rapidly expanding leaf, such a tracer will not be exported, but will move only to the leaf tip (Crafts 1956; Crafts and Yamaguchi, 1964).

Based on two lines of evidence, this work has been interpreted to substantiate the mass-flow mechanism of phloem transport in plants: (1) Since a number of labeled tracers undergo similar patterns of distribution that are unrelated to their concentrations or toxicities, but closely parallel food movement, it seems evident that they diffuse through the cuticle and migrate to the phloem. Once inside the sieve tubes, they ride along on the assimilate stream. (2) In moving along stems, such tracers go from source to sink of assimilates, bypass all mature leaves along the translocation route, and enter only roots and growing leaves, stem tips and buds.

In the studies reported here on comparative movement of labeled herbicide molecules in soybean seedlings, a new type of treatment was introduced that sheds further light on transport mechanism. Whereas previous treatment was limited to leaves and roots, radioactive herbicide molecules were applied here on the side of the epicotyl between the cotyledon and the primary leaves. Distribution of the four molecules from this location, which obviously is not a source in the true source-sink sense, produced some distinctive patterns that aid in further understanding of mechanism. This paper concerns these distribution patterns and the role played by the vascular anatomy of the stem.

METHODS

Seeds of soybean (*Glycine soja*) were germinated and grown in vermiculite for two weeks; from these, plants were selected for uniformity and transplanted to Mason jars containing singlestrength Hoagland solution. Two days

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² See "Literature Cited" for citations referred to in the text by author and date.



Fig. 1. Left, autoradiographs of plants to the right. Right, mounted plants of soybean treated on one cotyledon with labeled 2,4-D. Left plant, treated for two days; center and right plants treated for four days.

later, plants were chosen again for uniformity and each treated on one cotyledon with 2,4-D^{*2} at a rate of 0.05 μ c in a 0.01 ml drop; treatments lasted for periods of one, two, and four days. At this stage the primary leaves were fully grown, with the shoot tips starting to grow.

A second set of plants was selected three weeks after sowing, when the primary leaves were fully expanded and the first trifoliates were rapidly growing. Four of these were treated for three hours with applications of 2,4-D*, monuron*, amitrole* and MH* to one side of the epicotyl of each. Twelve of the same lot of plants were treated with these same four tracers; each tracer was applied to the cotyledon of one plant, the epicotyl of a second and the primary leaf of a third. Duration of treatment was two days.

A third lot of plants was treated six weeks after sowing. By this time one trifoliate leaf on some plants and two or three on others, had fully expanded. Two shoots had developed in the axils of the cotyledons, each with two or three trifoliate leaves. These plants received 2,4-D*—some on cotyledons, some on primary leaves, and some on trifoliate leaves. Duration of treatment was two days. A final lot of plants was grown and treated at the same stage as the second set. These received monuron*, amitrole* and MH* on one primary leaf each, and on roots of one plant each. Duration of treatment was six hours.

All applications to foliar parts were in lanolin rings. After the various treatment times, the plants were freezedried, mounted, and autoradiographed (autographed) according to methods described by Crafts and Yamaguchi (1964). The soybean plants used in these experiments were all grown in a greenhouse during the winter months when light intensity was low and days were short. These conditions caused a certain degree of etiolation, the hypocotyls and epicotyls being longer than they would have been if the plants had been grown in summer. However, the plants were healthy and grew rapidly.

RESULTS

Figure 1 shows the plants and their autographs from the first experiment in which 0.05 μ c of 2,4-D* was applied for two and four days to one cotyledon of each plant. The dose carried about 11 micrograms of 2,4-D, the formative effects of which can be readily noted in the twisting and bending of the epicotyls.

Translocation was rapid in these plants, the one-, two-, and four-day treatments all showing equal distribution. Movement was both acropetal to the shoot tips and basipetal into the total root systems. Opposite cotyledons and both primary leaves were completely bypassed, which indicates that these organs were all contributing to the assimilate stream. four compounds were applied at a rate of 0.1 μ mole each to epicotyls for three hours. The specific activities were as follows: 2,4-D*, 0.5; monuron*, 0.386; amitrole*, 0.94; and MH*, 0.5. Because the exposure (two weeks) of the mounted plants on the X-ray films was the same in all cases, the high activity of the amitrole* was reflected in a dark image. The other three images were normal.

Movement of 2,4-D* from the epicotyl treatment was both acropetal and basipetal, but, because of the short treatment time, this tracer did not reach the roots and stem tip. Movement was strictly symplastic.

Monuron* evidently penetrated the stem and moved upward in the xylem; no basipetal movement occurred in the adjustes radioactivity.

In the second experiment (fig. 2) the no basipetal mov ² The asterisk following names of compounds indicates radioactivity.



Fig. 2. Top, autoradiographs of plants below. Bottom, mounted plants. Treatments on far left with 2,4-D*; left center, monuron*; right center, amitrole*; far right, MH*. Epicotyls treated for three hours.

phloem. This confirmed previous observations of leaf applications: monuron is unable to enter the symplast and move rapidly in the phloem.

Amitrole^{*} moved strongly in the acropetal direction, weakly in the basipetal direction. Since both primary leaves were labeled, this compound evidently moved acropetally in the xylem. The distribution pattern in the trifoliate leaf and bud indicated simultaneous movement in the phloem.

MH^{*} entered the stem and moved both acropetally and basipetally; labeling, though weak was quite uniform in both directions. Movement through the phloem to the roots was the strongest with MH^{*} of any of the four compounds. Movement in the xylem accounted for labeling of the primary leaves.

For the third experiment, all four tracers were applied for two days to cotyledons, epicotyls, and primary leaves. This experiment presented some critical evidence of the mechanisms involved in bidirectional movement (Biddulph and Cory, 1960, 1965), and will be discussed in some detail.

First, the distinct contrast between monuron* and 2,4-D* distribution should be noted (fig. 3 a,b). Monuron*, seemingly unable to enter and move in the symplast, did not migrate out of the cotyledon. However, when applied to the epicotyl, it entered the tissues, moved via the apoplast to the xylem, and ascended to the primary and trifoliate leaves. While the concentration in the epicotyl appeared low, it should be remembered that the xylem strands are embedded deeply in the tissue. Intensity of labeling was intermediate in the primary leaves, low in the trifoliate. This probably reflected the relative transpiration rates from these two organs. Labeling of the terminal bud was also low. If transport of monuron* had taken place via the phloem, the reverse situation would be evident; labeling would have been intense in bud and growing trifoliate, and absent from the primary leaves.

Applied to one primary leaf, monuron* shows the typical wedge-shaped apoplastic pattern. Movement of the tracer was only via the cell-wall system with the transpiration water. No tracer was exported from the leaf via the phloem.

In contrast, 2,4-D*, in these soybean seedlings, apparently moves solely in the phloem. Uptake into the cotyledon resulted in symplastic movement, mainly to the roots, and in a somewhat lower intensity to the expanding trifoliate leaf and bud. Both primary leaves were completely bypassed.

Application on the side of the epi-

cotyl resulted in selective uptake into the phloem of the vascular strands and movement acropetally into the growing trifoliate leaf and terminal bud, and basipetally through the hypocotyl into the whole root system. Treatment on a primary leaf produced almost exactly the same distribution, except that some of the tracer traversed the veins of the treated leaf.

The autographs of plants in this experiment provide a graphic example of bidirectional movement. While 2,4-D* applied to the cotyledon moved acropetally to the shoot tip, that applied to the primary leaf moved basipetally through epicotyl, hypocotyl and root. Thus, two streams were moving in opposite directions at the same time through the epicotyl within phloem strands. Furthermore, as shown in the plant treated on the primary leaf, anatomy of the vascular strands of the epicotyl proves that the tracer moving to the shoot tip had to descend to within about 1 cm of the cotyledonary node, before it could ascend to the growing organs at the shoot tip.

Figure 4 shows a diagram of phloem distribution within a region extending from 1.3 cm below to 1.3 cm above the cotyledonary node as reconstructed from work by Bell (1934) and Weaver (1960). This shows how the phloem of this region would look if it were slit through the trace of Cotyledon Two (C_2) , opened, and flattened. The four radially arranged strands ascending from the root anastomose to form two broad crescents at the 0.85-mm level. then split to form the cotyledonary traces (C_1, C_2) and two crescent-shaped strands, each of which splits into three strands leading to Primary Leaves One and Two (P_1, P_2) . At a level 2 mm above the emergence of the cotyledonary traces, Strand c of P_1 divides giving off two branches. Likewise, Strand d of P_2 also divides giving off two branches. Two of these four branches anastomose within the next 6.0 mm to



Fig. 3a, b. Top, autoradiographs of mounted plants below. 3a. Two-day treatment with 2,4-D* to: left, one cotyledon; center, epicotyl; right, one primary leaf. 3b. Two-day treatment with monuron* to: left, one cotyledon; center, epicotyl; right, one primary leaf.

provide the central strand to Trifoliate One (T_1b) , the other two form the two side traces (T_1a,c) ; together these make up the phloem traces to Trifoliate Leaf One. Similarly, within the same 6.0 mm, traces of a of P_1 and f of P_2 divide and anastomose to form the three phloem strands to Trifoliate Two (T_2a,b,c) . Since all of this takes place within less than 1 cm of the epicotyl above the cotyledonary node, and no further anastomosis occurs within the epicotyl, it is apparent that transport of the 2.4-D* applied to a primary leaf must descend to a level within 1 cm of the cotyledonary node, before it can ascend to the trifoliate leaf. As shown in figure 3a (primary leaf treatment), an appre-



Fig. 3c,d. Plants treated as in 3a,b. Left with amitrole*, right with MH*.

ciable portion of the assimilate stream, exported from the primary leaf, moved continuously downward through epicotyl, hypocotyl, and roots. Probably, about an equal amount moved into the traces of Trifoliate One, joined the stream from Cotyledon One, and ascended to that growing trifoliate leaf. Probably, the streams moving in strands P_1b and P_2e were the ones that descend into the roots. In this case, there would have been bidirectional movement within the two phoem crescents from the level of insertion to the level at which the cotyledonary traces diverged. However, as stressed by Biddulph and Cory (1965), movement in opposite directions in this case was taking place in separate sieve-tube strands or groups. There was no evidence of



Fig. 4. (P = primary leaves, T = trifoliates, C = cotyledon.) Diagram of phloem distribution within a region 1.3 cm below to 1.3 cm above the emergence of the cotyledons. View as it would look if split through the trace of C_2 , opened and flattened.

bidirectional movement within a single file of sieve-tube elements.

Returning to the tracer study, the evidence on movement of amitrole* applied to the cotyledon and to one primary leaf supports the view that movement was confined to the phloem (fig. 3c). No labeling occurred in primary leaves in the first instance; in the second, no labeling occurred in the opposite primary leaf. However, when applied to the epicotyl, amitrole^{*} entered both phloem and xylem; basipetal movement into the roots was totally symplastic; transport into Primary Leaves One and Two was apoplastic via the xylem. In this respect, it is interesting to note that labeling intensity was strongest in the primary leaf directly above the point of application. Note also that labeling intensity here was highest in the terminal bud and intermediate in the trifoliate. This is evidence of phloem movement, and reflects the relative strength of the two sinks, a situation that contrasts with the monuron* pattern.

In this experiment, maleic hydrazide* showed its typical distribution pattern involving phloem-to-xylem migration. Both cotyledons and both primary leaves were apparent in all three autographs. This was leakage rather than secretion, since MH* appeared in the culture medium whenever plants received foliar application. Also, phloem movement exceeded xylem in these plants, as indicated by the relative labeling intensity of trifoliate and primary leaves; the high intensity in trifoliates and terminal buds (compare, for example, fig. 3b) indicates a strong symplastic movement into these active sinks.

One further point is of interest in the third experiment. In the roots of plants treated with 2,4-D* and MH*, the total lengths of roots have medium labeling with some increase in intensity at the tips. In contrast, amitrole* is very low

in intensity in the older portions of the roots and attains high intensity in the root tips. This indicates a certain retention throughout the transport channels for 2,4-D* and MH*. Amitrole* seems freely mobile and tends to pile up at the ends of these channels. This may be a key to the high herbicidal effectiveness of amitrole, particularly against seedlings and certain perennial species.

fourth experiment involved The plants grown for six weeks after sowing. These had developed strong shoots in the axils of the cotyledons as shown in figure 5a. Treated on the terminal leaflet of Trifoliate One of one of these axillary shoots with 2,4-D* and given a two-day treatment, the autograph of this plant shows strong tracer movement down the petiole, shoot, stem, and hypocotyl with gradual fading as it moved into the roots. This pattern is characteristic of 2,4-D* transport and gives evidence for its continuous uptake along the channels of movement observed in many such experiments. Notable also is the weak labeling of the young, expanding Trifoliate Three of the axillary shoot, and the complete bypassing of the side leaflets of Trifoliate One and of all leaflets of Trifoliate Two. The opposite axillary shoot was also bypassed, and no tracer moved into the epicotyl, primary leaves or Trifoliates One and Two of the main stemall evidence for the relative activities of the various sources and sinks in this plant.

A second plant (fig. 5b) was treated for two days on one primary leaf, after removal of the shoots in the axils of the cotyledons. Here movement of 2,4-D* was directly to the roots as might be suspected, since the primary leaves are the most basal organs in the food system of this plant. Distribution patterns in these two plants are characteristic of many involved in this experiment.

The final experiment in this series involved comparative tests on foliar and





Fig. 5a,b. Top, autoradiographs of mounted soybean seedlings below six weeks after planting. Left, plant treated with 2,4-D* on the terminal of Trifoliate One of the upper axillary shoot an example of two-way transport. Right, plant treated on one primary leaf showing unidirectional movement; shoots in axils of cotyledons removed.

root applications of monuron^{*}, amitrole^{*} and MH^{*} to soybean seedlings at the three-week stage, as in the second and third experiments. Each plant received 0.1 μ mole of the tracer either as a 10- μ l drop on one primary leaf or as 0.1 μ M dissolved in 100ml of culture solution (root treatment); treatment lasted for six hours. Figure 6a shows the plants and autographs of the monuron* treatment. As expected, only the apoplastic wedge showed tracer movement in the treated leaf; no monuron* was exported via the phloem. In the root

treatment, the tracer was absorbed and spread throughout the plant with greater concentration along the veins of cotyledons and leaves. Had the experiment run for 24 hours, the concentrations in these organs would have been greater and more uniform. In contrast, amitrole* (fig. 6b) was distributed throughout the plant that received the foliar application. There, the dense apoplastic wedge from the application spot indicated that this compound moves freely in the transpiration water. At the same time, the tracer moved out of the treated leaf with low concentrations in epicotyl, stem, terminal bud,

Trifoliate One and hypocotyl; it also showed faintly in the roots. Root application resulted in medium intensity in the root system and a faint image of the total foliar portion of the plant.

MH* (fig. 6c) has produced a prominent apoplastic wedge in the treated leaf; a dense image throughout the epicotyl, stem and terminal bud; and medium density in Trifoliate One, hypocotyl and roots. The opposite primary leaf and both cotyledons showed light images. Root application produced a medium density in the roots and a very faint image of the remainder of the plant.

DISCUSSION

Although interpretations of these experiments with four common herbicides may be directly applied in the field of weed control, light is also thrown on a basic tenet of translocation physiology. Bidirectional movement of solutes in the phloem was long considered to be a process that was unexplainable by the mass-flow mechanism (Mason and Phillis, 1936; Palmquist, 1938; Biddulph and Markle, 1944). In recent years Biddulph and Cory (1960, 1965) have shown that such movement may take place in different strands of the phloem, and in such cases cannot be considered as controverting the mass-flow mechanism.

Many mechanisms to explain bidirectional movement have been suggested through the years, but evidence from tracer studies such as these becomes more and more convincing that a massflow mechanism is responsible for rapid, long-distance transport of assimilates in the sieve tubes. Basically, however, only two concepts are in question: (1) Massflow which implies simultaneous movement of solutes and solvent in a stream, and (2) metabolic movement of some sort which implies independent movement of various solutes and of the solvent water. However, such independent movement of, for example, sucrose molecules through the sieve-tube system filled with static water at velocities up to 300 cm per hour (Anon., 1964) seems impossible to visualize. Given a molecular weight of 342, and carrying at least six molecules of water of hydration, plus many more loosely bound, exchangeable water molecules, it seems difficult to conceive of a mechanism that could successfully apply the necessary energy and dissipate the frictional heat. Words such as "activated diffusion," "interfacial movement," "polar movement," "exchange movement," or "metabolic movement" are frequently used in describing these alternative hypotheses. In no case are these words adequately defined, nor is evidence offered to support their use.

Meanwhile, research of the type described herein has proved that labeled tracers, including toxic herbicides, move with assimilates from sources in leaves to sinks in shoot and root tips, flowers, fruits, and the like; that they bypass mature, exporting leaves in their passage; and that often they accumulate in sinks to very high levels, because there are no means for their disposal. Such distribution patterns cannot be explained by any independent movement mechanism; they are readily explainable by mass flow. Only mass flow can account for the movement of 2,4-D*, amitrole*, MH*, trichlorobenzoic acid (TBA*) (Mason, 1960) and many other phloem-mobile compounds of varying degrees of toxicity from young, exporting leaves to shoot tips. Surely all of these compounds are not being moved by the same "metabolic" mechanism into the same sinks at comparable velocities. Instead, evidence indicates that having penetrated the cuticle and moved symplastically to the phloem, these compounds "ride along" on the assimilate stream and accumulate in young, growing organs. The shoot tips, so loaded with 2,4-D*, will show growth inhibition, formative effects and necrosis; amitrole* will cause growth inhibition; and TBA* stimulated respiration and necrosis.

To lend further support, evidence is accumulating (Esau and Cheadle, 1961; Duloy *et al.*, 1961; Evert and Murmanis, 1965; Engleman, 1965, and others) that the protoplasmic connections traversing sieve plates of functional sieve tubes are tubular. This problem of sieve-plate permeability has hampered the understanding of sievetube function for more than 100 years. Now, there seems little to hinder complete acceptance of mass-flow as the major mechanism responsible for food movement at rapid rates in plants.

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