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**COMPARATIVE TESTS ON THE UPTAKE
AND DISTRIBUTION OF LABELED
HERBICIDES BY ZEBRINA PENDULA AND
TRADESCANTIA FLUMINENSIS**

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From comparative autoradiographic studies in which labeled herbicides were applied to the leaves of *Zebrina pendula* and *Tradescantia fluminensis*, to barley seedlings, and to potato tuber tissue, the following conclusions were drawn:

1. Aminotriazole, maleic hydrazide, urea, 2,4-D, and 2,4,5-T applied to leaves may move out to other plant parts via the phloem; monuron is limited to xylem transport; compounds that are mobile in the phloem will not move out from chlorotic leaves.

2. The movement of 2,4-D seems to be restricted by its accumulation into living cells of the leaf, and its movement is apparently limited to phloem.

3. The movement of aminotriazole and maleic hydrazide is relatively free, and they may move in both phloem and xylem.

4. The establishment of active sinks for transport by means of growth stimulation may bring about movement into roots or into shoot tips or two-way movement into both roots and shoot tips.

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COMPARATIVE TESTS ON THE UPTAKE AND DISTRIBUTION OF LABELED HERBICIDES BY ZEBRINA PENDULA AND TRADESCANTIA FLUMINENSIS¹

A. S. CRAFTS AND S. YAMAGUCHI²

INTRODUCTION

TESTS WITH LABELED 2,4-D have shown that this herbicide is absorbed and translocated into the roots by bean plants in about three hours; movement into terminal buds took about six hours; after 24 hours radiation intensity decreased in roots and treated leaves (Crafts, 1956*a*). Comparative results with bean, cotton, cucumber, wild morning-glory, and a number of woody species seem best to fit a scheme involving movement with assimilates in a common stream in the phloem (Crafts, 1956*a*, 1956*b*; Leonard and Crafts, 1956).

Reports on the use of aminotriazole (ATA) and maleic hydrazide (MH) in the greenhouse and in the field indicate that these two chemicals have considerably different properties than 2,4-D (Hall, Johnson, and Leinweber, 1954; Hauser and Thompson, 1954; Crafts, Currier, and Drever, 1958). They are both active against grasses, as well as against certain broad-leaved plants; they apparently are very thoroughly distributed in plants, producing profound effects at some distance from the area of application; they have entirely different physiological actions. A potent dose of ATA causes a severe chlorosis on all further growth; MH causes a pronounced dormancy that may last a year or more (Crafts, Currier, and Drever, 1958). Both chemicals are apparently less subject than 2,4-D to breakdown in living plant tissues; the favorable treatment period may be much longer than for 2,4-D; the formative effects they cause are distinctly different from those of 2,4-D (Crafts, Currier, and Day, 1950).

Zebrina pendula and *Tradescantia fluminensis* are very useful plants for studies on absorption and translocation of herbicides. They propagate easily; they are healthy and vigorous under greenhouse conditions; they respond readily to nutrient culture conditions. Having stomata only on the lower leaf surfaces, they can be used to compare cuticular with stomatal uptake. Because of their having typical monocotyledonous structure, they can also be used to study action on intercalary meristems and monocot-type roots. Finally, they are closely related to *Commelina*, a genus that is a prominent weed in the tropics.

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METHODS

During the conduct of previously described work (Crafts, 1956*a*, 1956*b*; Leonard and Crafts, 1956), methods for applying 2,4-D*³ and autographing the treated plants were developed. Freeze-drying proved necessary for short-time translocation tests, and it also proved advantageous for all autoradiographic work (Pallas and Crafts, 1957). During the course of the present work, methods have been further improved. Many of them are described in detail in another paper (Yamaguchi and Crafts, 1958), which also contains details of plant preparation. The following labeled herbicides were used in these studies: 2,4-D, 2,4,5-T, aminotriazole, maleic hydrazide, urea, and monuron.⁴ Application has been by single-drop, multiple-drop, spraying, and brushing treatments of leaves and stems.

EXPERIMENTS AND RESULTS

Since the upper surface of a *Zebrina* leaf lacks stomata, it seemed important to make drop applications to both upper and lower leaf surfaces to ascertain the role of stomata in spray absorption. Two large potted plants, each having many branches, were used, and some 32 applications were made, using 5 μ g of 2,4-D* per treatment. Half of the applications were on upper leaf surfaces, half on the lower. Shoots treated 2½ and 4½ hours were oven-dried; others treated 2 and 4 hours were freeze-dried.

Freeze-dried shoots treated on upper surfaces for 4 hours showed movement of the 2,4-D* downward in the stems; in 2-hour treatments the tracer did not move out of the treated leaves. Similar shoots treated on lower leaf surfaces showed movement outward after 2 hours; after 4 hours movement was strong, and the stems were autographed for their entire length, which, however, averages only 2¼ inches (fig. 1*A*). There was movement from the treated spots to the outer tips of the treated leaves in the case of the 4-hour treatments on lower leaf surfaces.

In the case of the oven-dried plants, movement of the tracer both outward toward the leaf tip and downward toward and along the stem averaged more than in the freeze-dried plants (fig. 1*B*). Untreated leaves contained tracer, and where movement took place in stems the autographs were darker. However, translocation was erratic in both the 2½- and 4½-hour treatment periods, and there was strong evidence for movement during the drying period. Although absorption was somewhat greater through the lower leaf surfaces, the differences were slight. It has been concluded that the small difference observed is due to the thinner lower cuticle rather than to stomatal penetration. The latter phenomenon is observed in *Zebrina* leaves only when

³ The symbol 2,4-D* is used to designate 2,4-D-1-C*, that is, 2,4-D labeled in the carboxyl position with C¹⁴. Radioactive forms of the other compounds studied are similarly designated.

⁴ Carboxyl-carbon-labeled 2,4-D and 2,4,5-T and C¹⁴-labeled urea were purchased from Tracerlab, Inc. Aminotriazole (3-amino-1,2,4-triazole-5-C¹⁴) was supplied by the American Chemical Paint Co., ethylene-carbon-labeled maleic hydrazide by the Naugatuck Chemical Division of the U. S. Rubber Co., and ring-labeled monuron by E. I. du Pont de Nemours and Co., Inc.

the treatment is with concentrated alcohol or when the leaves are vacuum infiltrated.

Under ordinary conditions (absence of a pressure gradient), fluid stops moving as soon as it has penetrated the stomata to the smallest diameter. In fact, capillary forces require such stoppage, for mass movement into stomatal chambers would involve negative capillarity, something that was not observed in these leaves. When pure solvents penetrate stomata, as they do in the classical solvent test for stomatal opening, they move in either as thin films that are thinner than the radius of the minimal opening or by mass flow which occurs only with thorough wetting of the walls and expulsion of the included air.

Comparison of 2,4-D* application to upper and lower leaf surfaces. To further explore the differences between upper and lower leaf surfaces with

TABLE 1
DOWNWARD MOVEMENT OF 2,4-D*
IN *ZEBRINA* PLANTS

Hours	Upper surface	Lower surface
$\frac{1}{2}$	0" to $\frac{1}{4}$ "	$\frac{1}{2}$ " to 2"; average 1"
1	$\frac{1}{4}$ " to $\frac{1}{2}$ "	$3\frac{1}{2}$ " to $5\frac{1}{2}$ "; average $4\frac{1}{2}$ "
$2\frac{1}{2}$	$\frac{1}{4}$ "	6" into all roots; heavy in 2 out of 5
$4\frac{1}{2}$	0" to $1\frac{1}{2}$ "	6" into all roots; heavy in all roots
8	$\frac{1}{2}$ " to 2"	6" into all roots; heavy in all roots

respect to penetration of 2,4-D*, some 52 individually potted *Zebrina* plants were treated and autoradiographed. The dose was $5\mu\text{g}$ applied in a $10\text{-}\mu\text{l}$ drop of 50 per cent alcohol, with 0.1 per cent Nonic 218 as a surfactant. Treatment times were $\frac{1}{2}$, 1, $2\frac{1}{2}$, $4\frac{1}{2}$, and 8 hours (fig. 2A and B). The plants were oven-dried. Table 1 presents the results of this experiment.

It is evident from table 1 that 2,4-D* penetrates the lower leaf surface (fig. 2B) more readily than the upper (fig. 2A); in fact, in the case of upper surface treatment, downward movement from the treated spots was almost lacking; the tracer did move out to the tips of the treated leaves, following the vascular bundles.

In the longer treatment periods it was observed that movement of 2,4-D* from upper leaves of these plants was predominantly upward toward the tips; from lower leaves it was predominantly downward into the roots. This agrees with Clor's (1951) observations on cotton and previous experience with wild morning-glory (Crafts, 1956b).

Failure of movement during the 8-hour treatment in this experiment seems difficult to understand in terms of slowness of movement, particularly since the tracer seemed to be present in the veins of the treated leaves after one hour. Examination of the autographs shows that the tracer was accumulated in the cells surrounding the vascular elements. In view of the slower penetration, a possible explanation seems to be that accumulation by living cells with-

drew the tracer from the channels of movement as fast as it entered through the thick upper cuticle. This topic is discussed more fully later.

Because previous work with beans (Crafts, 1956a) had shown that high dosage resulted in greater distribution than low, and because the limited area covered by the 10- μ l drop might also restrict movement, an experiment was designed to explore higher dosage and increased area of exposure to the tracer. Individually potted *Zebrina* plants were used. Three plants served as controls, and there were two experimental lots of 15 plants each. The latter were divided into five treatment groups of 3 plants each; 15 plants were treated on the upper surfaces of two leaves each, 15 on lower surfaces. Dosage was from 30 to 60 μ g, and in each case the solution was spread over the entire

TABLE 2
DOWNWARD (BASIPETAL) TRANSPORT OF 2,4-D* IN *ZEBRINA* PLANTS
TREATED BY SPREADING 30 TO 60 μ G OF THE TRACER OVER TWO LEAF
SURFACES. CONCENTRATION OF TREATMENT SOLUTION 1500 PPM IN
50 PER CENT ALCOHOL WITH 0.1 PER CENT NONIC 218

Hours	Upper surface	Lower surface
$\frac{1}{2}$	0" - 1"	0" - 6", variable
1	5" average. Light intensity in roots of 2 out of 3 plants	5" average. Light intensity in roots of 2 out of 3 plants
$2\frac{1}{2}$	6" average. Light intensity in roots of all 3 plants	6" Medium intensity in roots of all 3 plants
4	6" average. Medium intensity in roots of all 3 plants	6" Heavy intensity in roots of all 3 plants
8	6" average. Medium intensity in roots of all 3 plants	6" Heavy intensity in roots of all 3 plants

leaf surface. The five treatments constituted a time series, the treatment times being $\frac{1}{2}$, 1, $2\frac{1}{2}$, 4, and 8 hours. Table 2 summarizes the results of this experiment.

Here again, the movement through the upper cuticle was somewhat slower than through the lower. However, with the great increase in dosage, enough 2,4-D* actually traversed the upper cuticle to move throughout the roots in medium intensity (fig. 3). This indicates the possibility that accumulation of 2,4-D to a certain level is an inherent property of living cells, and that when this chemical is moving across tissues such accumulation constitutes a threshold which must be exceeded for translocation to be maintained. Undoubtedly the accumulative level of the tissues of a leaf would be a function of the species, and probably of the metabolic level of the leaf. This puts a new light on 2,4-D dosages in relation to particular plants, and it may explain many cases of 2,4-D tolerance by certain species.

Movement of 2,4-D out of green and chlorotic leaves. Since it had been shown by Mitchell and Brown (1946) and Weaver and De Rose (1946) that 2,4-D movement is correlated with movement of organic foods in the bean, and by Rohrbaugh and Rice (1949) and Weintraub and Brown (1950) that application of sugar will induce 2,4-D movement in starved bean plants, it

seemed desirable to test movement of applied 2,4-D* out of green and chlorotic leaves. Variegated plants of *Tradescantia fluminensis* having some completely chlorotic branches were used. Potted plants were treated with 5 μ g of 2,4-D* in 10 μ l of solution and allowed treatment periods of 1 and 3 hours. Some treatments were on green leaves, some on chlorotic leaves, and some on variegated leaves having varying ratios of green and white tissue. The plants were freeze-dried.

This experiment showed without exception no movement of 2,4-D* from chlorotic leaves, normal movement from green leaves, and somewhat less than normal movement from variegated leaves, depending on the amount of green tissue present (fig. 4). Similar results have been obtained with ATA* and MH*.

Route followed by 2,4-D within the plant. To obtain a clear picture of the distribution of 2,4-D in plants and the relation of distribution to final action, it is necessary to know where the chemical goes after it enters the plant and whether or not there is a tendency for it to localize in certain regions. For instance, van Overbeek (1947) found in Puerto Rico that 2,4-D treatment killed the intercalary meristem regions of the stem of *Commelina* before it affected other parts of the stem. It seems important to find out if this results from localization of the 2,4-D along the stem or if it is simply a localized response.

A number of experiments designed to prove this point were performed on *Zebrina* plants, using 2,4-D at concentrations ranging from 0 to 6,000 ppm. In none of these were the intercalary meristems killed. Next, mixtures of the emulsifiable acid formulation of 2,4-D were applied in drop treatments; concentrations of the treatment solutions ranged from 0 to 20,000 ppm. The lower concentrations in this series caused swelling of the nodes, and at all concentrations there was a tendency for abscission at the swollen nodes. Intercalary meristems were not killed. In another attempt the concentrations were lowered to 2,500, 5,000, and 10,000 ppm and the upper surfaces of *Zebrina* leaves were sprayed to runoff. Again the nodes swelled and some plants died, but none showed killing of the intercalary meristems.

In a final test the emulsifiable acid formulation was mixed at 20,000 ppm; 15 per cent alcohol and 5 per cent propylene glycol were added to improve penetration. The solution was applied by spraying at 800, 4,000, and 20,000 ppm, and single, double, and quadruple applications were made at half-hour and at one-day intervals. A number of the plants treated with the 20,000-ppm concentration developed collapsed intercalary meristems at nodes and leaf bases typical of the 2,4-D injury described by van Overbeek (1947). Since in the case of hundreds of 2,4-D*-treated plants the autoradiographs have failed to show accumulation of the tracer in the intercalary region, it seems reasonable to assume that this collapse of the meristems is a localized response and not the result of localized accumulation. This last experiment showed further that four applications at 800 ppm were much more effective than one at 4,000 ppm. In fact, two applications at 800 ppm with the one-day interval were more effective than a single application at 4,000 ppm.

Another experiment involving the use of a sample of 2,4-D* having a specific activity of 6 mc per mM gives further information on the absorption

of 2,4-D. The total dose of radiation was $0.55 \mu\text{c}$ confined by a lanolin ring to a spot 6 mm in diameter on the lower surface of a *Zebrina* leaf. Treatment time was 3 hours, and the plant was freeze-dried. Two autographs were made from the mounted plant—one exposed for $2\frac{1}{2}$ days, a second exposed for 26 days. Figure 5 shows the autographs and the treated portion of the plant. In the short exposure (fig. 5A and C) the vascular bundles of the leaf stand out distinctly, and both the long main veins and the short laterals have accumulated the tracer, presumably in the active border parenchyma cells that enclose the conduits of the bundles. This indicates that as soon as 2,4-D* starts moving from the point of application via the vascular elements, it is taken up by living cells, and any that moves over an appreciable distance constitutes a

TABLE 3
PENETRATION AND MOVEMENT OF 2,4-D* AND ATA* IN ZEBRINA LEAVES
THROUGH THE UPPER AND LOWER SURFACES

Chemical	Dosage	Upper surface			Lower surface		
	μg per drop	Penetration*	Movement		Penetration*	Movement	
			Upward	Downward		Upward	Downward
2,4-D*	1	1	0"	0"	2	$\frac{1}{4}$ "	1"
	5	4	0"	0"	8	1"	1"
	25	4	$\frac{1}{2}$ "	1"	8	1"	1"
ATA*	1	2	0"	0"	2	0"	0"
	5	4	1"	0"	4	1"	0"
	25	6	1"	0"	12	1"	1"
	125	10	$1\frac{1}{2}$ "	$\frac{1}{2}$ "	10	1"	1"

* Penetration was judged visually in terms of a set of empirical values from 1 to 12.

residue over and above that held by the active cells. Figure 5B and D, the 26-day autograph, reveals details of the vascularization of the stems, but details in the treated leaf are blotted out by the diffuse radiation from overexposure.

Comparison of 2,4-D* and ATA* applied to upper and lower leaf surfaces. A later experiment tested the relative penetration of 2,4-D* and ATA* through upper and lower leaf surfaces of *Zebrina*. The plants were mature, slow-growing pot cultures. Treatment time was $2\frac{1}{6}$ hours. Concentrations were: 2,4-D*, 100, 500, and 2,500 ppm (1, 5, and 25 μg per 10- μl drop) in 50 per cent alcohol; ATA*, 100, 500, 2,500, and 12,500 ppm, aqueous. No surfactant was used. In this short period, used to test penetration, the difference between upper and lower leaf surface was again demonstrated, this time for both 2,4-D* and ATA*. The images were very dense in the case of the 125- μg dose of ATA* through both upper and lower leaf surfaces, and in the case of the 25- μg dose through the lower surface.

Table 3 gives the results of this experiment in terms of penetration and movement. From this table it is apparent that penetration of the lower surface

somewhat exceeded that of the upper in the short period; that translocation through the upper surface took place only from applications of 25 μ g and more of 2,4-D* and 125 μ g and more of ATA*; and that for translocation of ATA* through the lower surface a dose of at least 25 μ g was needed. Where translocation occurred, the tracers traveled about the same distances at all dosages, but increase in dosage brought about increase in the amount moved.

Leakage of 2,4-D from treated plant. In 1951 M. A. Clor found that 2,4-D applied to a cotyledon of a cotton plant would move down into the root and leak into the culture solution, whence it could be picked up by a second cotton plant growing in the same culture, and would produce symptoms in young leaves (Clor, 1951). To find if 2,4-D would do this in *Zebrina* cultures, an experiment was designed involving *Zebrina* and cotton plants paired in culture jars; the leaves of the *Zebrina* plants were sprayed with 2,4-D at 800, 4,000, and 20,000 ppm, while the cotton plants were carefully shielded. Seven daily applications were made of the 800-ppm and the 4,000-ppm solutions, but only two of the 20,000-ppm solution because of its toxicity to *Zebrina* plants. After 12 days the *Zebrina* plants showed 2,4-D symptoms and the cotton plants had cupped upper leaves, indicating the presence of 2,4-D in the culture solutions. The cultures were in pairs, one pair for each concentration of 2,4-D. Of these pairs, one culture received the 2,4-D made up in 15 per cent alcohol and containing 0.05 per cent Vatsol OT. The second culture received the same solution to which glucose had been added.

With 2,4-D at 800 ppm, only the culture with the added glucose produced symptoms on the cotton plant; at 4,000 and 20,000 ppm, the plants in both cultures showed symptoms, but those with glucose produced the greater formative effects. This experiment shows that 2,4-D will move from treated leaves of *Zebrina* downward into roots and leak into the medium in appreciable quantities.

Effect of different levels of nutrition on rate and direction of 2,4-D* movement. To further explore the factors that determine the rate and direction of 2,4-D* movement, eight *Zebrina* plants were provided with different levels of mineral nutrition. Two plants were kept in tap water for 4 weeks and two for 2 weeks; two were in $\frac{1}{16}$ Hoagland's solution for 2 weeks, and two were in $\frac{1}{2}$ Hoagland's solution for 2 weeks. Trials had proved that tap water would maintain *Zebrina* cultures in a mature, healthy condition for some time, but that by the end of one month practically all growth would cease; that two weeks in tap water would reduce growth to a very low rate; that $\frac{1}{16}$ Hoagland's solution would permit limited top growth and vigorous root growth; and that $\frac{1}{2}$ Hoagland's solution would permit extensive top growth but only limited root growth.

One day before treatment one of each pair of plants was shaded by applying a black paper cap to the upper portion of the plant above the leaf to be treated. Treatment consisted of applying 0.6 μ c of 2,4-D* in two drops one day apart to one leaf near the base of the plant. Treatment time was 4 days from the first application.

In the case of the plants cultured for one month in tap water, there was practically no movement out of the treated leaves; shading had no effect (fig. 6A). In the plants cultured for two weeks in tap water there was light but

appreciable movement into the roots; shading again had no effect (fig. 6B). The plants in $\frac{1}{16}$ Hoagland's solution moved enough 2,4-D* into the roots to produce strong images (fig. 6C); in the shaded plant there was also movement upward through two internodes (fig. 7A). In the plants in $\frac{1}{2}$ Hoagland's solution, movement of 2,4-D* into the roots was somewhat less but enough for autographs of medium density (fig. 7B); in the shaded plant upward movement occurred through two internodes at medium concentration and through two more in detectable quantity (fig. 7B).

From a number of considerations—failure of upward movement in the shaded, slowly growing or nongrowing plants, observation of normal movement in nutrient-deficient barley plants that were still growing,⁵ and the fact that a slight image of 2,4-D* can be detected throughout the roots in the autograph of figure 6, proving that slow phloem movement was actually taking place—we interpret these results as indicating that 2,4-D* will move in *Zebrina* in quantity if photosynthesis is active and if active sinks are present so that translocation is rapid. Where transport is slow, as in mature, static plants, accumulation seems to withdraw the tracer before it moves any distance. Mineral nutrition is apparently important through its effect on growth and the utilization of foods.

The most intense autograph yet produced with *Zebrina* involved a plant cultured for two weeks in $\frac{1}{2}$ Hoagland's solution, at which time the root was growing very actively (fig. 7C). The treatment consisted of two drops of 2,4-D* solution spaced one day apart and totaling 0.65 μ c. Treatment time was 4 days from the first drop. The top of the plant was not shaded, and translocation was entirely basipetal into the roots. Notable also is the fact that the one leaf basipetal to the treated leaf was completely bypassed by the tracer. This indicates that there was no leakage to the xylem during the treatment period.

In distinct contrast to figure 7C is an autograph shown in figure 8. The plant from which this was made is one of seven that were cultured in $\frac{1}{2}$ Hoagland's solution for one month, when all expanded leaves except the three lowest healthy ones were removed; the shoot tip was left intact. Treatment consisted of two drops on separate leaves of 2,000-ppm solution of 2,4-D* of specific activity of 6 mc per mM, totaling 1.1 μ c. Plant No. 1 (fig. 8A) had a treatment time of 9 days, followed by freeze-drying. Plant No. 2 (fig. 8B) had a treatment time of 3 days. While plant No. 1 shows strong movement both acropetally and basipetally, plant No. 2 shows mainly basipetal movement.

The remaining five plants received the same application, but it was followed on later days with drops of nonradioactive 2,4-D solutions, which were applied with the idea that they would maintain a supply of the radioactive material for movement farther into the plant extremities. Plant No. 5, which received 2,4-D* on the first day of the experiment (comparable with plant No. 1), followed on the 8 succeeding days by nonradioactive 2,4-D treatment, is shown in figure 8C. Evidence of a washing-out action is apparent in the treated leaves; intensity of radioactivity in the acropetal and basipetal parts is less than in plant No. 1. Possibly the tracer in the

⁵ A. S. Crafts, unpublished data, 1958.

roots moved out into the culture solution. If any of it moved into the upper portion of the plant, it must have been metabolized, because intensity there is relatively low. Such an artificial method of moving 2,4-D is apparently much less effective than the creation of sinks for movement by inducing active growth.

Movement of 2,4-D* from tip-growth application. To show that tip leaves will move 2,4-D* for long distances in a basipetal direction, providing a proper sink is present, an experiment was prepared involving four tall *Zebrina* plants cultured in $\frac{1}{2}$ Hoagland's solution for one month. All fully expanded leaves except the topmost two were removed and tip growth beyond the topmost expanded leaf was also removed. One lower branch was left on each plant. The roots were actively growing.

Treatment was the same as in the previous experiment. Figure 9A shows the plant and autograph of the 9-day treatment. Treatment on 8 successive days with nonradioactive 2,4-D plus 0.5 per cent glucose solution failed to increase the intensity of the autograph of plant No. 2. The extensive movement into the side shoot and roots, shown in figure 9B, is evidence for the great effectiveness of active sinks provided by vigorous growth.

Comparison of 2,4-D* and ATA* mobility. To study the relative mobility of 2,4-D* and ATA*, a group of mature, branched, variegated *Tradescantia fluminensis* plants was treated with these two herbicides. Five plants were treated with each chemical and one plant served as a control. The 2,4-D* was applied in 50 per cent alcohol solution with 0.1 per cent Nonic 218; one drop at 3,000 ppm ($0.167 \mu\text{c}$) was applied to a completely chlorotic leaf of one plant and three drops were applied to three leaves ($0.5 \mu\text{c}$ total) of each of four plants, two of which had treatment times of 2 days and two of 9 days. Of the latter pairs the 2-day treatments were on upper leaves and lower leaves; the 9-day treatments were on upper middle leaves and lower leaves. The ATA* treatments were the same except that the concentration was 5,000 ppm in distilled water and no surfactant was used. All treatments were on lower leaf surfaces.

As in previous tests, no movement occurred from entirely chlorotic leaves; this proved true of ATA* as well as of 2,4-D*. Of the 2-day treatments, 2,4-D* treatment on middle leaves resulted in light movement along the main stem into the roots; on lower leaves movement again was predominantly downward and into interspersed chlorotic shoots. Tips on these plants were not growing rapidly, and 2,4-D* mostly remained in the treated leaves.

Of the 2-day treatments with ATA*, movement from upper middle leaves was mostly upward into the shoot tip, which happened to have several chlorotic leaves; from middle leaves movement was both upward and downward, and the tracer concentrated in the young tips of interspersed shoots, particularly those that were chlorotic.

In the case of the 9-day treatments, 2,4-D* moved from upper middle leaves to the growing shoot tip, from lower leaves downward into roots. Accumulation in the treated leaves was high and concentration in the stems was faint, partly because of contact injury. ATA* in the 9-day treatments moved from upper middle leaves primarily upward to the shoot tip; some of it also moved long distances in the opposite direction and concentrated

in the young growing tips of many chlorotic branches. From lower leaves the tracer moved mostly downward to the tips of many chlorotic lateral shoots.

This experiment indicated for the first time the great difference in mobility between 2,4-D* and ATA*. The latter proved much more mobile in these plants and characteristically accumulated in growing shoot tips far removed from the treated leaves. The 2,4-D*, on the other hand, remained in high concentration in the treated leaves and adjacent stem tissues, and failed to move in large amounts. Figure 10A and B shows some of these plants.

Comparative tests of 2,4-D*, ATA*, and MH*. For a further comparative study, six large *Zebrina* plants in pots in a depleted soil were treated with 2,4-D*, ATA*, and MH*. Each plant received three 10- μ l drops, one drop each on the lower surface of three separate leaves at concentrations that resulted in each plant receiving 0.5 μ c. Treatment time for three plants was 7 hours; for the other three it was 4 days.

In the case of the 7-hour treatments, autographs of the three plants looked very similar; the treated leaves were dense, but little or no translocation had taken place. Autographs of the 4-day treatments were very different, and they served to show the great inherent differences between the three compounds. The 2,4-D* remained very intense in the three treated leaves and adjacent internodes; very little tracer was present in the stems beyond the treated leaves; there was no accumulation in the intercalary meristem regions. In 4 days the ATA* had moved throughout the plant and was present in a light and rather uniform concentration in all stems and in the roots. It was accumulated in three growing tips at concentrations above those in intervening stems. It bypassed many mature leaves.

In 4 days MH* had moved throughout the plant; it was present in medium intensity in the stem, from the treated leaves up to the tip of the shoot, and in the young expanding leaves; it was also in the stem from the treated region downward into the roots. Intensity of radiation in the side shoots was lower, but the MH* was present at low intensity in all mature untreated leaves in contrast to ATA* which had bypassed such leaves on its way to growing shoot tips. MH* intensity in the treated leaves was distinctly lower than in the 2,4-D*-treated leaves; those on the ATA*-treated plants were intermediate. Figure 11A, B, and C shows the plants and autographs of the 4-day treatments.

Several conclusions are evident from this experiment. (1) Of these three chemicals, MH moves most freely throughout the plant, ATA is intermediate, and 2,4-D is least readily moved. (2) Because MH moved so freely in these plants, it seems that lack of movement on the part of 2,4-D reflects not failure of the transport mechanism, but rather the strong accumulative capacity of the living plant tissue. Accumulation apparently immobilized the chemical before it could be moved any distance by the slow-moving assimilate stream of these inactive plants. (3) ATA is apparently freely mobile, but under the conditions of this experiment it was not released from the symplast. (4) MH, because of its distribution in mature untreated leaves, must move from phloem to xylem and migrate via this tissue in the transpiration stream. The very uniform distribution of radioactivity in these mature

leaves indicates little tendency for the MH to be held in living cells. In fact, MH may behave much as phosphate is known to do; it seems very possible that MH may circulate in the plant, moving downward in the phloem and upward in the xylem. Such behavior would explain its known ability to maintain dormancy for such long periods in treated plants.

As a further check on the relative mobility of these three labeled compounds, the solutions were applied to the stems of three mature *Tradescantia* plants. For the stem application a small cup of aluminum foil was shaped to each stem and sealed with lanolin to prevent leakage. Each cup received 30 μ l of the treatment solutions made to a uniform strength of 0.45 mc per mM. This amounted to 0.15 μ c per treatment. Treatment time was 4 hours.

In this experiment the 2,4-D* moved downward in greater quantity than upward and was confined to the stem. However, the image was faint throughout with the exception of the treated spot, which was dark. ATA* moved predominantly upward, and all green leaves above the treatment autographed at a medium intensity, with the veins showing prominently. MH* moved both upward and downward into the roots. Image intensity was heavy in the stem near the treatment area and graded to medium at the shoot tip. All leaves above the treatment region autographed with a tendency for greater intensity in the green portions, less in the chlorotic. All leaves from the region of treatment to the roots were bypassed, indicating that the downward movement was restricted to the phloem. Figure 12 shows the plants and autographs.

From a careful study of the autographs from this experiment and consideration of the normal direction of sap movement in the xylem and phloem of plants, it appears that the 2,4-D* movement was largely confined to the phloem; that of ATA* and MH* in the upward direction was in the xylem; and MH* moved downward in the phloem as well. This experiment points out the complexity of the problem of movement of chemicals in plants; it poses the question of leakage or exchange between the two major transport systems; and it emphasizes our need for more knowledge of the detailed functioning of vascular tissues.

Comparative tests with barley plants. Two final experiments will be described to complete the comparative picture of herbicide transport. The first involved 3-week-old barley plants growing with their roots on filter paper saturated with full-strength Hoagland's solution. Six carbon¹⁴-labeled chemicals were used—2,4-D, 2,4,5-T, ATA, MH, monuron, and urea. Dosage was 0.05 μ c and 0.2 μ c on paired plants and treatment time was 2 days. Each plant was treated within a lanolin ring placed in the middle of the second leaf. There were three expanded leaves on each plant. The plants were killed with pulverized dry ice and freeze-dried under vacuum.

The results of this study, some of which are illustrated in figure 13A, B, and C, show some wide differences between these herbicides with respect to distribution. On the basis of the autographs, the chemicals can be placed in three groups:

1. Urea*, MH* (fig. 13C), and ATA* (fig. 13B) moved throughout the plants, particularly at the higher doses. In the roots in all three instances the tracers were concentrated in the growing extremities and were less con-

centrated in the older regions. ATA* was concentrated in the treated leaves, from the regions of application to the tips (fig. 13B); urea* and MH* were less concentrated in the tips of the treated leaves; all three could be seen in both the mature first leaves and in the developing third leaves of all plants.

2. 2,4-D* (fig. 13A) and 2,4,5-T* moved from the treatment spots downward to the crown; here a sharp break in concentration occurred, the roots autographing very lightly. Either the phenoxy compounds had been metabolized within the roots or they did not move into them. Although the whole root system of the plant that received $0.2 \mu\text{c}$ of 2,4-D* can be seen, the radiation intensity is very low; the first and third leaves were completely bypassed.

3. Monuron* moved only in an acropetal direction in the barley leaves; apparently it cannot enter the phloem, and it moves with the transpiration stream. This agrees with the results reported on tomato by Haun and Peterson (1954).

The failure of 2,4-D* and 2,4,5-T* to move in appreciable quantity to the top of the barley plant parallels a result observed by Wilkinson (1956) with dalapon. Treatment of the second or third leaf of young barley plants resulted in this same limited movement; treatment of the first leaf resulted in movement into roots and young expanding leaves.

Subsequent work with 2,4-D* on barley plants of different ages proves that strong movement into roots may follow application to mature leaves, whereas little or no movement takes place from leaves that are still growing actively. The 2,4-D* applied to such leaves may move from the point of application to the intercalary meristem at the leaf base, but it will not pass this meristem in quantity if active growth is still in progress.^a

Apparently dalapon and the phenoxy compounds undergo the same type of distribution in barley seedlings. Considering the work on *Zebrina*, this distribution apparently depends upon the relative effects of accumulation by living cells and upon the velocity of translocation in the phloem. If the treated leaves in the experiment under consideration had barely passed the compensation point and hence were using the bulk of their own assimilates in growth, export might have been very slow; under these conditions, accumulation in the growing zones could have accounted for the restricted distribution of 2,4-D* and 2,4,5-T*.

Because the untreated leaves of the latter plants were bypassed, it seems that the movement of the phenoxy compounds was limited to the phloem. Presence of the labeled tracers in untreated leaves in the case of urea*, MH*, and ATA* would seem to indicate leakage into the xylem.

Comparative tests with potato tubers. The final experiment involved tests with urea*, MH*, ATA*, 2,4-D*, 2,4,5-T*, and monuron* applied to blocks of potato tuber. This was designed to throw some light on the movement of these tracers in parenchyma tissue, as contrasted with vascular tissue. Figure 14 shows the results. Dosage was $0.05 \mu\text{c}$ applied in a $10\text{-}\mu\text{l}$ drop to a $\frac{1}{4}$ -inch spot, confined by a lanolin ring, on the cut surface of a block of tuber tissue approximately $1.5 \times 1.5 \times 1.5$ inches. Treatment time was 4 days. At the end of the treatment time a slice $\frac{1}{8}$ inch thick was cut from the treated

^a A. S. Crafts, unpublished data, 1958.

surface; this was freeze-dried, then slightly dampened, and pasted, treated surface down, to the paper backing. The sheet of mounted slices was pressed flat and autographed for 28 days.

As with the barley plants, there is a pattern of behavior, with only one notable exception. The urea* and MH* moved freely and were so thoroughly distributed that only a faint spot shows in the treatment position. ATA* shows much more restricted movement, and the treated area is represented by a spot about $\frac{3}{4}$ inch in diameter; 2,4-D* and 2,4,5-T* are even more limited, the spots being only a bit over $\frac{1}{2}$ inch in diameter. Monuron*, in contrast to the other tracers, disappeared from the treated area, but the whole block has a distinctly darker shade than the others. Apparently it was uniformly distributed throughout the treated blocks. It seems that in this tissue 2,4-D and 2,4,5-T are accumulated and held in living cells, ATA moves somewhat more freely, MH and urea move quite freely, and monuron is uniformly spread throughout the tissue and is not held in cells or on cell walls. These final experiments emphasize the great usefulness of the comparative method for studying mobility of compounds in plants.

DISCUSSION

The experiments described in the foregoing pages present some new concepts concerning the movement of materials in plants. Previous reports (Crafts, 1956*a*, 1956*b*; Leonard and Crafts, 1956) gave the impression that 2,4-D moves relatively freely in plants, accompanying foods in their passage from regions of their synthesis to regions of their utilization. The comparative tests reported here prove that maleic hydrazide, aminotriazole, and urea move relatively freely; that 2,4-D and 2,4,5-T are somewhat restricted in their distribution; and that monuron, though readily mobile in the transpiration stream via the xylem, will not enter the phloem following leaf application. These three distinct types of behavior are undoubtedly related to the structure of the molecules concerned, and they provide extremely useful tools for translocation study.

The role of accumulation or retention by living cells in the over-all pattern of distribution is important in the use of herbicides as it is in an understanding of food distribution. Past views of phloem transport in plants have tended toward oversimplification in that the search for the motive force causing rapid movement has focused attention on the sieve tubes of the phloem. Important as these elements are as conduits for the rapid phase of movement, the role of parenchyma cells of cortex, phloem, cambium, and xylem, as stressed in the present work, should not be overlooked.

When a chemical is applied to the leaf surface for the purpose of bringing about systemic herbicidal, fungicidal, or insecticidal effects, it must traverse a variety of cells and tissues before it arrives at the phloem for rapid transport. Among these are epidermis with its specialized cuticle layer, chlorenchyma, spongy parenchyma, border parenchyma, and phloem parenchyma. The applied chemical may also encounter sclerides of various forms, secretory systems such as latex or resin ducts, and living cells of the xylem parenchyma. The most logical interpretation that can be given

certain aspects of the work reported here is that these various categories of cells, individually or in combination, may accumulate or retain within their protoplasts the applied chemical and so limit its further movement.

Furthermore, once a chemical has entered the sieve-tube system it is subject to withdrawal by the living parenchyma cells that surround the sieve elements throughout their length. And from these cells it may accompany foods on their way to cambium, xylem, or cortex so that an outward gradient may be maintained.

Finally, the chemical may move through the phloem to roots, tubers, fruits, or other storage organs where foods are being used in growth or retained for future use. Here, if it is not metabolized, it may accumulate as in the case of ATA* and MH* in the roots of barley (fig. 13*B* and *C*).

The comparative studies reported here re-emphasize the concept of the symplast as the interconnected, integrated living system of the plant, and they point out that the phloem represents simply the highly specialized mechanism by which rapid longitudinal transport is accomplished. The total distribution system includes all living cells of the plant, and its proper working requires active functioning in the regions of synthesis (source) and regions of utilization (sink). Certain chemicals, such as MH, can apparently enter this system and move relatively freely from source to sink with little or no interference from the accumulative propensities of various parenchyma tissues. Others, such as 2,4-D and 2,4,5-T, may be retained during entry; they may be held and metabolized by living cells along the route of movement; or they may be stored or broken down in mature storage tissues. Finally, some, such as monuron, are unable to enter the phloem system and hence are not useful for foliage application.

Less is known of uptake by roots and transport in the xylem, but apparently all of the chemicals used in this study are readily absorbed from the culture medium and distributed via the transpiration stream. Careful study of autographs of plants receiving root treatment reveals characteristic differences between such distribution and that which follows foliage application. Distribution via the xylem tends to be uniform throughout the leafy portion of the plant; if differences exist, the concentration is higher in fully expanded, rapidly transpiring leaves. The roots may retain the labeled compounds at a distinctly higher concentration than is found in the tops.⁷

Phloem movement, as emphasized in figures 8, 9, 10, 11, and 13, is far from uniform and usually follows a definite pattern illustrating the source-to-sink relationship. Concentrations are often much greater at shoot tips, buds, and root tips than in intervening tissues.

Finally, the findings of Clor (1951) and Crafts and the experiments illustrated by figures 11 and 13 provide much evidence that some chemicals may migrate from conduits of phloem to those of xylem or leak to the external culture medium. Evidence for this is the uniform marking of untreated leaves, as in figures 11*C* and 13*B* and *C*, and the production of symptoms on untreated plants growing in cultures with treated ones (Clor, 1951).

From the above considerations and the experiments herein reported, it seems (1) that 2,4-D will move out of green leaves but not out of chlorotic

⁷ A. S. Crafts, unpublished data, 1958.

ones; (2) that it will move out of variegated leaves roughly in proportion to the amount of green tissue in them; (3) that in mature, slow-growing or nongrowing plants little or no outward movement of 2,4-D from mature leaves may occur; (4) that establishment of sinks for transport by stimulating growth may bring about movement into roots or shoot tips, or two-way movement into both roots and shoot tips; (5) that when 2,4-D, ATA, and MH are tested comparatively, 2,4-D is the least mobile, ATA is intermediate, and MH is highly mobile; (6) that movement of 2,4-D is largely or completely limited to the phloem, that ATA may move from source to sink in the phloem or with the transpiration stream in the xylem, and that MH, by passing from one vascular system to the other, may circulate in the plant.

The works of Mitchell and Brown (1946), Weaver and De Rose (1946), Rice (1948), Linder, Brown, and Mitchell (1949), Rohrbaugh and Rice (1949), Weintraub and Brown (1950), Mitchell and Linder (1950), Davis and Smith (1950), and Day (1950) seem to clearly indicate a relationship between movement of 2,4-D and food movement in the plant. The results enumerated above seem most readily explained on the same basis. And the most satisfactory mechanism for reconciling these many results would seem to be movement of the 2,4-D in a common stream of assimilates, moving from the source in green leaves to various sinks in the plants that are maintained by metabolism, growth, and storage.

Results of the tests reported here further indicate that 2,4-D is accumulated by and held in living cells of leaves and stems. Where this accumulation is rapid and translocation is slow, 2,4-D fails to move over appreciable distances. Where translocation is rapid as a result of rapid growth, movement into the growing region or regions may be great.

The requirement of growth for 2,4-D transport was emphasized in a previous paper (Leonard and Crafts, 1956). In the present studies this is re-emphasized, and the role of mineral nutrition is clearly established. In a fertile soil and under favorable conditions of temperature and light, root growth is inherent in plants; depletion of soil moisture is the most common limiting factor. In brush areas in California, soil moisture is probably the most important requirement that becomes limiting (Crafts and Leonard, 1956). In regions of frequent summer rainfall, soil moisture is usually adequate, but nutrient supply is much more likely to become deficient. Since 2,4-D transport apparently stops when growth slows down, it seems important to recognize the possibility of failure and to withhold 2,4-D treatment until a more favorable time.

According to the comparative tests, the movement of ATA and MH is much less dependent upon rapid food transport; treatment later in the season may, therefore, still be effective. Field experience bears this out. Furthermore, tests on woody species with labeled herbicides prove that ATA* and MH* may move extensively in plants at a time when 2,4-D* movement has ceased.⁸

There is evidence in the horticultural literature that in entering the green leaf, urea* may be split into NH_3 and radioactive CO_2 . The latter may be rapidly synthesized into radioactive sugars and amino acids. Hence, the

⁸ O. A. Leonard, personal communication, 1957.

distribution of urea* mentioned on page 431 may actually represent movement of foods rather than urea.

Ten years of intensive research with 2,4-D have brought many improvements in formulation and spray practice, so that species that would not respond to the early formulations may now be controlled. Possibly even more improvement may be made. However, in view of the results with ATA* and MH* obtained in the studies reported here, it seems that continued search for new, highly mobile compounds is justified. A compound with the toxicity of 2,4-D and the mobility of MH would control many of our hard-to-kill perennials.

Dalapon, 2,4-D, and dinitro compounds all seem to respond to formulation practices that reduce ionization of the basic chemicals. In every case, however, these compounds are subject to accumulation and detoxification by living cells. ATA, MH, and monuron are not responsive to reduction in dissociation; they apparently enter plants along a completely aqueous route; they are all highly polar compounds. If we could establish that nonpolar compounds entering the leaf along a lipoid route are subject to accumulation, while nonpolar compounds entering via an aqueous route are not accumulated, much would have been accomplished toward a clearer understanding of the relation of molecular structure to movement of herbicides.

The experiments reported in this paper would seem to have considerable significance in relation to two recent papers of Hay and Thimann (1956a, 1956b). These papers purport to show that 2,4-D is not translocated into the roots of bean plants, that 2,4-D transport involves a metabolic component, and that the mass-flow hypothesis is invalid. If one considers the role of growth in 2,4-D transport as illustrated in our work, it seems quite likely that because of the methods used by Hay and Thimann most of their work was completely irrelevant.

In the first place, they grew their bean seedlings in tap water for 14 days, and then they removed "the cotyledons, terminal bud, and one primary leaf." In short, they grew the plants in the absence of nutrients until the roots were at a standstill; then they removed the terminal bud, so that all sinks for transport were gone.

Next they cut the leaf to be treated so as to make a flap, which they immersed in the treatment solution in a vial. It has long been known that the phloem tissue seals itself almost immediately upon injury, so uptake of the treatment solution was presumably by the cut xylem elements, and distribution, as shown by their figures, was primarily into the treated leaf. Here migration from xylem to phloem probably took place because ringing materially reduced subsequent movement into the upper hypocotyl (Hay and Thimann, 1956b, table IV). However, with the plants in such a condition as these were, transport must necessarily have been slow, and accumulation apparently removed what little 2,4-D got into the phloem tissue. This would be comparable to the movement of 2,4-D in our figure 11A.

Sugar application seemed to increase 2,4-D transport in the plants in the dark; mannitol, arabinose, and urea failed to do this. However, although the latter compounds were probably absorbed by leaf cells, there is no evidence that they ever entered the phloem. In view of Wanner's (1952) evi-

dence on sugar transformation in the phloem, it seems highly unlikely that the enzymes involved in transforming hexoses to sucrose would function in the case of mannitol and arabinose. Urea could well have been split into carbonate and ammonia.

The statement of Hay and Thimann (1956*b*) that "if sucrose and 2,4-D were moving simultaneously in response to a differential in osmotic pressure, the transport should be relatively insensitive to temperature changes" seems not to consider the effects of low temperature on viscosity of sugar solutions (Crafts, 1932, p. 203) or its possible role in reducing thermal agitation and hence permeability of the protoplasm of the protoplasmic connections of the sieve plates. Crafts postulated in 1948 that the ability of the sieve-plate protoplasm to conduct assimilates might depend upon loss of lipid and polar groups along protein chains (Crafts, 1948). The sieve-plate strands, with their remaining skeletons of longitudinally oriented polypeptide chains, could serve as effective conduits so long as thermal agitation kept these chains in lively motion. Slowing of this agitation would rapidly result in clogging and retardation of transport. This would apply to transport of any sort along the interconnecting protoplasmic strands between the sieve-tube elements, and we know of no other channels through which rapid phloem transport, from one sieve element to the next, could take place.

To state that "it is deduced that transport of 2,4-D is not due to simple osmotic forces as in the mass flow hypothesis, but involves a metabolic component" is to overlook many statements emphasizing the role of cell metabolism in phloem transport. The mass-flow mechanism necessarily involves many metabolic components (Crafts, 1931, p. 31; 1932, p. 215). As viewed by modern physiologists, the mass-flow mechanism involves an energy-requiring polar concentration of sucrose within the sieve tubes; it requires a metabolically controlled permeable condition of the protoplasmic strands traversing the sieve plate; and it visualizes an active uptake of assimilates in regions of active growth (roots, tubers, fruits, buds, cambium, etc.). It differs from other less accurately defined theories mainly in not postulating some unknown process within the sieve tubes capable of accelerating movement by non-osmotic means far beyond that of any known physical phenomenon.

SUMMARY

Labeled 2,4-D (2,4-D*) moves into *Zebrina* leaves more rapidly and in greater quantity through cuticle on lower leaf surfaces than on upper.

Apparently 2,4-D* is accumulated by living cells of the leaf. This restricts translocation and limits the amount that moves to stems and roots. Tests on variegated *Tradescantia* plants prove that 2,4-D*, ATA*, and MH* fail to move out of chlorotic leaves but move readily from green leaves.

In solution cultures containing both *Zebrina* and cotton plants, treatment of *Zebrina* leaves with 2,4-D resulted in movement of the chemical into the roots, leakage into the culture solution, and uptake by the cotton. This confirms Clor's results of 1951.

Treatment of *Zebrina* plants growing at different nutrient levels with 2,4-D* proved that active root growth, as determined by nutrient level, is

essential to the translocation of this compound from leaves to roots. Shading the plant tops above the point of application resulted in movement both upward and downward in plants having active root growth.

Removal of mature leaves along the stems of *Zebrina* plants, followed by treatment of two remaining basal leaves, resulted in movement both upward to growing shoot tips and downward into growing roots. Mature leaves below the treated ones were bypassed by the tracer.

Removal of mature leaves along stems of *Zebrina* plants, removal of growing tips, and treatment on two fully expanded upper leaves resulted in strong movement of 2,4-D* into roots and lateral shoots that were young and active. Roots were in an active state of growth.

Comparative tests on *Zebrina* with 2,4-D*, ATA*, and MH* proved that 2,4-D* may be restricted in its movement by accumulation into living cells; that ATA* moves relatively freely but in this species remains limited to phloem; and that MH* moves freely in phloem and apparently leaks into the xylem and circulates in the plant. The same pattern of distribution is found in barley plants, except that ATA* seems to leak into the xylem to a limited extent, urea* moves freely into roots, and monuron* moves from the point of application only to the leaf tip via the xylem.

Comparative tests on potato tuber tissue with six labeled tracers gave the following results: 2,4-D* and 2,4,5-T* showed very restricted movement; ATA* showed somewhat less restricted movement; MH* and urea* showed relatively free movement; and monuron* disappeared from the point of application and apparently moved uniformly throughout the treated tissue block.

In figures 1 to 4 and 6 to 13 the upper row of illustrations consists of autoradiographs; below each is a photograph of the treated plant, black arrows indicating the area of application.

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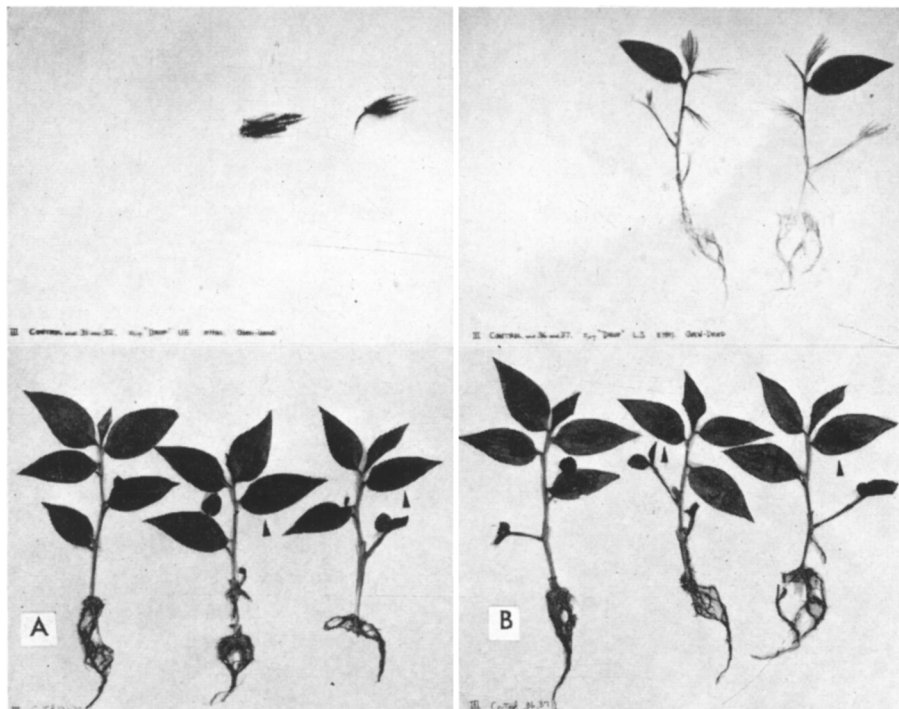


Fig. 2. Differences of penetration of 2,4-D* applied to upper and lower leaf surfaces of *Zebrina*. Application to upper surfaces (A) permitted very limited absorption, and no translocation had occurred at the end of 8 hours. Application to lower surfaces (B) permitted continued absorption and translocation.

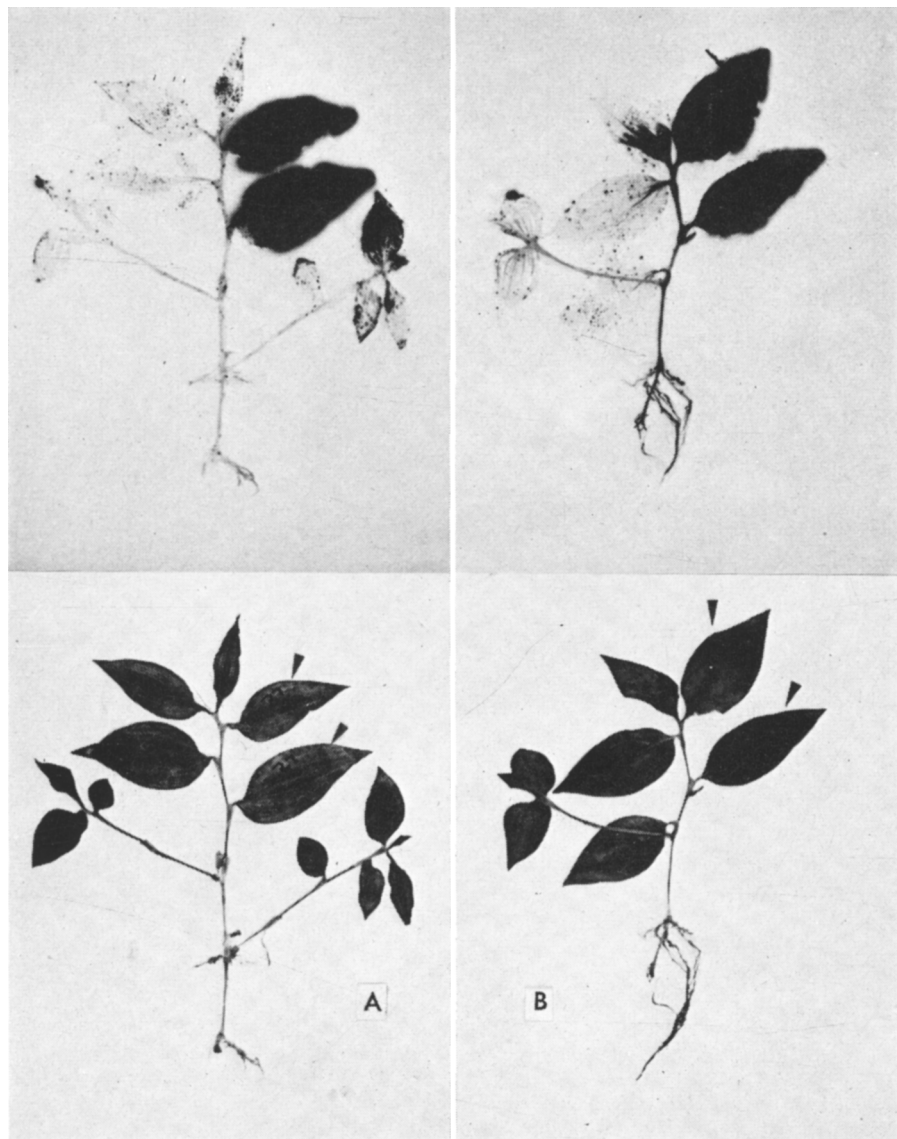


Fig. 3. High concentrations of 2,4-D* applied to the upper surface (A) and lower surface (B) of *Zebrina* leaves. The autograph on the left shows that when a high enough dose is applied to the upper surface of the leaf, a certain amount of 2,4-D does translocate.

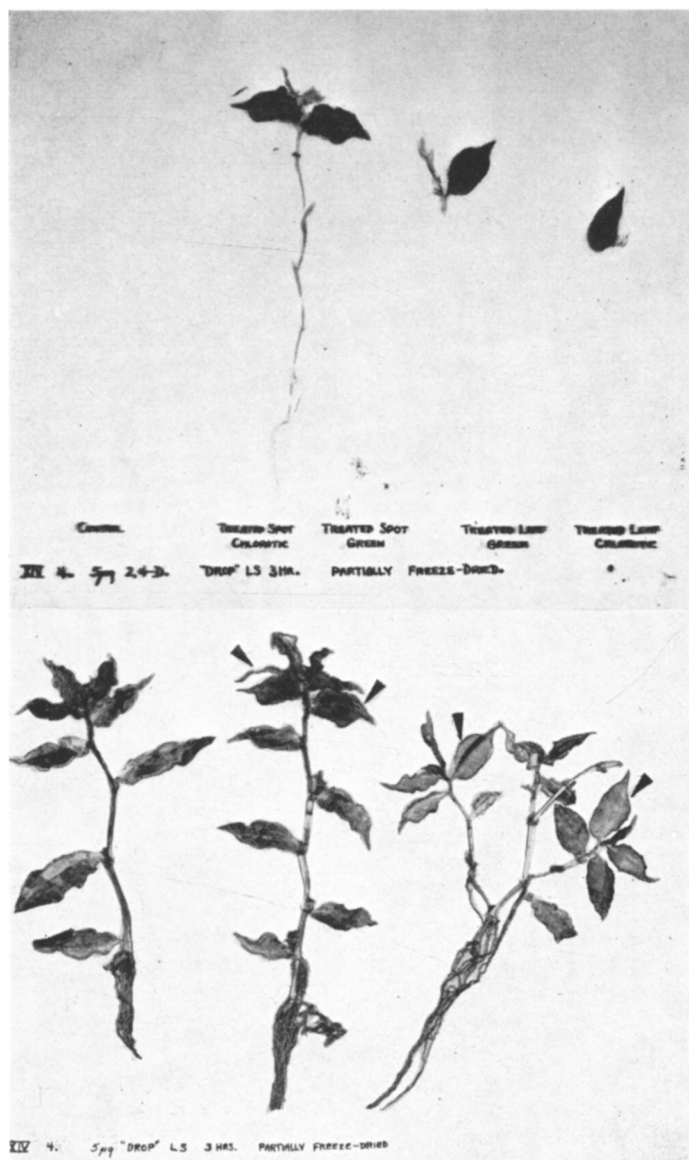


Fig. 4. Translocation from green and chlorotic leaves of *Tradescantia fluminensis*. The autograph of the chlorotic leaf (right) shows movement only within the treated leaf. The other treated leaves, which are green or variegated, show outward movement. Treatment: $5\mu\text{g}$ of 2,4-D* applied to lower surface of leaf; treatment time, 3 hours; freeze-dried.

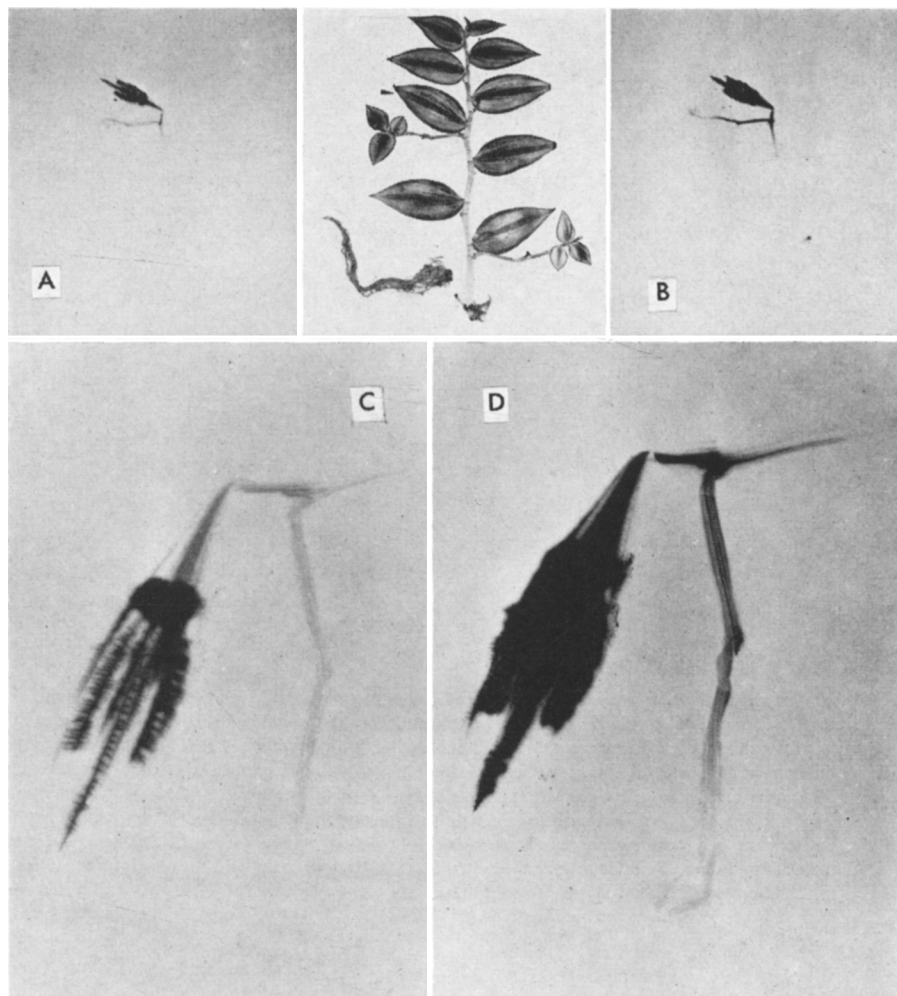


Fig. 5. The early phase of movement and absorption of 2,4-D* in *Zebrina*. 2,4-D moves from the treated spot toward the tip of the leaf, apparently by way of xylem (A), and is absorbed by parenchyma cells immediately adjacent to the vascular tissue. Such absorption also occurs when 2,4-D* is translocated by phloem through the stem, producing a more intense tracing of the vascular tissue and the stem near the treated leaf and a lighter tracing in the direction of translocation (B). The treated plant is shown between the two upper autographs (arrow indicates the area of application); C and D are enlargements of A and B. Treatment: 20 μg (0.55 μc) of 2,4-D* (6.0 mc per mM) in 10 μl of solution, applied to the lower surface of leaf; treatment time 3 hours; freeze-dried.

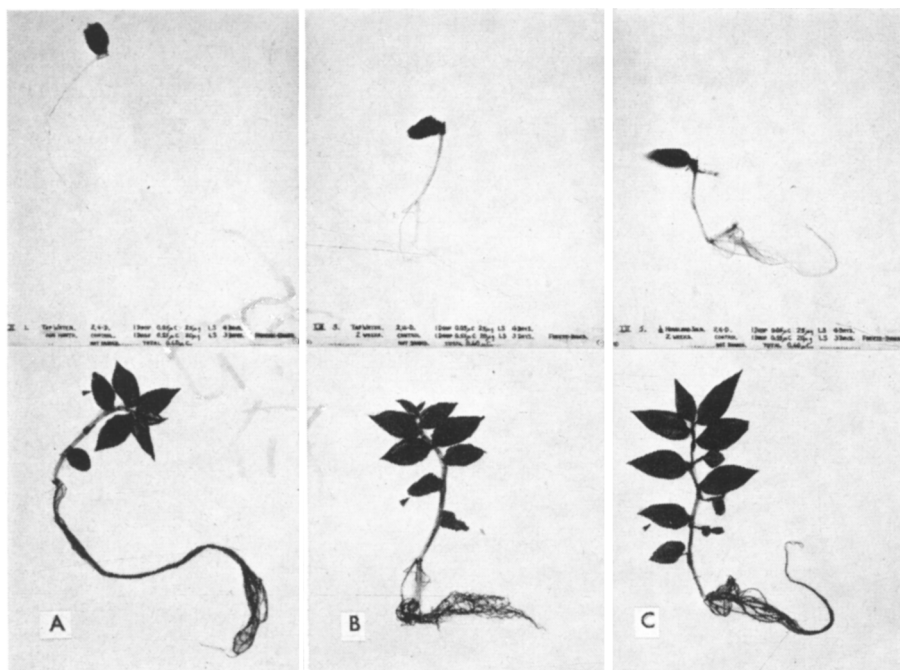


Fig. 6. Translocation of 2,4-D* as affected by growth conditions under various levels of mineral nutrient. The plant grown for a month in tap water (A) shows essentially no translocation of 2,4-D* from the treated leaf. The long root system is typical of plants in low-nitrogen cultures. This growth took place early in the month's exposure to tap water. Growth had virtually ceased at the time of 2,4-D* treatment. The other two plants (B, two weeks in tap water; C, two weeks in 1/16 Hoagland's solution) show greater amounts of translocation, corresponding to the rate of growth in the roots at the time of treatment. Treatment: $0.6 \mu\text{c}$ in $45 \mu\text{g}$ of 2,4-D* applied to the lower surface of the leaf; treatment time 4 days; freeze-dried.

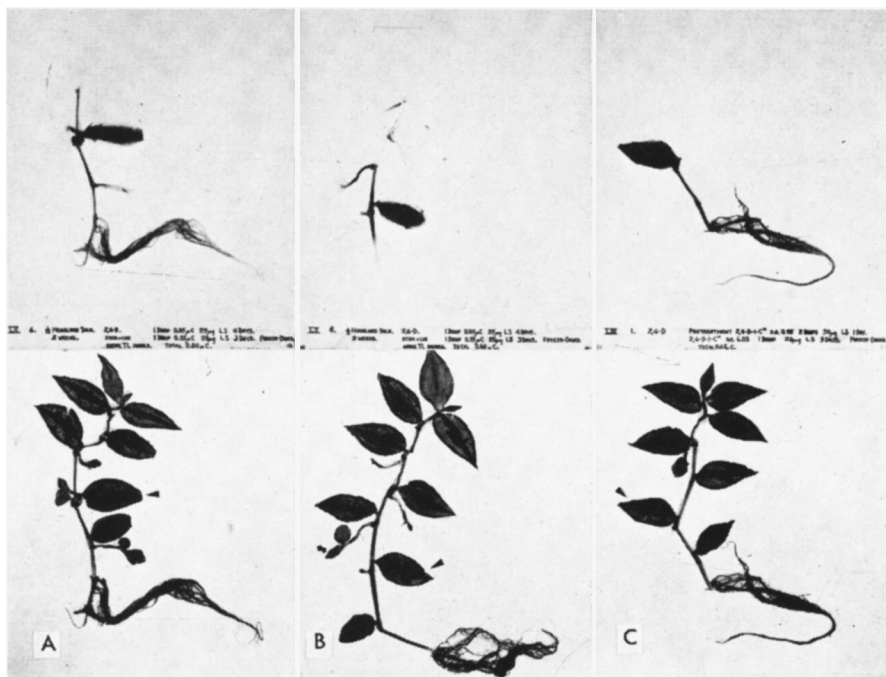


Fig. 7. Translocation of 2,4-D* as affected by growth conditions and shading. Translocation from a lower mature leaf is usually toward the growing roots, but if all regions above the treated leaf are shaded it can be partly in the direction of the rapidly growing stem tip (*A* and *B*). Treatment for these two plants was the same as for those in figure 6. *C* shows heavy movement of 2,4-D* toward the roots of a plant still exhibiting exceptional root growth two weeks after transfer from tap water to $\frac{1}{2}$ Hoagland's solution. Treatment: $0.65 \mu\text{c}$ in $70 \mu\text{g}$ of 2,4-D*, applied to lower surface of leaf; treatment time 4 days; freeze-dried.

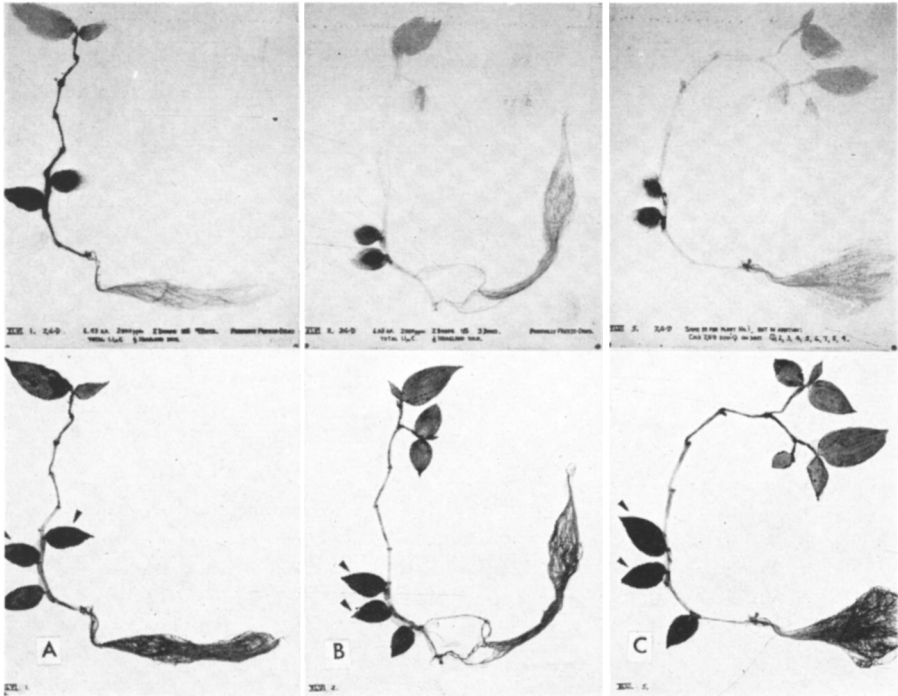


Fig. 8. Upward translocation from lower leaves, achieved by removing all upper leaves. The upper leaves maintain a continuous flow of photosynthates to the stem tip, thereby holding a pressure against upward translocation from the lower leaves. When these upper leaves were removed, upward translocation occurred from the lower leaves. The plants were grown in $\frac{1}{2}$ Hoagland's solution for very rapid growth. The large leaves at the stem tips are growth made during the 9-day treatment period. Treatment for *A* and *B*: $1.1 \mu\text{c}$ in $40 \mu\text{g}$ of 2,4-D*, applied to the upper leaf surface; treatment times 9 and 3 days, respectively; freeze-dried. Treatment for *C* was the same as for *A*, except that eight additional daily applications of $50 \mu\text{g}$ of unlabeled 2,4-D were made.

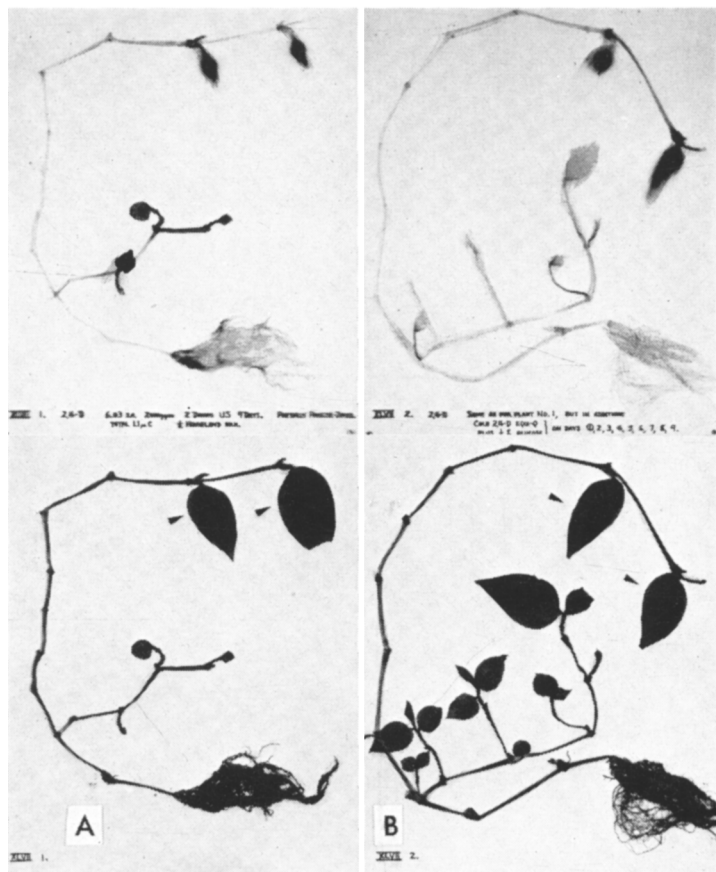


Fig. 9. Downward translocation from upper leaves, achieved by removing all lower leaves and stem tips. Here again, as in figure 8, the direction of translocation was reversed over a distance of several internodes by removing all intervening leaves. *A* and *B* both show good translocation over distances unusually long for 2,4-D in *Zebrina*. Note that the tracing of the new growth in the branches is even darker than that of the intervening stem. The plants were grown in $\frac{1}{2}$ Hoagland's solution for a very rapid rate of growth. Treatment: $1.1 \mu\text{g}$ in $40 \mu\text{g}$ of 2,4-D*, applied to the upper surface of a leaf; treatment time 9 days; freeze-dried. *B* received eight additional daily applications of $40 \mu\text{g}$ of unlabeled 2,4-D, plus 0.5 per cent glucose.

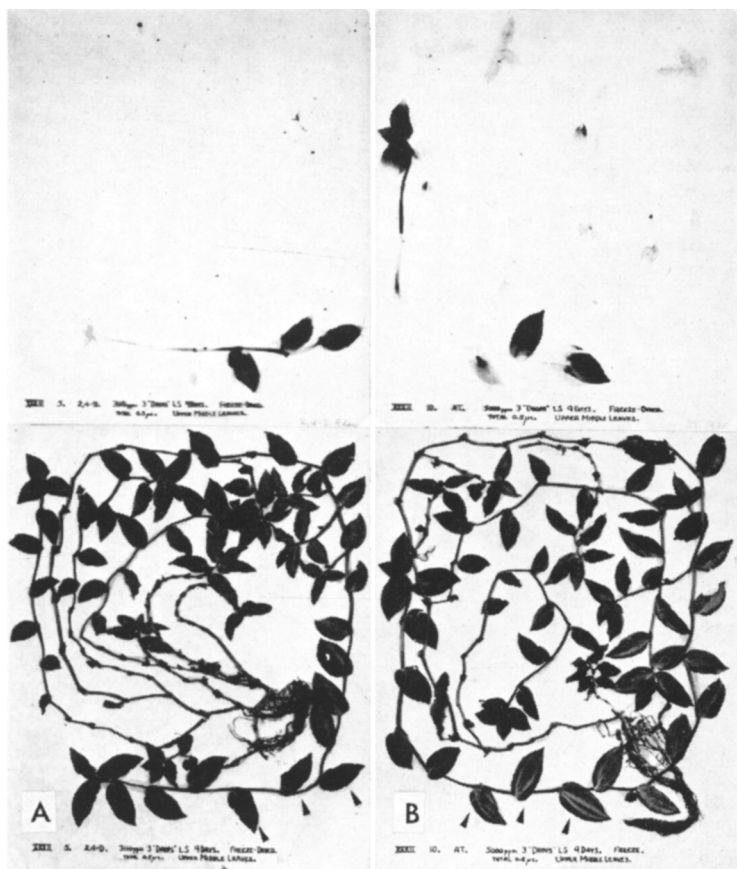


Fig. 10. Comparison of the mobility of 2,4-D* and ATA*. Translocated 2,4-D* was mostly absorbed by the first two internodes (A). ATA* was translocated mostly to the growing tips (B). The plants were grown in tap water supplemented with Hoagland's solution. Treatment: 0.5 μ c (in 93 μ g of 2,4-D* and in 150 μ g of ATA*), applied to the lower surface of leaf; treatment time 9 days; freeze-dried. The injury in the treated leaves in A was caused by 2,4-D.

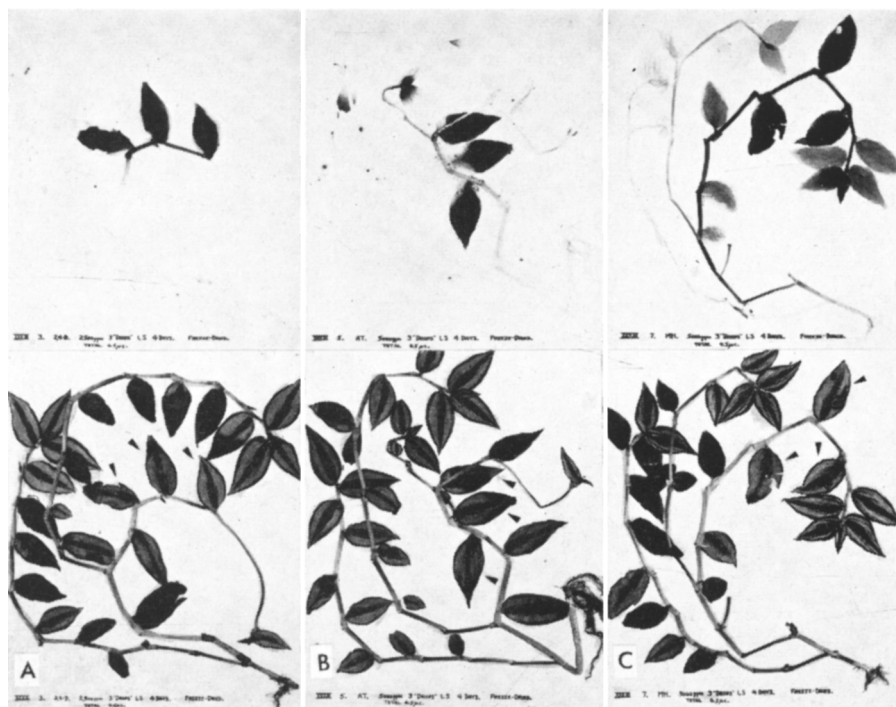


Fig. 11. Comparison of the mobility of 2,4-D* (A), ATA* (B), and MH* (C) in *Zebrina*, autographs arranged in the order of increasing mobility of the chemical. The plants were grown for several months in depleted soil in pots. Treatment: $0.5 \mu c$ (in $75 \mu g$ of 2,4-D* and in $150 \mu g$ of ATA* and MH*), applied to the lower surface of leaf; treatment time 4 days; freeze-dried.

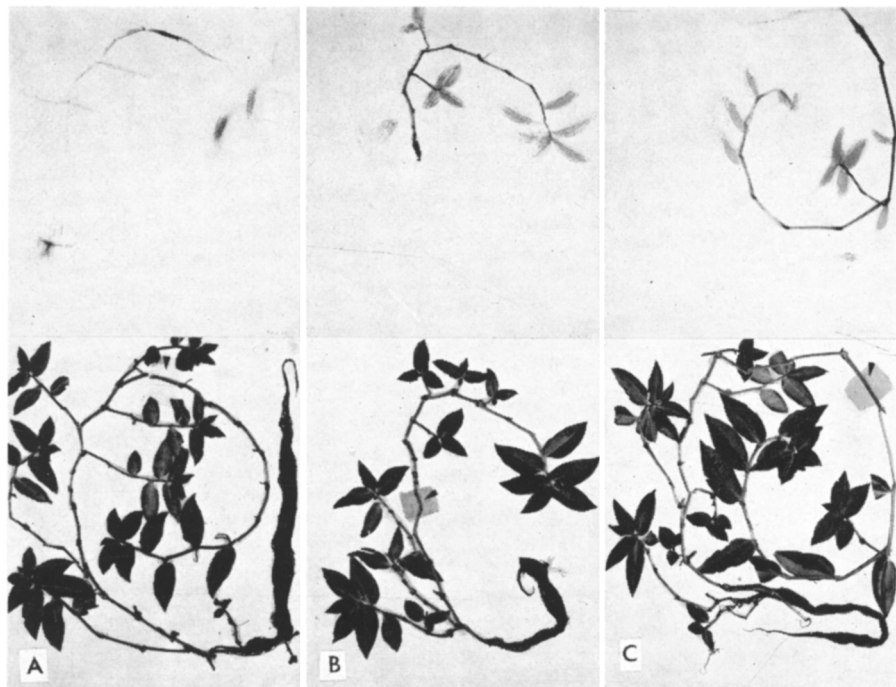


Fig. 12. Comparison of the mobility of 2,4-D* (A), ATA* (B), and MH* (C) in *Tradescantia fluminensis*, autographs arranged in the order of increasing mobility of the chemical. ATA* and MH* exhibit a greater tendency than 2,4-D* toward free mobility in the transpiration stream. The plants were cultured in tap water supplemented with Hoagland's solution. Treatment: $0.15 \mu\text{c}$ of carbon¹⁴-labeled chemical adjusted to 0.45 mc per mM in $30 \mu\text{l}$ of solution, applied to the internode and kept in place by means of a retainer built around the stem; treatment time 4 hours; freeze-dried.

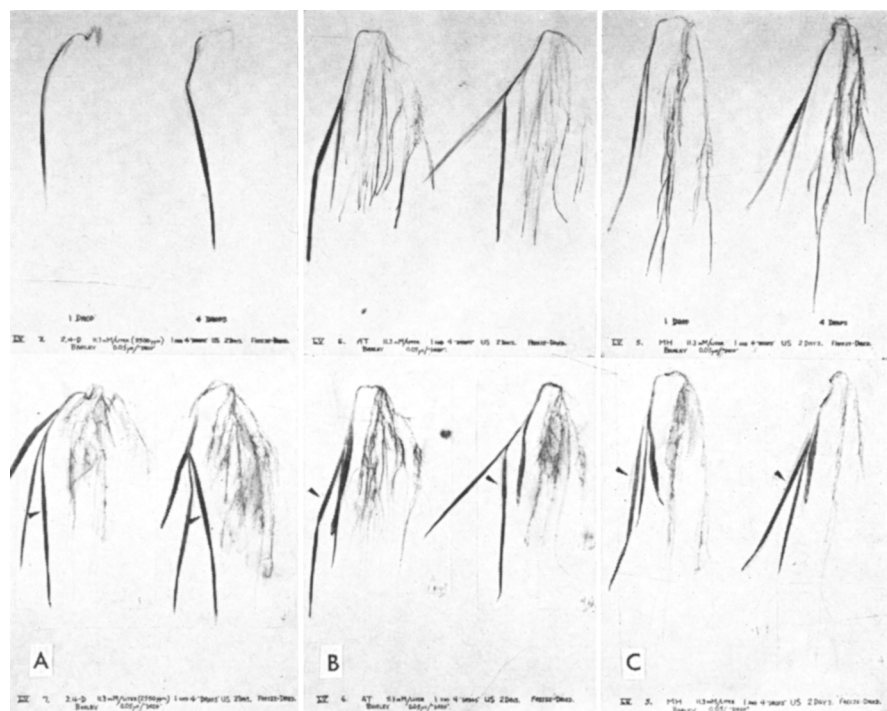


Fig. 13. Comparison of the mobility of 2,4-D* (A), ATA* (B), and MH* (C) in barley plants, autographs arranged in the order of increasing mobility of the chemical. The plants were grown on filter paper saturated with full-strength Hoagland's solution. Two levels of concentration were used for each chemical: 0.05 μc (left) and 0.2 μc (right) of carbon¹⁴-labeled chemicals adjusted to 0.45 mc per mM, applied to the middle of the second leaf. Treatment time 2 days; freeze-dried.

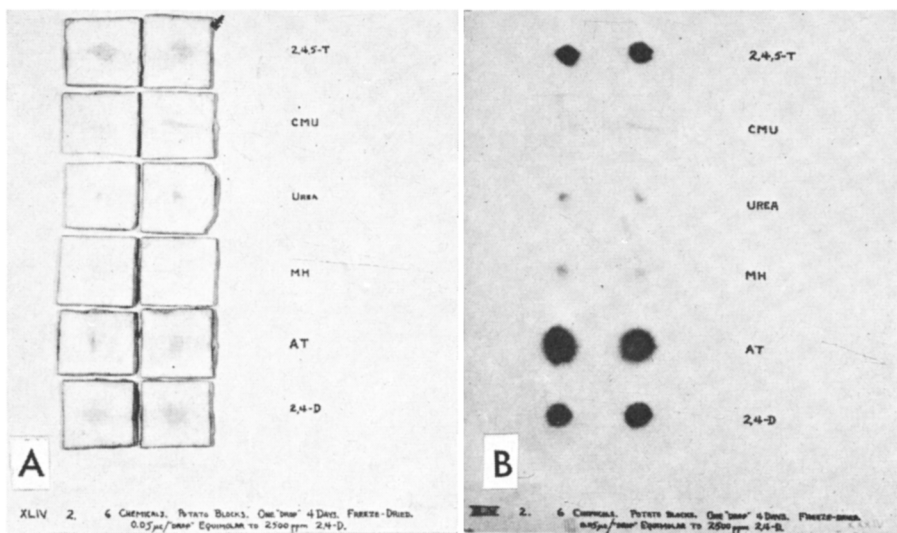


Fig. 14. Comparative mobility of six C^{14} -labeled chemicals in potato tuber blocks. *A*, slices from which the autoradiograph was taken (two identically treated slices for each chemical); *B*, autoradiographs showing the distribution of the chemicals after 4 days' treatment time in moist chamber at about 25° C. Each treatment was 0.05 μ c of chemical adjusted to 0.45 mc per mM, applied to a spot confined by a $\frac{1}{4}$ -inch lanolin ring in the middle of the potato tuber block from which a slice was taken at the end of 4 days' treatment time. The slices were freeze-dried.

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