HILGARDIA

TRANSLOCATION OF HERBICIDES

I. THE MECHANISM OF TRANSLOCATION: METHODS OF STUDY WITH C^{14}-LABELED 2,4-D

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THE MECHANISM OF TRANSLOCATION

Tracer studies with radioactive 2,4-D in bean, cotton, and cucumber plants confirmed previous findings that 2,4-D penetrates the cuticle of sprayed leaves, migrates to the phloem, and is transported in that tissue along with food materials in the plant. Freeze-dried plants were radioautographed to determine the rate and direction of translocation and the amount of chemical moved to various parts of the plant.

WILD MORNING-GLORY RESPONSE TO RADIOACTIVE 2,4-D

Radioactive 2,4-D was applied first to greenhouse-grown morning-glory seedlings to compare various formulations. Emulsifiable acid and heavy ester formulations proved superior to the older salts and light esters, and the addition of a surfactant was found to increase absorption and translocation.

Field studies revealed that 2,4-D moves most actively in plants growing in moist soil, that movement is most rapid and extensive in plants in the seedling stage, and that 2,4-D moves where foods are moving.

BRUSH SPECIES AND RADIOACTIVE 2,4-D

Further evidence of the correlation between 2,4-D movement and food movement in plants was provided by tracer studies in seven species of woody plants common to California: coyote brush, arroyo willow, wedge-leaf ceanothus, manzanita, toyon, blue oak, and live oak. In addition to detailed analyses of the tracer studies in these species, the following general conclusions are presented:

Contact injury is a major hindrance to the uptake and transport of 2,4-D.

Soil moisture and root growth are important to 2,4-D transport and response.

In evergreen species the chemical may move throughout the plant for many months, whereas in deciduous species it may move only for relatively short periods.

Different species require different treatments; a single application cannot be expected to control mixed brush populations under California conditions.

I. THE MECHANISM OF TRANSLOCATION: METHODS OF STUDY WITH C14-LABELED 2,4-D[1]

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INTRODUCTION

AN IDEAL HERBICIDE has long been regarded as one that would move from the foliage of weeds into the roots following application. Although in some situations it is preferable to treat weeds through the soil, such treatment involves possible reactions of the herbicide with the soil; even where such reactions do not occur, the dilution effects of the soil, which weighs over 3 million pounds per acre-foot, are an obvious disadvantage. Much time and effort have been put into the search for translocated herbicides, particularly for use against perennial weeds (Robbins, Crafts, and Raynor, 1942; Crafts and Harvey, 1949; Crafts, 1953a, 1953b).[3]

As discussed by Robbins, Crafts, and Raynor (1942), the acid-arsenical solution, sodium chlorate, and ammonium sulfamate, though translocated, left much to be desired. Introduction of 2,4-D changed the picture, for in this compound we have an organic chemical of extreme potency that moves readily through plants, apparently in association with food materials. And such are the selectivities of the cells and tissues of many plants that 2,4-D may be absorbed and moved through living cells of the foliage at concentrations that are lethal to roots. The treated foliage is not killed, at least not until enough of the toxicant has been moved to destroy whole root systems. In fact, use of excessive amounts of the chemical or inclusion of toxic additives in the spray solution may bring about such rapid killing of the foliage that movement into and injury of roots is lessened.

Not only is 2,4-D an excellent translocated herbicide; it is as well a useful translocation indicator for use in purely physiological studies. And 2,4-D carrying carbon 14 as a radioactive tracer is even more valuable. With the synthesis of 2,4-D carrying carbon 14, it seemed that the long search for an ideal translocation indicator was at an end, and that this complex function of plants would soon be completely elucidated. The following pages, however, will point out the great complexity of the problems involved and

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[3] See "Literature Cited" for citations referred to in text by author and date.

some of the reasons why a complete answer is still lacking. It is hoped that they will, as well, point out substantial progress, and that they may serve as an inspiration to further research on the problem. While it should be obvious to any well-trained plant physiologist that the ideal chemical that will kill every weed to which it is applied may never be found, it is heartening that so much has been learned about the processes of absorption and translocation and the conditions that promote them. At least, as a result of the many studies being carried on, we are able to explain why many applications of translocated herbicides fail. And to a limited extent, by describing the ideal conditions for absorption and translocation, and by fitting formulations to the needs of these two essential functions, much improvement has been made in the practice, particularly so far as certain hard-to-kill species are concerned. Experiments described in this paper have contributed to some of this improvement.

Translocation of water, salts, and organic compounds in plants was one of the first functions to arouse the interest of plant physiologists. And although the mechanics of absorption and translocation of water and salts is fairly well understood, the movement of organic materials has been the subject of much controversy. Even at the present time, after the introduction and use of the hormone indicators and radioactive tracers, agreement has not been reached on the basic mechanisms involved in organic solute movement. The phloem is undoubtedly the principal tissue involved in rapid longitudinal transport, but whether movement of solutes and the solvent water together in a stream constitute the essential mechanism, or whether the solutes move independently of the water and of each other, is not agreed upon.

Final solution of this problem is important in two ways. Once the basic mechanism has been described, demonstrated, and agreed upon, effort now being expended in attempts to settle the controversy can be turned to studies on the nature of the translocation process in various plants, and the role of various factors in modifying it. Once the latter studies have been made, the results can be immediately applied in the practical use of translocated herbicides in the field.

As an example of the nature of this problem, if 2,4-D, after absorption, is moved with food materials in the so-called assimilate stream, then for effective killing of roots, application should be made after the spring flush of growth when replenishment of the root reserves has started but before meristematic activity in the roots has ceased, for, as van Overbeek (1947) has stressed, 2,4-D is most active against cells in the meristematic state. On the other hand, if 2,4-D translocation is independent of food movement and dependent only on 2,4-D gradient, then application would better be made during the spring flush of growth while meristematic activity throughout the plant is at a maximum.

Much of the evidence now at hand indicates that translocation of 2,4-D and similar compounds takes place via the phloem in the so-called assimilate stream. The present writer is in sympathy with this view, and many of the experiments reported in this paper were designed to clarify the mechanism and substantiate this theory. The forces responsible for the functioning of this mechanism have been described (Münch, 1930, 1943; Huber, Schmidt,

and Jahnel, 1937; Huber and Rouschal, 1938; Rouschal, 1941; Crafts, 1951, 1953a, 1953b).

Briefly, the phloem is visualized as an extended and ramifying osmotic system in which exists, because of metabolic processes, a gradient, or gradients, of concentration of osmotically active substances (assimilates). Because the phloem system has a common source of water in the xylem, absorption of water in regions of high concentration where synthesis is taking place brings about increased hydrostatic pressure, whereas utilization of assimilates in growth, storage, and respiration results in lowered concentration and hence lowered hydrostatic pressure where these activities are going on. Because of the gradients of hydrostatic pressure so created, solution moves from regions of synthesis to regions of utilization through the sieve tubes of the phloem. And any solute present in this stream is carried with it. The accumulation of evidence that 2,4-D moves by such a mechanism in plants (Crafts, 1953b) substantiates this concept. Recent evidence that the protoplasts of the sieve tubes are plasmolyzable (Currier, Esau, and Cheadle, 1955) will have to be considered in any detailed visualization of such a mechanism.

METHODS

Many methods have been used in carrying on the studies. In the earlier tests, single-drop applications of 2,4-D solution were made to leaves of greenhouse-grown plants, and the expression of 2,4-D symptoms was used as evidence for the final distribution of the chemical. Later, the bean-bending test was used for studies on the role of pH, surfactants, and other factors in absorption and translocation of 2,4-D (Day, 1950, 1952).

With the provision of labeled 2,4-D, this material has been used as a tracer, and autographing has been the principal means of detection, though some counting has been done. Paper chromatography has also been used. Details of methods will be given with the description of experiments.

DETAILS AND RESULTS OF EXPERIMENTS[4]
Background Work

After comprehensive studies on translocation of 2,4-D in the bean plant were completed (Day, 1950, 1952), a study was undertaken of the distribution of absorbed 2,4-D in greenhouse-grown cotton plants of differing sizes and states of maturity (Clor, 1951). Treatment, in most cases, consisted of applying 0.01 ml of a solution of 2,4-D acid in 2 per cent ethyl alcohol to the center of a cotyledon or leaf. In general, the droplet was dry in 15 to 60 minutes.

From results obtained it seemed justifiable to assume that absorption of 2,4-D by different leaves was constant and that lack of appearance of symptoms on older plants was not caused by lack of absorption. When older plants were treated on different leaves, subsequent manipulation, such as pruning, which induced growth of axillary shoots, brought about expression of 2,4-D symptoms on these young growing shoots. As Gifford has since shown (1953), expression of 2,4-D symptoms results from changes in young leaves that

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[4] The research reported in this paper represents the efforts of a number of students as well as of the writer. The credit due these workers is indicated as the results are reported.

are undergoing rapid differentiation at the time of treatment or immediately after. Leaves that are mature at the time of treatment are not affected, and after a certain period of response leaves subsequently produced may show diminishing effects. The duration and extent of 2,4-D injury to cotton depend primarily upon dosage, and low dosages may give only temporary response followed by complete recovery.

When these experiments were planned it was hoped that injury to roots from translocated 2,4-D would be measurable. It proved impossible to measure the immediate effects on growing roots; although large doses actually killed the roots, small amounts translocated from treated leaves caused no measurable injury. In two experiments dosages of 16 μg to cotyledons and first leaves produced swelling of the hypocotyl just above the root zone.

Briefly, Clor's studies showed that about 8 hours were required for absorption by cotyledons of cotton and translocation through the petiole to the stem; treatment of young plants (3 to 4 weeks old) resulted in movement both upward and downward from lower leaves; movement from upper leaves (third and fourth) occurred mostly in an upward direction; with older plants (6 weeks or more) movement from upper leaves was acropetal, and no movement toward the apex occurred from lower leaves; downward movement of 2,4-D from the upper leaves of old plants, as well as upward movement from lower leaves, took place when sinks for food utilization were created above or below the treated leaves, respectively.

The growth substance did not move downward when the stem was steam-ringed below the treated leaf; it moved upward past a ring only when it was applied in high concentration. When the growth regulator was added to the nutrient solution it was readily absorbed by roots and carried to upper parts of the plant in the transpiration stream. Injury to the upper portions was proportional to the amount of 2,4-D added to the nutrient solution.

When cotyledons and first leaves of young plants were treated with 8 μg or more of 2,4-D, most of the substance moved down to the root and caused distinct swelling of the lower portion of the hypocotyl. When only the cotyledons of such plants were treated with 8 μg or more of 2,4-D, the substance moved to the root and leaked out to the nutrient solution where it was absorbed by the roots of untreated control plants growing in the same jar. The latter developed symptoms in their upper leaves about two weeks after the appearance of symptoms on the treated plants (fig. 1).

Clor's final conclusions from these experiments were that the movement of foliar-applied 2,4-D takes place primarily in the phloem and that the movement is dependent upon the movement of food materials in the plant and not upon the 2,4-D gradient. These conclusions are in agreement with Day (1950, 1952) and other workers in this field (Crafts, 1951).

Following Clor's work, a series of tests was conducted in the greenhouse,[5] designed to clarify the relations of pH of the applied solution, formulation of the compound, and presence of surfactants and buffers to absorption and translocation of 2,4-D and similar compounds. Work with the dinitro herbicides had proved that acidification of the spray solution materially enhances the toxicity, and chemical reasoning indicated that association of the dinitro-substituted-phenol molecules increases their solubility in lipoids and hence their permeation of the cuticle (Crafts and Reiber, 1945). Similar reason-

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[5] Conducted by Emelio Levi.

ing applied to data on 2,4-D indicated that with this toxicant too, acidification of the salts or use of the parent acid should enhance the toxicity (Crafts, 1948).

To substantiate this reasoning, five experiments were run, using the bean-bending test, to find the effect of pH on absorption of 2,4-D and 2,4,5-T. All five tests and one subsequently performed[6] were in agreement; between pH 10 and pH 2 there was a regular increase in the rate of absorption and the quantity absorbed. Of the six experiments, three used kidney bean plants, three used black-eyed peas. One involved 2,4,5-T, five were run with 2,4-D. Table 1 presents data from one of the tests. In this one, black-eyed peas

TABLE 1

ABSORPTION AND TRANSLOCATION OF 2,4-D AS INDICATED BY BENDING OF BLACK-EYED PEA PLANTS TREATED BY APPLICATION TO ONE UNIFOLIOLATE LEAF

pH of solution	Bending (in degrees) at end of:								
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.	28 hrs.
0.5	0	0	1.0	3.0	1.5	2.5	7.0	10.0	11.0
1.0	0	0	2.0	2.5	1.5	1.5	4.5	5.5	7.0
2.0	0	0	5.5	21.5	65.5	100.5	102.5	94.0	91.5
3.0	0	0	4.5	27.0	58.0	88.5	94.0	87.5	92.5
4.0	0	0	0.0	15.0	54.5	94.5	96.0	81.0	84.0
5.0	0	0	0	11.0	46.0	87.5	100.0	88.0	86.0
6.0	0	0	1.5	12.0	44.5	90.5	100.0	91.0	79.5
7.0	0	0	0	1.0	9.0	32.0	70.5	56.5	82.5
8.0	0	0	0	1.0	1.0	10.0	24.5	72.5	76.5
9.0	0	0	0	0	1.5	10.5	39.0	70.0	91.5
10.0	0	0	0	0	1.0	12.5	40.0	68.0	86.5

(*Vigna sinensis*) were used; the dosage was 2.5 μg of 2,4-D per treatment; the solution contained 0.1 per cent Trem 615 as a surfactant. Phosphoric acid and trisodium phosphate were used to buffer the solutions within the range covered by these compounds. Hydrochloric acid was used to obtain the pH values of 0.5 and 1.0.

As noted in the table, solutions of pH 0.5 and 1.0 were so acid that they produced rapid burning of the treated area. Evidently the cells were killed and translocation was inhibited. Though slight injury occurred at pH 2.0, it was not enough to inhibit absorption, and translocation was normal.

One notable exception to this pH relation occurred in an experiment where acetate, phosphate, and borate buffers were compared. Whereas a regular reduction in absorption occurred with phosphate-buffered solutions through the range 7 to 10, with borate buffer absorption was quite constant and about midway between that from phosphate-buffered solutions at pH 6 and pH 7. Evidently borate ion in some way enhances the uptake of 2,4-D through the cuticle. Figure 2 shows the relationship of these two absorption rates.

It has been recognized for years that inclusion of a surfactant in a 2,4-D solution increases its effectiveness. To confirm this view and to find the relation of pH to this enhanced uptake, 2,4-D acid and the sodium salt of 2,4-D were compared with and without surfactant. In this case Trem 615

[6] Conducted by Charles McCarthy.

was used at 0.1 per cent. Figure 3 presents the results of this test. There seems to be no question as to the increase in 2,4-D uptake when a surfactant is used, and apparently the effect is present with the sodium salt (pH 8.0 at the concentration used) as well as with the acid (pH 3.3 at the concentration used).

Our next experiment tested the relative effectiveness of several concentrations of two surfactant formulations, Trem 615, a nonionic polyhydric alcohol with ester linkage, and Multifilm L, a mixed formulation containing a hydrocarbon fraction and free and combined fatty acids. Figure 4 shows that between the concentrations of 0.05 and 0.8 per cent there was little difference in effects. In all subsequent tests a concentration of 0.1 per cent has been used for the surfactant content of 2,4-D test solutions.

Five screening tests on commercial surfactants were run in 1950 and 1951, four by Levi and one by McCarthy. Out of some 17 materials, Nonic 218 brought about the most rapid absorption of 2,4-D acid in two trials; it equaled an experimental material in one trial; and it was inferior to several materials in two trials. Since a nonionic surfactant is desirable to use in experiments with 2,4-D acid, because it has no effect on the pH of the solution, Nonic 218 has been selected for use in subsequent studies. Other nonionic surfactants with similar properties would probably serve as well. From much testing of surfactants it seems clear that they vary widely in chemical properties (Snell, 1949), in wetting ability (Currier, 1954), and in toxicity to plant tissues. And they are extremely variable in their effects on 2,4-D absorption—so much so that it is impossible to rate any number of them in the order of their effectiveness because successive experiments give differing values. Nonic 218 was used in many of the tests to be described because it has a definite composition (not blended), it is one of the most effective tried, it is low in toxicity, and it is nonionic and relatively nonreactive.

The 2,4-D molecule can be combined into a great number of compounds having widely differing properties. It has already been shown that the acid is more effectively absorbed than the sodium salt. According to chemical reasoning (Crafts, 1948), the alkyl esters should also be readily absorbed, but their partition from the cuticle and other lipoid fractions of the leaf is questionable. A great number of alkylamine and alkanolamine salts have been used. Again, the balance of charges in the molecule and its partition between lipid and aqueous phases should determine its initial absorption and subsequent translocation. Levi tested several 2,4-D compounds and formulations. The results of one test involving three alkyl esters are presented in figure 5. Probably the mobility of the ester molecules as related to their size and weight is the factor responsible for the differences shown. As a herbicide in the field, the hexyl ester is probably as effective as the methyl ester, if not more so, and the octadecyl ester will give a high degree of bending within 24 hours. The molecular weights of these esters are 234, 304, and 444, respectively. The value of this experiment lies in its demonstration that physical laws do apply to the plant functions being studied.

The next test compared several formulations of 2,4-D, namely the emulsified acid, the isopropyl ester, the propyleneglycolbutylether ester, and the butoxyethanol ester. As shown in figure 6, these molecules were all rapidly absorbed and translocated into the epicotyls of the bean plants.

A comparison of 2,4-D and 2,4,5-T acids was made using 10 μg per application and including 0.1 per cent Trem 615 as a surfactant. Figure 7, showing the results, indicates that 2,4,5-T enters the plant somewhat more slowly than 2,4-D. The relative molecular weights are 220 and 254, respectively. Theoretically, the chlorine substitutions in these molecules are lipophilic, and the third chlorine might hinder the partition of the molecule from the lipid phase of the leaf or cell surface into the aqueous medium of the living cell.

One further test was run comparing additional formulations of 2,4-D and including some 2,4,5-T formulations. Figures 7 and 8 present the results. Again the ester formulations are superior to the salts, and, in general, 2,4-D is absorbed and translocated somewhat more effectively than 2,4,5-T. It should be emphasized that the plants for these tests were grown at different dates in the greenhouse with only natural light, no control over humidity, and only rough temperature regulation. Comparisons within a test are valid, but different tests should not be compared. In the tests reported in figures 4, 5, and 7, 5 μg of 2,4-D, on the acid-equivalent basis, constituted the dose. Surfactant was included in all test solutions.

Although 2,4-D was the best translocation indicator that had been introduced into plant physiological research, it still left some questions unanswered. For instance, is it 2,4-D per se or is it a breakdown product, an association complex, or some secondary compound produced in the plant as a result of the stimulus of 2,4-D that causes bending, inhibition of leaf development, and formative effects? Is the 2,4-D translocated as the acid, the ion, or as a sugar ester when it moves with assimilates in the plant? When 2,4-D with carbon 14 in the molecule was synthesized, a more effective tool was at hand for studying both the biophysical and the biochemical aspects of 2,4-D action. With the receipt of an Atomic Energy Commission contract in June, 1951, studies using carboxyl-labeled 2,4-D were initiated, and the primary function of this paper is to report on some of these studies.

Studies Using C^{14}-labeled 2,4-D

Mode of Killing and Treatment Periods. With carboxyl-labeled 2,4-D (hereafter referred to as 2,4-D*) obtained from Tracerlab (specific activity of 1.24 mc per mM), a stock solution was prepared to give 50 μg of 2,4-D acid per 0.01 ml of solution. Because the bean-bending test had proved very erratic during the winter in the greenhouse, a bank consisting of twenty-four 8-foot slim-line tubes and sixteen 75-watt incandescent bulbs was installed to provide supplementary illumination. Fairly reproducible bending could be obtained during cloudy weather if these lights were used.

Our first experiment was exploratory. Bean plants were grown in 4-inch pots until the unifoliolate leaves were fully expanded. They were treated, usually in the morning, by placing one drop of 2,4-D* solution inside a lanolin ring at the base of the lamina of one unifoliolate leaf. In this experiment a 50-μg dosage was used, consisting of a 0.01-ml droplet of a 0.5 per cent 2,4-D* solution in 50 per cent alcohol and containing 0.1 per cent Nonic 218.

At the end of the specified treatment period the plants were removed from the pots, their roots were washed free of soil, and they were killed.

This first experiment concerned the method of killing the plants at the end of the experimental period of treatment, and the time required for auto-graphing. Some of the plants were killed by quick-freezing between blocks of dry ice and dried between hot, dry blotters; the remainder were dried between hot, dry blotters by the method commonly used in preparing her-barium specimens. Exposure periods of the dried plants on the film were 1, 2, 4, and 8 weeks. Kodak no-screen X-ray safety film was used.

Killing of the plants started after a 4-hour period of treatment, and all plants were drying in the press within 2 hours. The dried plants were placed on the X-ray film and bundles of the films with the plants in position and separated by blotters were bound as in an ordinary plant press and placed in the dark for the requisite exposure period. The films were developed in Kodak X-ray developer and replenisher and fixed with the Kodak pre-pared fixer.

As for exposure period, one week produced good autographs, 2-week auto-graphs had somewhat more detail, 4-week autographs were somewhat darker but contained no more detail, and 8-week exposures were excessive, produc-ing very intense images that were somewhat blurred at the edges. With more experience, we have found that use of less radioactive 2,4-D in the initial treatment (5 to 10 μg), and 4-week autographing give the best results under average conditions.

This first set of autographs had many blurs and spots that proved to result from leakage of the radiation through the blotters used to separate the films. We now separate films with separators made by cementing together two blotters with a sheet of aluminum foil between them. No further trouble with leakage has occurred.

The greatest differences shown by this experiment were between the plants killed by quick-freezing and drying compared with those killed by the slower drying procedure. The quick-killed plants gave sharp, clear autographs with the tracer well distributed throughout the petiole of the treated leaf and the stem, and, in almost every plant, present well down into the root system. In many plants it was present in the petiole and main veins of the opposite leaf. In no case was there a high concentration in the terminal bud.

In the slowly killed plants (killed between hot blotters) the concentration of tracer was less in the veins of the treated leaf and in the roots of the plants. It was not present in the petiole and veins of the opposite leaf, but it was highly concentrated in the terminal bud. These differences are illus-trated in figure 9 *A* and *B*.

At the time that these experiments were conducted (January, 1952), we were at a loss to explain these differences. Now, after much work, including tests using freeze-drying, we offer the following explanation. With the dry-ice treatment all liquid in the plant is rapidly frozen. The plants come from the dry-ice treatment stiff and extremely brittle. As the tissues thaw they become extremely permeable and 2,4-D from the treated area can diffuse into the xylem and there be carried to any region where drying is taking place. This probably explains its presence in the untreated leaf and its very extensive distribution throughout the roots of the treated plants. From the standpoint of the use of radioactive 2,4-D as a tracer of phloem transport, this constitutes an artifact and gives a distorted picture that must be con-sidered in interpreting autographs of plants killed by this method.

In the plants killed by slow drying, there is undoubtedly a considerable extension of the period of treatment during which phloem transport continues. This is shown by the high intensity of tracer in the terminal buds. On the other hand, the restricted distribution in roots, the lack of tracer in the untreated leaf, and the intensification of the image of the terminal bud all indicate that in this killing procedure restriction of transport to the phloem indicates normal movement of 2,4-D*. The undetermined extension of the treatment period is the most serious drawback to the method.

Dosage Series. A short exploratory experiment using a 2-hour treatment period, dosages of 1, 5, 10, 20, and 50 μg of radioactive 2,4-D, and killing by the quick-freezing method proved that fewer than 5 μg of tracer were insufficient under the conditions of the experiment, 10 and 20 μg gave excellent autographs, and 50 μg were about a maximum for obtaining clear autographs.

Time Series. Our next experiment was an extensive one designed to explore a wide range of treatment times. The dosage was 50 μg and the times were as follows: ¼, ½, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 36, 48, 72, 96, 120, and 144 hours. The quick-killing method was used, and in interpreting the results this has to be taken into account.

The 15-minute test in this experiment showed little movement beyond the treated spot (fig. 10); the 30-minute test showed the tracer present in the roots of the plants, but its presence in the opposite leaf indicates that this is probably the result of xylem movement after killing (fig. 11). Day (1952) found that absorption and movement into the phloem required about an hour and that seems to be true in this case also (fig. 12). In the 2-hour test, four out of five plants had 2,4-D* in the roots; in the 3-hour test, five out of five had 2,4-D* in the roots and four of the plants were bent (fig. 13); however, in 4- and 5-hour tests, one plant in each lot failed to translocate 2,4-D* into the roots. Beyond the 5-hour treatment period all plants had 2,4-D* in their roots, and beginning with the 6-hour period, 2,4-D* was present in the terminal buds, increasing in concentration with increasing treatment time (fig. 14). Since all these plants were quick-killed, they should give a critical measure of the time required for movement into this meristematic tissue. Apparently in the first experiment (killed by drying between blotters) the large difference in intensity of the images of the terminal buds represented movement into the bud after the plants were removed from the soil and placed in the press. The image intensity of the terminal buds in the plants dried without quick killing corresponded with that of the quick-killed plants of the time experiment having a treatment period of 24 hours.

In this treatment-time experiment, bending of the epicotyls was evident in two out of five plants treated for 2 hours; in four out of five treated 3 hours, and to varying degrees in nearly all plants treated for longer periods. Wherever there was bending, 2,4-D* was present in the epicotyl.

An outstanding result in this time series was the gradual loss of intensity in the roots, treated leaves, and opposite leaves after 24 hours. This probably represents actual loss of the C^{14} in the form of $C^{14}O_2$ from the plants as found by Weintraub et al. (1952). Figures 15, 16, and 17 show autographs of some of the plants described. Figure 18, *A* and *B*, shows an effect noted in the ½-hour and 1-hour tests. Our interpretation of this phenomenon is that there was leakage through the cuticle directly into the

epidermis and mesophyll cells and thence into the xylem, and distribution
to the leaf tip in the transpiration stream. After 1 hour, such 2,4-D* should
be picked up and moved out via the phloem. The fact that this one-way
movement is not shown in any of the longer treatment times seems to justify
this view.

Soil Moisture. In his preliminary studies on the "bean test," Day (1950,
1952) found that it was necessary to water his plants fairly soon before
treating them because they would not bend if the soil moisture was approach-
ing the permanent wilting percentage. To find out if this was caused by
lack of absorption and translocation or just by lack of the bending response,
an experiment was set up to determine the relation of soil moisture to the
movement of 2,4-D*. In this experiment plants grown as usual were watered
48, 24, and 3 hours before treatment. The dosage was 50 μg of 2,4-D* with
Nonic 218; treatment time was 2 hours. The roots were cleaned as well as
possible without washing. As the plants were harvested they were cut into
(1) treated leaf plus petiole, (2) untreated leaf plus petiole, (3) epicotyl
and hypocotyl, and (4) roots. The fractions were quick-frozen and dried
in the usual fashion. At time of harvest the plants watered 3 hours previ-
ously were bent; the other two groups had straight epicotyls.

In studying the autographs of this experiment, three features were promi-
nent: (1) in no case was there any 2,4-D* in the opposite leaf or in the
roots; (2) 2,4-D* was present in the stems of straight as well as bent plants;
(3) translocation of 2,4-D* into the hypocotyls of the plants having ample
soil moisture was more prominent than in those from which water had been
withheld, but the differences were small. Evidently dissection of the plants
had eliminated the artifact of xylem transport experienced in the other tests.

A second experiment on the role of plant-water relations in 2,4-D* trans-
port involved three sets of plants. All plants were grown as usual except
that water was withheld for 24 hours before treatment. In this experiment
the dosage was 50 μg of 2,4-D* with 0.1 per cent Nonic 218; treatment
period was 4 hours. Immediately after application of the 2,4-D* five plants
were placed in a humid chamber with a glass top, five were left under the
bank of lights in the open greenhouse, and five were placed outside on the
south side of the greenhouse in the bright sun. This latter constituted a
low-humidity treatment. These plants were harvested whole, quick-frozen,
and dried by the usual method. At the time of harvest the plants from the
humid chamber were bent, one of five from the greenhouse bench was slightly
bent, and the five from out-of-doors were straight. Figure 19, *A, B,* and *C,*
shows the autograph of one plant from each environment. These were chosen
to illustrate the average condition. All plants had 2,4-D* in the untreated
leaves and in the roots. However, those from the outside had the greatest
intensity of 2,4-D* in the epicotyl and hypocotyl; those under greenhouse
conditions and those from the humid chamber slightly less. The 2,4-D* had
moved into the epicotyls and hypocotyls of unbent as well as bent plants.

Thus, in two separate experiments bending was shown to be a function
of the water balance of the plant as well as an indication of the presence of
2,4-D*. Because this last experiment had a treatment time of 4 hours, pres-
ence of 2,4-D* in the roots probably represented phloem transport; as in the
stems, the amount in the roots was less in the plants under dry conditions, but

the differences were not great. The velocity of movement was apparently the same in all three conditions, but the amounts moved depended upon environmental conditions. One factor of importance here was the rate of drying; plants in the moist chamber retained the 2,4-D* in solution for much longer periods than did those in the open air. Figure 19, *A,* showing the high intensity of radioactivity in the apical portion of the treated leaf, reflects this favorable condition for prolonged absorption.

Placement Series. The next experiment concerned the placement of the droplet in treating the bean plant. Day and Levi, in earlier tests, had shown that treating young, rapidly expanding leaves resulted in little or no bending. And Day (1950) showed that placing the droplet over the main veins at the base of the lamina gave the most pronounced bending response. Treating the underside of the leaf was more effective than treating the upper surface, but this imposes a practical hardship; since treatment on the upper surface gives satisfactory response, this method has been made standard practice.

McCarthy performed two experiments on placement of the droplet on the leaf or bud and one on application to roots. In the leaf treatments, droplets were placed (1) at the base of the lamina over the main veins, (2) at the same place on both leaves of each plant, (3) in the center of the leaf over the midrib, (4) over the midrib at the apex of the leaf, (5) on one spot at the edge of the leaf, and (6) on five spots around the periphery of the leaf. There was also a treatment in which application was made on the terminal bud. Treatment time was 4 hours; some treatments were at 50 μg, others at 20 μg. Killing was by quick-freezing.

Plants treated through the roots were grown in Hoagland's solution until ready for the experiment. They were then removed; their roots were rinsed in distilled water and placed in a 1 ppm solution of 2,4-D* for periods varying from $\frac{1}{2}$ hour to 8 hours. They were removed, rinsed twice in distilled water, quick-frozen, dried, and autographed.

Plants treated at the base of one or both leaves showed the same pattern of distribution of radioactivity, namely, movement throughout the stem and well into the roots. Intensity in the stems was high (fig. 20 *A* and *B*). Treatment over the midrib in the center of the leaf was equally effective during the 4-hour treatment period (fig. 21). Treatment at the apex of the leaf, on one basal lobe, or at five different positions around the periphery resulted in about the same distribution into the stem and roots, but in all cases the intensity of the autographs was materially reduced (figs. 22 *A, B;* 23).

Plants with their roots in 1 ppm 2,4-D* for 4 and 8 hours had the tracer throughout the roots, and some, but not all, had it up the stems and into the leaves. Evidently this compound is not rapidly absorbed and concentrated in bean plants, and more concentrated bathing solutions will be needed to give good autographs. The problem is to get absorption from solutions that will not cause serious injury of root cells. This was accomplished in later tests.

Experiments involving the presence or absence of wetting agent proved that these additives enhance 2,4-D* absorption and give more intense autographs, other factors being the same. Distribution of the tracer was not changed. Other wetting-agent tests showed that concentrations of 1.0 and 0.1 per cent made no difference in concentration or distribution of absorbed 2,4-D*.

Transport from Different Leaves. One experiment with bean plants attempted a study of transport from different leaves. The plants were allowed to grow until the two unifoliolate leaves and one trifoliolate leaf were fully expanded. Applications were of 5 μg of 2,4-D* in 50 per cent alcohol with 0.1 per cent Nonic 218. The treatment period was 2 hours. The plants were quick-frozen and dried between blotters.

Treatments on the terminal buds and on young trifoliolate leaves resulted in no movement from the treated regions. On the second trifoliolate leaf, treatment resulted in movement only within the leaf. On the first trifoliolate leaf, treatment resulted in movement into the roots in low concentration; on unifoliolate leaves, movement into roots was more prominent; the tracer also moved out into the young stems and leaves at the tips of the plants.

Several attempts to check on movement during thawing and drying, by fractionating the plants in the frozen state, indicate less absorption when the plant is so fractionated, but loss of sap from the thawed plant parts when they are pressed between blotters obscures the results.

Killing and Drying Methods. A number of experiments have been conducted attempting to evaluate the artifact of xylem transport during drying of quick-frozen plants. In these, freeze-dried plants have been compared with plants dried between warm blotters after quick-freezing. McCarthy performed one such experiment, and though he was not certain that the plants had not thawed during drying, through inadequacy of the freeze-drying equipment, he did find that much less 2,4-D* was present in the freeze-dried plants. The treatment period was 4 hours, and distribution was about the same in both sets of plants.

Later freeze-dry experiments[7] confirm the finding that less 2,4-D* is moved when the plants are dried in the frozen condition. The first freeze-drying experiment compared four freeze-dried plants with four that were cut into leaves, petioles, stem, and roots while frozen and subsequently dried as usual, and four that were quick-frozen and dried between warm blotters. All plants were treated for 1 hour with 5 μg of 2,4-D*. The fractionated plants were placed between sheets of filter paper, and the spots on these sheets were autographed. The freeze-dried plants were treated for 1 hour, quick-frozen, placed between sheets of $\frac{1}{8}$-inch mesh hardware cloth, and immediately covered with pulverized dry ice. Four plants were stacked and placed within a precooled steel vacuum chamber containing calcium hydride, and the chamber was then placed within the freezing unit of a domestic refrigerator. A vacuum pump attached to the vacuum chamber was started and allowed to run throughout the drying period of 5 days. A thermocouple inside the vacuum chamber was used to check on the temperature, which did not rise above –7°C during the 5-day period. The vacuum maintained after the dry ice had sublimed averaged around 3 mm of mercury. The plants came out of the chamber in a very dry condition.

Autographing revealed the intensity in the stems to be very low, and while the roots could be seen on the autographs they were at the lower limit of visibility. The fractionated plants had even less intensity in the stems, but autographing of the filter papers between which they were dried showed appreciable leakage from the cut ends. The quick-frozen, blotter-dried plants had more 2,4-D* throughout their stems and roots. Considering that the

[7] Conducted by James E. Pallas, Jr.

treatment lasted only 1 hour, the period of time that Day (1952) had found necessary for absorption by the leaf, these results are to be expected. They prove that the higher concentrations of tracer found in blotter-dried plants, and the very rapid distribution found in certain experiments (Crafts, 1953a, also fig. 12), are the result of the killing process, or, more specifically, the thawing and drying following quick-freezing. This places a definite limitation on the interpretation of autographs of plants killed by such methods. Later experiments with treatments lasting as long as 24 hours show that 2,4-D* is present in the roots in 4-hour-treated plants, and in 24-hour treatments the roots autograph very strongly as shown in figures 25 A, B, and 26.

Cotton Plants. Because cotton is highly sensitive to 2,4-D and gives pronounced formative effects, it was used for translocation studies by Clor (1951) as reported above. In order to check on Clor's results, McCarthy performed three experiments on young cotton plants, using the labeled 2,4-D*.

The first used plants 2, 3, 4, and 7 inches high; treatment was with 10 μg in some instances and 50 in others, the droplets being placed on one cotyledon in each case. The treatment period was 4 hours and killing was by quick-freezing. Results were as follows: (1) in small plants (2 inches) with cotyledons still expanding, outward movement was slight; (2) with larger plants with cotyledons fully expanded and green there was strong movement into the stem and root (fig. 27); (3) with plants somewhat larger and the true leaves starting to expand, transport was strong and some 2,4-D* moved into the expanding leaves (fig. 28); (4) in the case of 7-inch plants with four leaves expanded above the cotyledons, translocation from a cotyledon was outward and both downward to roots and upward into expanding leaves (fig. 29).

In the next experiment, where treatment was on the second and fourth expanded leaves, movement from the second leaf was both downward and upward (fig. 30), whereas movement from the fourth leaf, which was still expanding, was very slight in the downward direction (fig. 31).

In a third experiment, where the treatment time was 2½ days, movement from the cotyledon of 4-inch plants was both downward into the roots and upward into expanding leaves. When application was upon the second expanded leaf, it was both downward into the hypocotyl and roots and upward into young expanding leaves.

In general, the results confirm Clor's studies (1951) indicating that translocation of 2,4-D* accompanies assimilates in their movement from regions of synthesis to regions of utilization.

Cucumber Plants. For further confirmation of this translocation pattern a parallel experiment was run with cucumber seedlings.[8] Seeds were started in sand and, after one week, transferred to full-strength Hoagland's solution in aerated culture tanks. Growth was rapid, and at 10 days the cotyledons were fully expanded and green; at 14 days the first true leaf was expanded; at 17 days the second true leaf was expanded and the plants were of correct size to autograph on 10 × 12-inch film. Applications were made in four locations: cotyledon, first leaf, second leaf, and third leaf. Treatment times were ¼, ½, 1, 2, and 4 hours. Dosage was 5 μg of 2,4-D* with a specific activity of 1.24 mc per mM. The 2,4-D* was dissolved in 50 per cent alcohol at a concentration of 500 ppm, and the solution contained

[8] Conducted by James E. Pallas, Jr.

0.1 per cent Nonic 218. A lanolin ring was used to confine the droplet. At the end of the treatment time each plant was removed from the culture solution, quick-frozen, and dried between blotters.

Movement in these plants was rapid. Although the possible artifact of xylem movement has to be taken into account in the ¼- and ½-hour treatments, in the 1-, 2-, and 4-hour periods movement into the roots from the cotyledon and first leaf was prominent, from the second leaf it was much less, and from the small third leaf it was very slight. When movement from the young third leaf was prominent, the petiole and adjacent stem were very-dense, indicating possible spread of the treating solution from the leaf onto the stem. The lanolin ring used to confine the solution is difficult to make on such young leaves as they are still folded and very hairy.

All treatments were made in duplicate and figures 32, 33, 34, and 35 show one of each of the 1-hour treatments. It is very evident that translocation into the roots is much more prominent from the cotyledon and first-leaf treatments. This result is borne out by the other autographs of this experiment and serves to indicate again that 2,4-D* moves with assimilates in this species and not along an independent gradient.

DISCUSSION AND CONCLUSIONS

The foregoing presentation of experimental results of studies with radioactive 2,4-D as a translocation tracer emphasizes the difficulties involved in this type of research. Despite the many virtues of this material as a tracer, unless the experiments are carefully designed and correctly interpreted, one can be led into false conclusions. Probably the best way to indicate the difficulties is to analyze carefully the many possible reactions that may result from the simple procedure of placing a droplet of the tracer solution on a leaf of the test plant.

Colwell (1942) showed that when a large area of a squash leaf is wet with an aqueous solution of radioactive phosphorus, the tracer solution is drawn into the leaf and down through the petiole and stem via the xylem. To avoid this, he had to limit the size of area treated so that the water in the applied solution was evaporated from the treated leaf. Under these conditions, the movement of the tracer out of the leaf was confined to the phloem as indicated by its failure to move through a steamed petiole. Biddulph and Markle (1944) showed that even though the whole vein including the xylem was cut to introduce radiophosphorus into the cotton leaf, by limiting the absorption time of the test solution to 5 minutes, outward transport was limited to the phloem. Here, the method of cutting the vein so that initial movement of the test solution was toward the periphery of the leaf, and the time limitation in absorption were so controlled that backflow through the xylem was prevented. Carrying the analysis further, if a test solution is applied to a leaf with intact cuticle having no stomates in the treated area, absorption through the cuticle should confine the tracer to the fat-like cuticle and such cell phases as could receive it by partition. Theoretically, such a tracer could be confined to living cells of the epidermis and mesophyll and could move via these cells to the phloem for transport from the leaf. In short, uptake and transport would be confined to the symplast (Münch, 1930).

When one considers that evaporation of water from the leaf by trans-

piration takes place from moist cell walls of the mesophyll, and further, that the continuous-wall phase, the apoplast, is a hydrated colloidal medium in dyamic equilibrium through imbibitional forces with water in the xylem, it is evident that any source of water at atmospheric pressure that comes into physical contact with the apoplast at any point will serve as a source for uptake and flow. Hence, any aperture in the cuticle layer, or any open stomates, or even any cuticle surface that is not completely isolated from the hydrated apoplast may serve as an opening through which liquid may reach the xylem. However, this phenomenon is probably relatively unimportant except where there is severe injury to the leaf such as occurs following quick-freezing. While open stomates are not ports of entry for aqueous solutions under normal conditions, when the test solution being applied to a leaf contains alcohol and a surfactant, as was the case in many of the solutions used in the experiments herein described, entry may be effected, and once the tracer is inside the stomatal chamber it can reach the xylem and move in it. Lambertz (1954) has recently shown that the cuticle of many species is pierced by innumerable plasmodesmata. These, too, could serve as ports of entry for chemicals.

The upper surface of the bean leaf has stomates and so, with this plant, the cuticle cannot bring about isolation of a test solution containing surfactant. However, an application of only 0.01 ml of solution, which often dries within 15 minutes, would not seem enough to allow backflow from the leaf via the xylem. That this is true is shown by the pH studies (table 1, fig. 2), which indicate that 2,4-D enters the plant across a lipoid barrier—the intact cuticle of cutinized or suberized mesophyll walls (Scott, 1948). If this is true, the tracer, to move as it does, must be taken up by living cells and moved out of the leaf via the phloem. The many evidences for movement with assimilates (see review in Crafts, 1952) strengthen this view.

The above conclusions apply to intact living plants as used in the bean-curvature test. Proceeding now to tests with the radioactive tracer, when carbon 14 is used, as it was in the 2,4-D* used in the present work, detection or measurement of the radiation requires extraction and counting, or autographing of the plants. Both of these processes require killing the plants and this presents difficulties. In normal greenhouse plants the xylem operates under a greatly reduced pressure, and the phloem under a fairly high positive pressure. Therefore, when a plant is cut, movement within these systems is extremely rapid (Kennedy and Crafts, 1930; Crafts, 1936), and the fractions of the cut plant do not represent the state of the intact one. For this reason, any technique involving cutting or grinding is objectionable. Additional objections to the counting method, as far as this problem is concerned, are the difficulties with extraction and the laboriousness of preparation. Counting was actually used in our studies to check concentrations found in autographs, to analyze extracts that were chromatographed to identify 2,4-D, and to calibrate test solutions. For the bulk of our work, however, for the reasons noted above, autographing was used.

In order to detect and measure the radioactive tracer in a plant by the autographing method, it is necessary to kill and dry the plant so that it may be placed on X-ray film to give the exposure necessary. We used drying between warm blotters, quick-freezing with dry ice, followed by drying between blotters for our initial studies. The objection to the first method is

the time required for killing the plant and stopping all physiological processes. Our tests indicated that the heating probably speeded up the processes of translocation until drying of the tissues finally stopped them. This means that the treatment time was prolonged for an unknown period.

The quick-freezing method gave excellent, sharp autographs and a number of experiments were completed (Crafts, 1953a) before it was fully appreciated that transport was considerably more extensive in these plants than in plants in which all activity was stopped immediately (freeze-dried plants). A careful analysis explains why this is so. When the plant is quick-frozen, all moisture in the tissues is solidified and the plant comes from the treatment stiff and rigid. Soon it thaws, and after a few minutes between filter papers backed by warm blotters it is perfectly flaccid. Pressing will then remove considerable quantities of sap from the tissues. In the case of 2,4-D*-treated plants, this sap is contaminated and will produce an autograph. Figure 36 shows a normal autograph and figure 37 shows the autograph produced by placing the filter paper backing upon a film for four weeks.

Freezing, in this case, kills the cells of the plant and renders them completely permeable. As the plant thaws, solution moves through the tissues to satisfy gradients established by drying. And apparently 2,4-D* in solution in the sap of mesophyll of treated leaves may move into the open xylem tubes and translocate rapidly to the tip of the treated leaf, down the petiole to the stem and root, and via the anastomosed bundles of the nodes to the opposite leaf. Careful study suggests that a strong image of the veins of the opposite (untreated) leaf of bean indicates such xylem transport. A light, blurred image may result from 2,4-D* carried in through the phloem when conditions are right for import of assimilates. Movement from the node subtending the unifoliolate leaves upward into the bud or young expanding shoot is also phloem-limited, at least while this young shoot is only about 1 cm in length.

From this analysis it is evident that with the killing of the cells the symplast is destroyed as an osmotic system and the apoplast becomes continuous throughout the plant. Liquid within cells, or within tissues such as the phloem and xylem, becomes free to follow hydrostatic gradients not related to the previous functioning of the living plant but related only to the drying process. Distribution of a tracer by this system therefore becomes an artifact that may not be directly related to normal transport and hence is unreliable as an index of normal function. How serious is this as an error in the experiments previously reported (Crafts, 1953a) ?

Obviously, absorption through the leaf surface, migration across the mesophyll, and transport through the phloem are three distinct processes, each requiring a definite time. According to Day (1950, 1952), absorption and migration to the phloem require about 60 minutes. Careful study of many autographs made in the studies being reported indicates that from 1 to 2 hours were required in these tests. Translocation within the phloem is known to be quite rapid (Crafts, 1951; Biddulph, 1954). Day found an average velocity of about 50 cm per hour. This would indicate that it takes about 10 to 15 minutes for 2,4-D* to move from the treated leaf to the epicotyl and two to three times as long for it to move throughout the roots. From this it seems evident that the movement indicated in figures 6 and 8 of

an earlier paper (Crafts, 1953a) was brought about by xylem transport during drying and not by phloem transport during the 30-minute treatment period. The presence of 2,4-D* in the petiole of the opposite leaf is evidence for this same effect. While the autographs in figures 9 to 13 may also contain this artifact, it evidently is not responsible for the differences shown because all plants were killed by the same method. Careful studies of several hundred autographs indicate that, with the exception of the untreated leaf, distribution of 2,4-D* in plants given treatment periods of 2 hours or more is accurately shown in autographs. It is in the short-time exeriments that the artifact is prominent, and autographs of plants in these experiments should be viewed with this in mind. In this connection it should be pointed out that the test involving the plant shown in figures 6 and 8 of the 1953 paper included five plants only two of which showed such extensive movement. In the other three the 2,4-D* had reached only into the petioles. If Day (1950, 1952) was correct in concluding that absorption and migration to the phloem take about 60 minutes, and this conclusion was based on well replicated tests, then even these other three plants give an erroneous picture. The problem here is plant variability, and the most certain thing about these tests is that the plants are quite variable. Plants dried in the frozen condition should give a true picture of phloem transport of the tracer. As shown by figures 25 and 26, transport into the roots of bean plants occurs in 4 hours, and distribution is complete in 24 hours.

Turning now to the tests involving longer treatment periods, it seems evident that these studies with 2,4-D* substantiate the view that this chemical can penetrate leaf tissues and move readily with assimilates in the phloem. Several practical applications can be made of these findings. First, with regard to penetration, the studies on pH and the tests with esters and with surfactants all show that where translocation of the 2,4-D is desirable, formulation is of extreme importance. The pH studies show that somewhere in the leaf a lipoid barrier must be passed, and here the fat solubility of the acid and esters has a bearing. The comparative test on esters points out the influence of chain length on mobility in the lipoid phase and on partition characteristics on the aqueous side. And the surfactant studies show the benefits of reducing the interfacial tension at the leaf surface to allow very intimate contact of the herbicide molecules with the cuticle. Since adsorption and penetration of the 2,4-D depend on this intimate contact, a suitable surfactant is a necessary constituent of a 2,4-D formulation that is designed for penetration and translocation (Orgell, 1954). One gains the impression, in talking to salesmen and company representatives, that the inclusion of surfactants has been mainly aimed at low cost and convenient emulsion stability. These studies indicate a much more important function and one wonders if this has been kept in view in testing surfactants for 2,4-D formulation.

When the differences in penetration and movement of different esters are studied, one wonders again if these factors have been sufficiently considered in testing esters. This is particularly pertinent with respect to the heavy esters now being formulated. Hundreds of esters and hundreds of surfactants could be and actually are being synthesized.

Some of the newer emulsifiable acid and heavy ester formulations are killing weed species that simply would not respond to the old salt and

alkyl ester products. This proves the importance of formulation and empha-
sizes the role of balanced solubility, lowered interfacial tension, and proper
molecular configuration in herbicidal function. And after the ideal mix-
tures for the common weed species have been found, there remains a host
of special problems relating, for example, to odd tree and brush species,
to riparian vegetation, to some of the legume species, such as aruba, broom,
and gorse, to fern and cycad species in the tropics. After more than 100
years of agricultural research we are still investigating the use of simple
chemicals like nitrate fertilizers. Undoubtedly it will take as long or longer
to work out all of the details in the most effective use of 2,4-D. And as the
studies reported herein show, the use of radioactive isotopes as tools in this
research will not solve these problems overnight. Although these tools are
time-saving and convenient, they must be used with caution, and with a
full realization of all possible sources of error.

In addition to formulation problems, there remain those relating to the
physiology of the treated plants. And in the case of selective action, the
physiology of both weed and crop species must be considered.

From the studies on cotton and cucumber plants it is evident that ap-
plied 2,4-D may be transported to the roots or to the shoot, it may move
to both root and shoot, and under certain conditions it may not leave the
treated leaf. When the whole plant is sprayed, the problem is somewhat
simplified. Will the bulk of the applied chemical go to the roots, will it go
to the shoots, or will it stay in or on the treated leaf? Obviously, this is im-
portant in the control of perennial weeds.

In considering the movement of assimilates in perennial weeds, it has
been found that, in the early spring, carbohydrate reserves in the roots
move up to provide for the nutrition of the growing shoots (Robbins, Crafts,
and Raynor, 1952). Spraying young foliage with 2,4-D has proved com-
pletely ineffective in killing the roots. About the time that shoot buds are
forming on many perennials, the assimilate stream moves from the foliage
to the roots, and the roots are still actively growing. At this time and until
early blossoming, spraying is usually very effective in producing root kill.
During late blossoming and seed maturation the assimilate stream moves to
the roots, but these are mature and apical growth often has ceased. Trans-
location of 2,4-D may be effective, but the mature root tissues are not killed.
This tells us why 2,4-D treatments must be properly timed, and the work
with radioactive 2,4-D has clarified the picture.

Concerning application methods, the experiments illustrated in figures
20, 21, 22, and 23 show that the actual size and location of spray droplets
can be important. This is reflected in the common recommendation that
sprays be applied to weeds in the form of coarse droplets; particularly is
this true in treating brush (Fisher, 1952). Coarse-droplet application is
essential only in application of translocated herbicides. This contrasts with
the recommended method for applying contact herbicides and certain in-
secticides where complete coverage is essential.

Concerning the mechanism involved in the movement of 2,4-D in the
phloem, recent proof that the sieve tubes in this tissue retain the property
of semipermeability and plasmolyzability (Currier, Esau, and Cheadle,
1955) indicates the very special properties that must be attributed to this
herbicide. Apparently 2,4-D and similar translocated compounds move

through these highly specialized cells in concentrations that kill roots without rapidly injuring the living conduits. Eventually there may be phloem necrosis (Eames, 1950), but during the hours when translocation is taking place, injury is not sufficient to prevent functioning of the phloem system.

This is a further indication that dosage must be regulated to minimize contact injury where translocation is essential to successful use of the method.

SUMMARY

Translocation of an herbicide is essential if the roots of a plant are to be killed by foliage spraying. Early work indicates that 2,4-D permeates the cuticle of sprayed leaves, migrates to the phloem, and is transported in this tissue, along with food materials in the plant. This paper reports work with labeled 2,4-D, and tends to substantiate the early conclusions.

When radioactive 2,4-D (2,4-D*) is used as a tracer, the methods of killing and drying the plants for autographing are critical. Freeze-drying proved to be the most reliable method. In bean plants, 2,4-D* was absorbed and translocated into the roots in about three hours. Bending of the epicotyls took two to three hours, and 2,4-D* was present in all bent epicotyls. Movement into terminal buds took about six hours. After 24 hours, intensity of 2,4-D* in autographs decreased in roots, treated leaves, and opposite leaves. This probably represents metabolism of the chemical and loss in the form of CO_2.

Translocation of 2,4-D* took place in plants that did not bend because of water deficiency. Placement of the 2,4-D* on the leaf determined the relative amount of chemical moved; over the midrib at the base of the leaf was the most favorable position. Absorption of 2,4-D* was enhanced by the presence of a surfactant in the applied droplet. In many-leafed bean plants, absorption and translocation were greatest from the lower unifoliolate leaves, less from successively higher leaves.

Translocation of 2,4-D* into stems and roots of cotton and cucumber plants was greatest from cotyledons, less from successively higher leaves. No 2,4-D* was transported from young leaves that were still importing foods from more mature parts of the plants.

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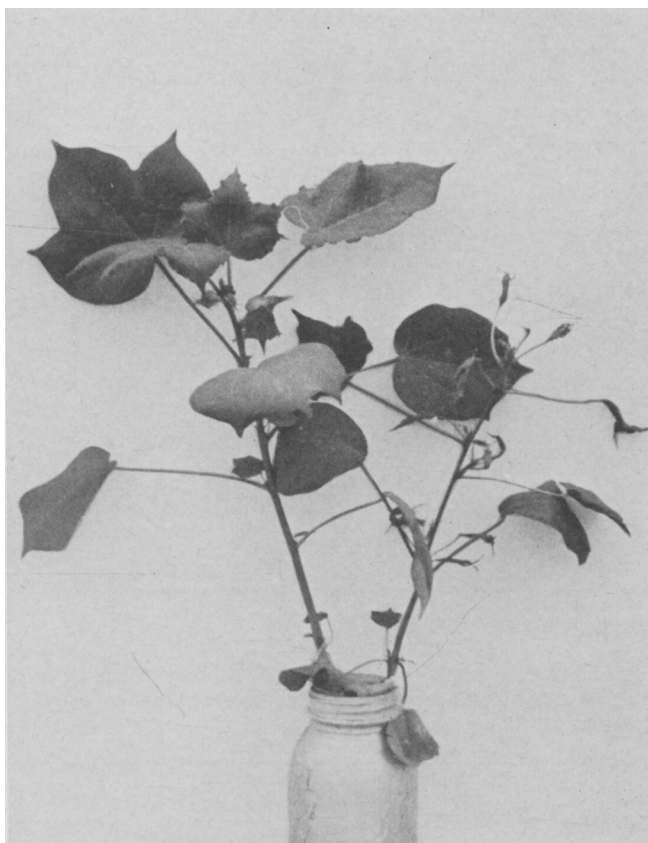
Fig. 1. Effect of 2,4-D on cotton. Symptoms (curling) on the leaves of the untreated plant (left). The plant to the right was treated with 16 μg of 2,4-D.
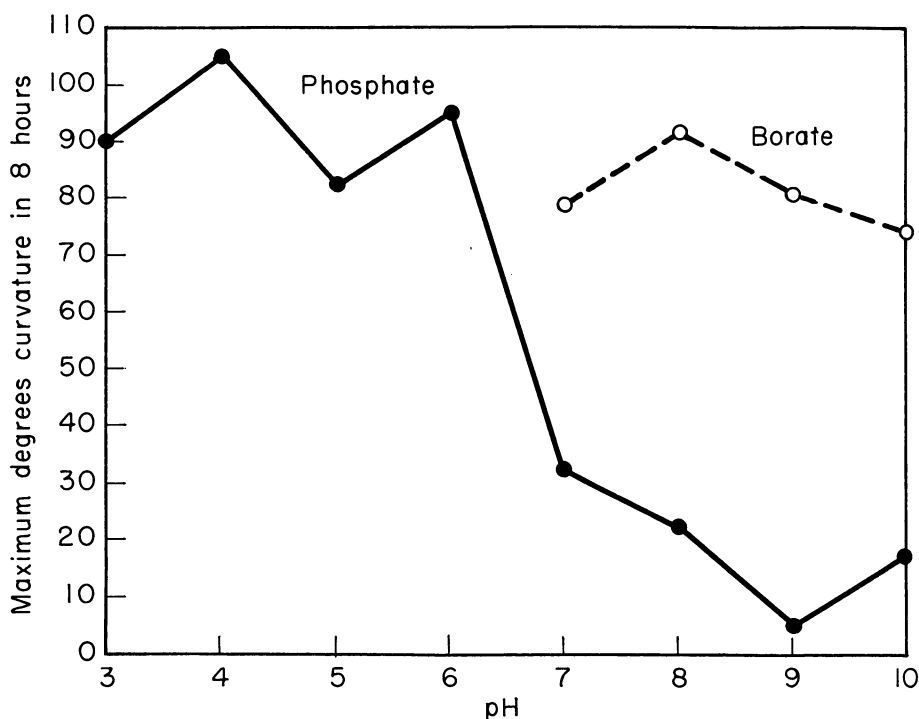
Fig. 2. Comparison of curvatures at high pH with 2,4-D solutions buffered with phosphate and with borate. Black-eyed peas, 2 replicates for the phosphate and 8 for the borate. Wetting agent, Trem 615, 0.1 per cent; 1.0 μg 2,4-D.
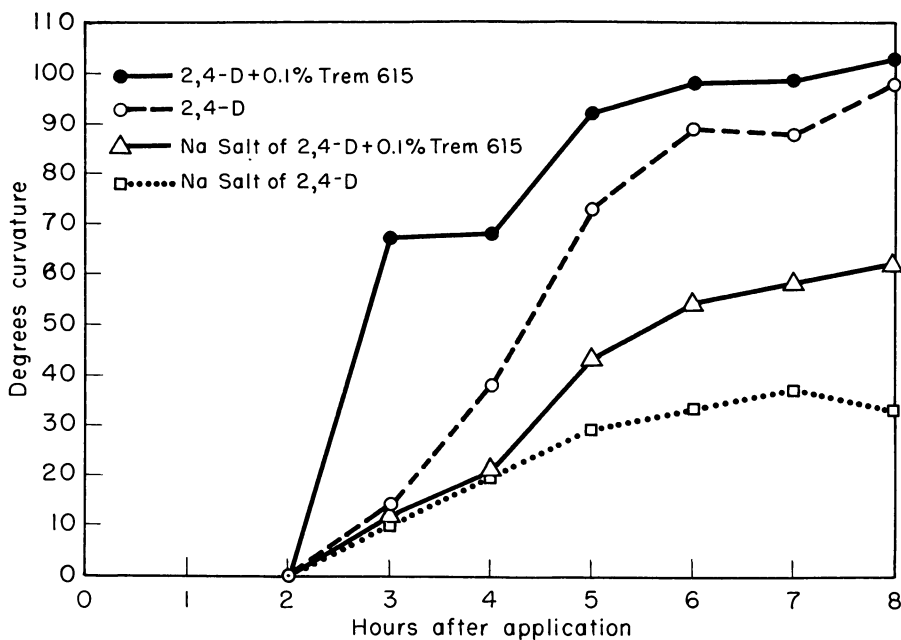
Fig. 3. Comparison of curvatures of bean plants from 2,4-D acid and its sodium salt, with and without wetting agent. Five replicates; 10 μg of acid equivalent per leaf.
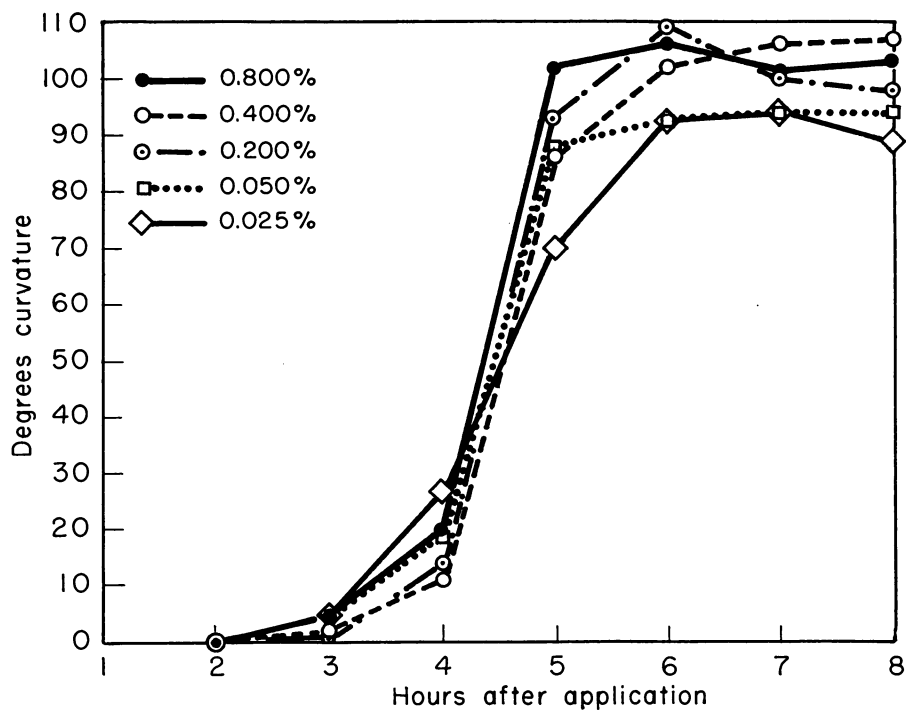
Fig. 4. Comparison of curvature at 10.0 μg of 2,4-D with different concentrations of wetting agent, Trem 615. Beans, 5 replicates.
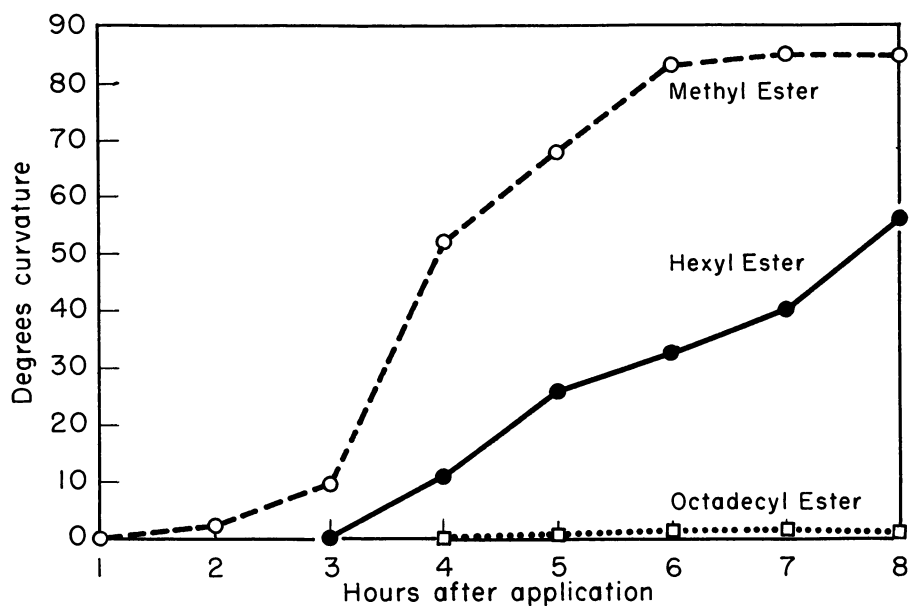
Fig. 5. Comparison of curvatures with three alkyl esters of 2,4-D. Beans, 5 replicates; 5.0 μg of acid equivalent; wetting agent, Mersox (Monsanto Chemical Co.), 0.1 per cent.
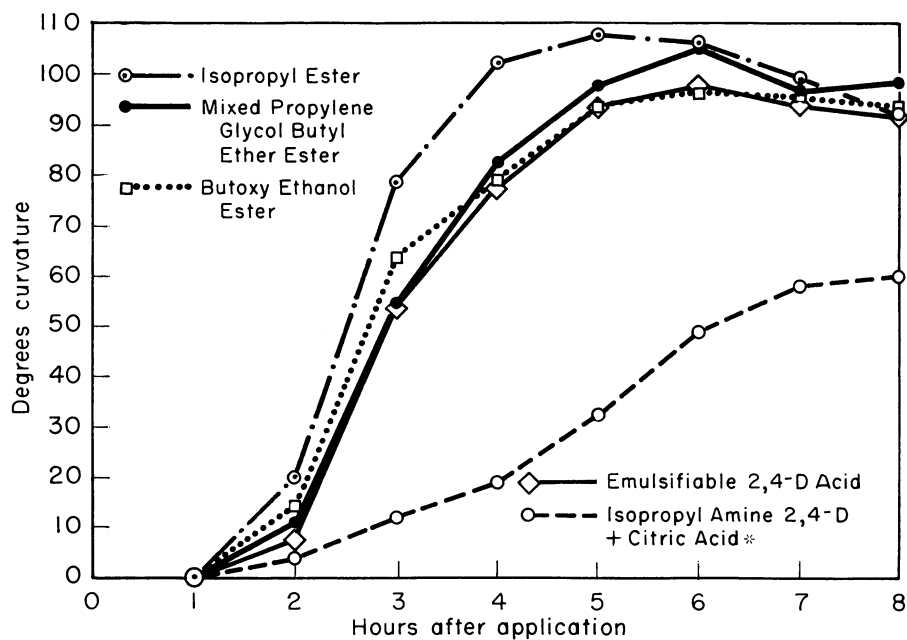
Fig. 6. Comparison of different formulations of 2,4-D by bean curvature. Beans, 10 replicates; 5.0 μg of acid equivalent. Formulations originally contained wetting agent. * was not formulated and 0.1 per cent Trem 615 was added.
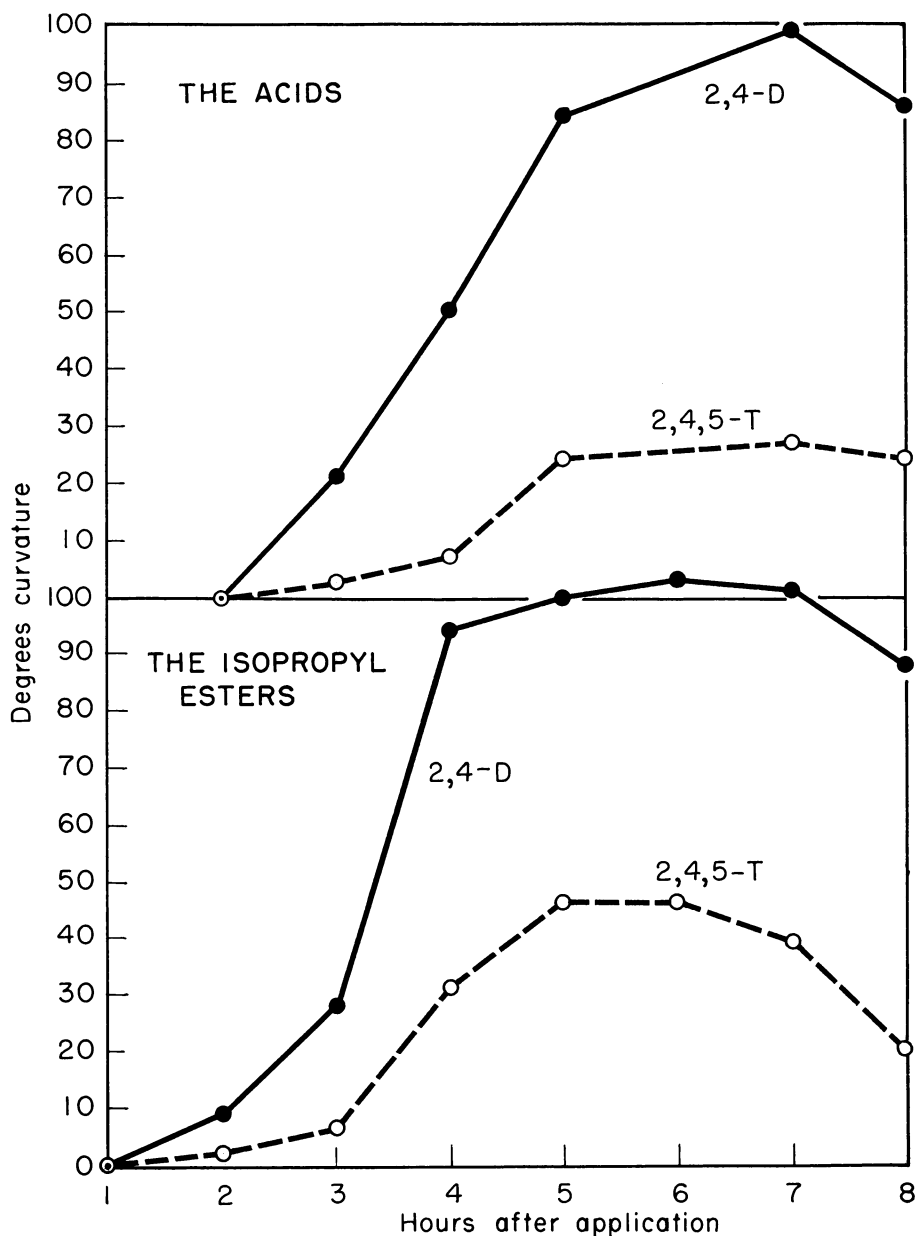
Fig. 7. Comparison of corresponding compounds of 2,4-D and 2,4,5-T using bean curvature. Beans, 5 replicates; 5.0 μg of acid equivalent. Formulations originally contained wetting agent.
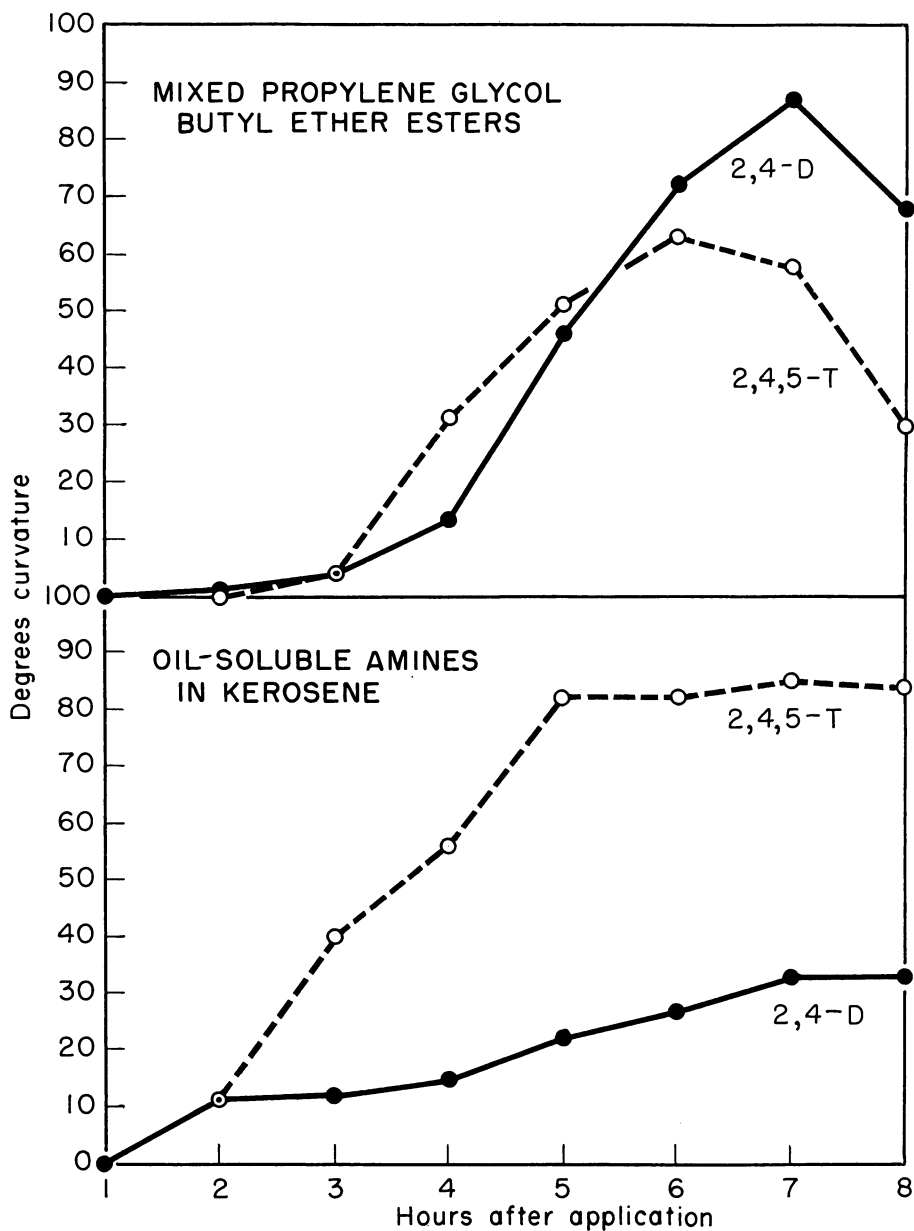
Fig. 8. Comparison of curvatures with corresponding compounds of 2,4-D and 2,4,5-T. Beans, 5 replicates. 5.0 μg of acid equivalent. Formulations originally contained wetting agent.

A   B
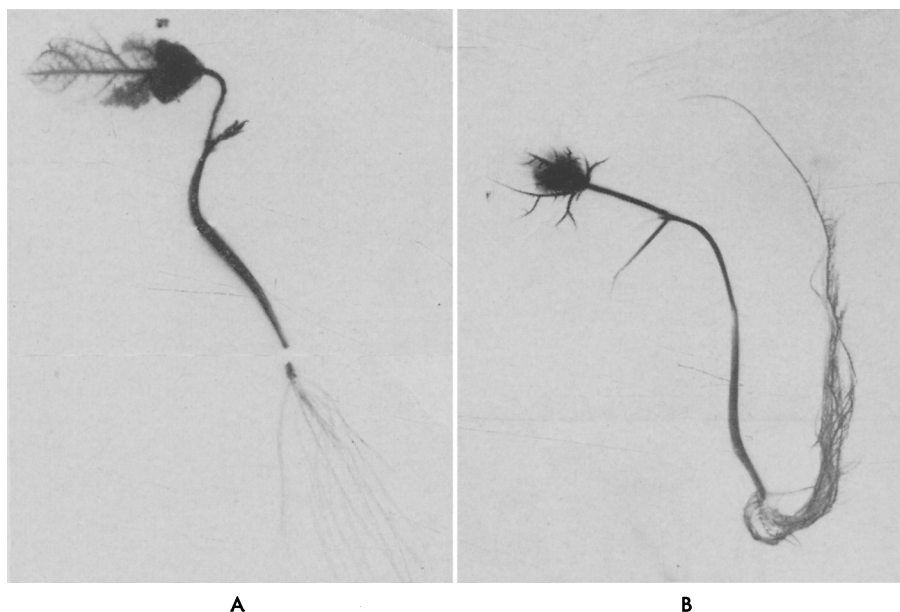
Fig. 9. *A*, radioautograph of a bean plant killed by drying between hot, dry blotters. Dosage 50 μg of 2,4-D*, treatment 4 hours, exposure 4 weeks. *B*, radioautograph of a bean plant killed between blocks of dry ice and dried between blotters. Dosage 50 μg, treatment 4 hours, exposure 4 weeks.

Fig. 10. Radioautograph of plant killed by quick-freezing and blotter-drying. Dosage 50 μg, treatment 15 minutes, exposure 4 weeks. Left, ozalid print of the dried plant; right, radioautograph.
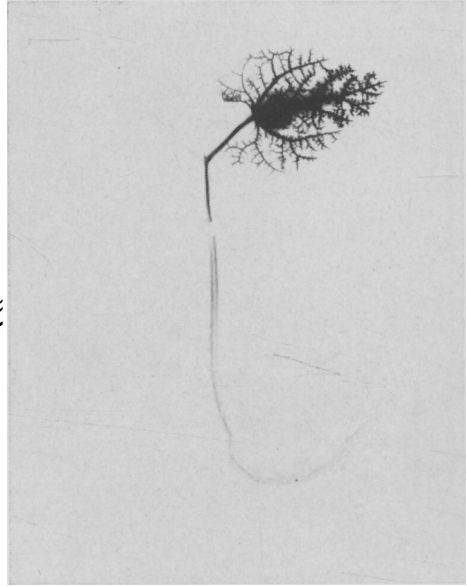
Fig. 11. A 30-minute treatment; other conditions as in figure 10.
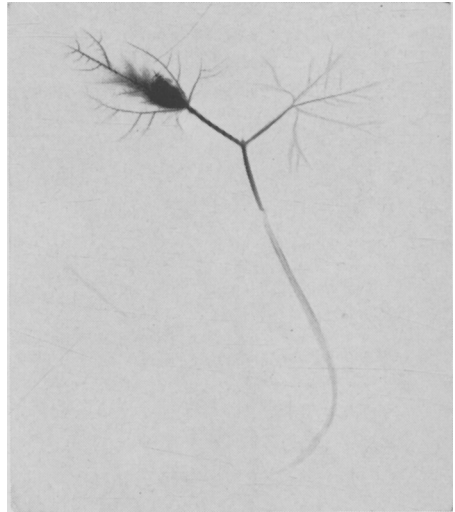
Fig. 12. Radioautograph of plant with 1-hour treatment period.
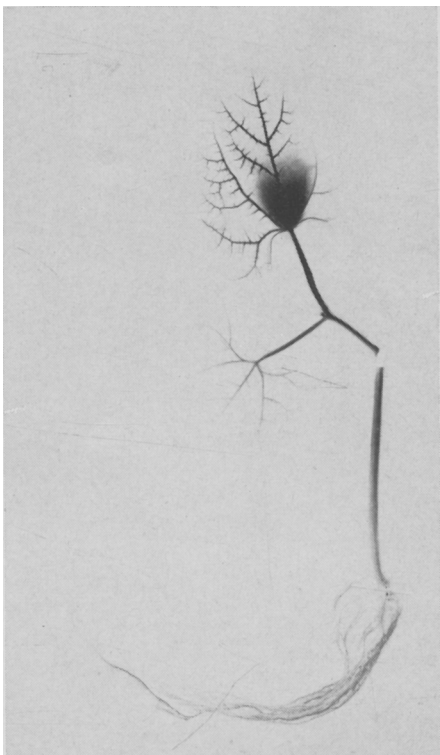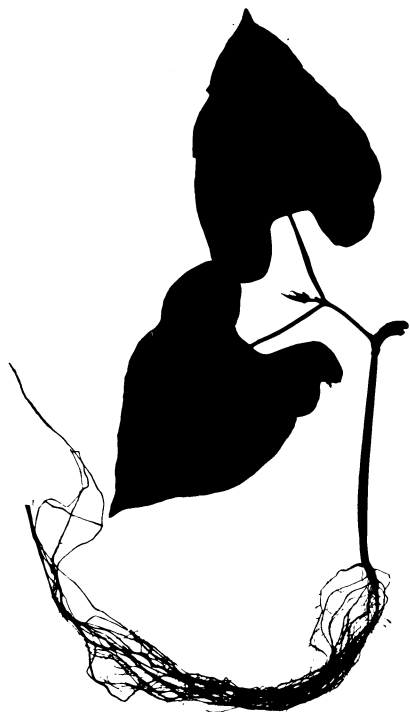
Fig. 13. Treatment period 3 hours.
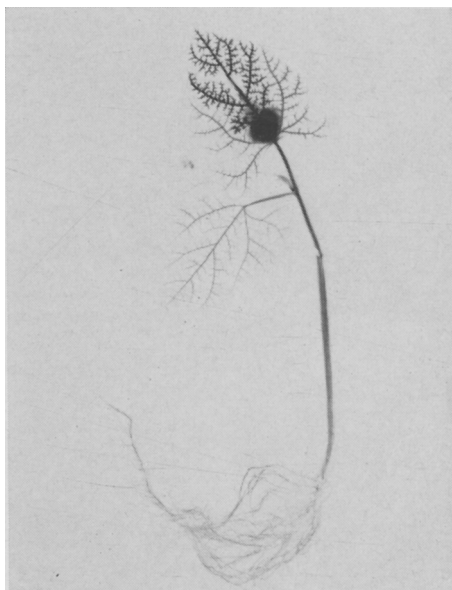
Fig. 14. Treatment period 8 hours.
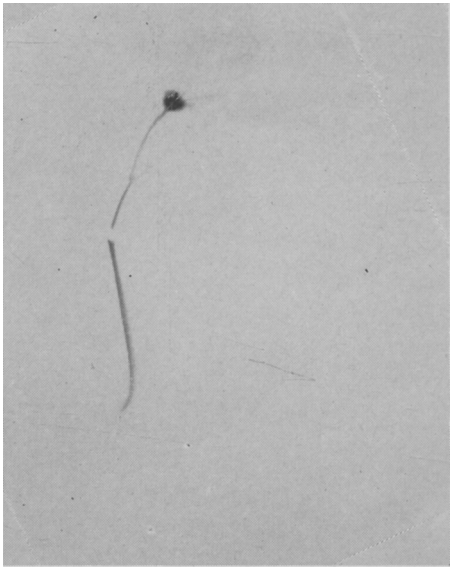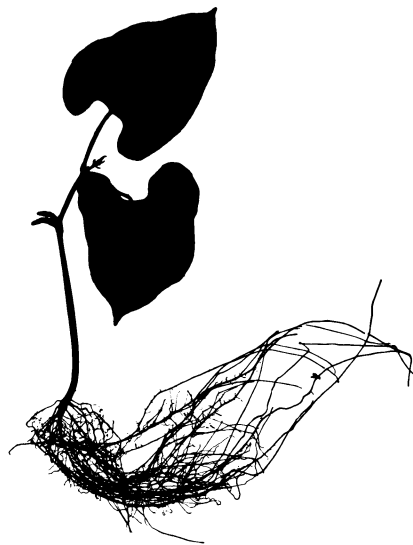
Fig. 15. Treatment period 24 hours.
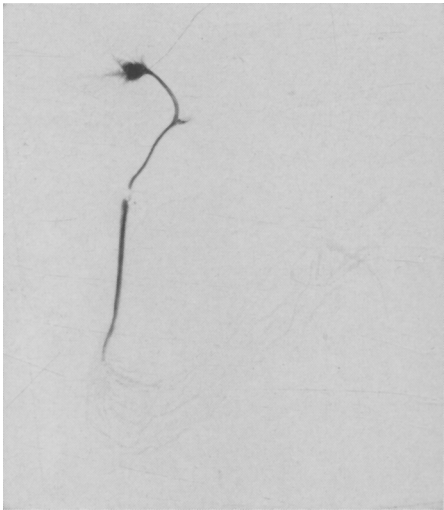
Fig. 16. Treatment period 72 hours.
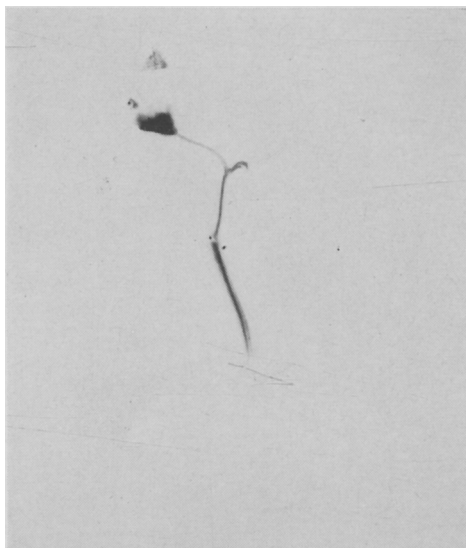
Fig. 17. Treatment period 144 hours.
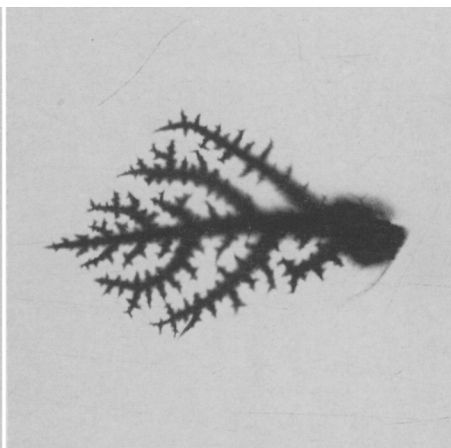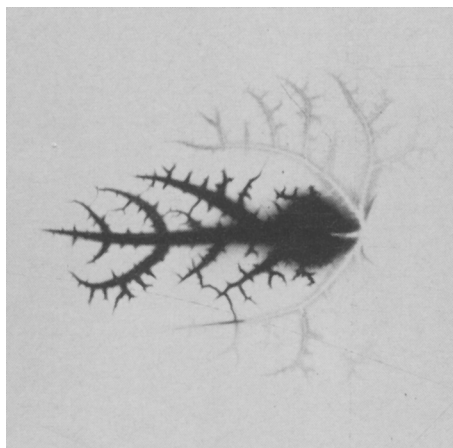
Fig. 18. Radioautographs of bean leaves of plants with dosage 50 μg, treatment period 30 minutes, exposure 4 weeks. Movement here has been only in the acropetal direction in the treated leaves.
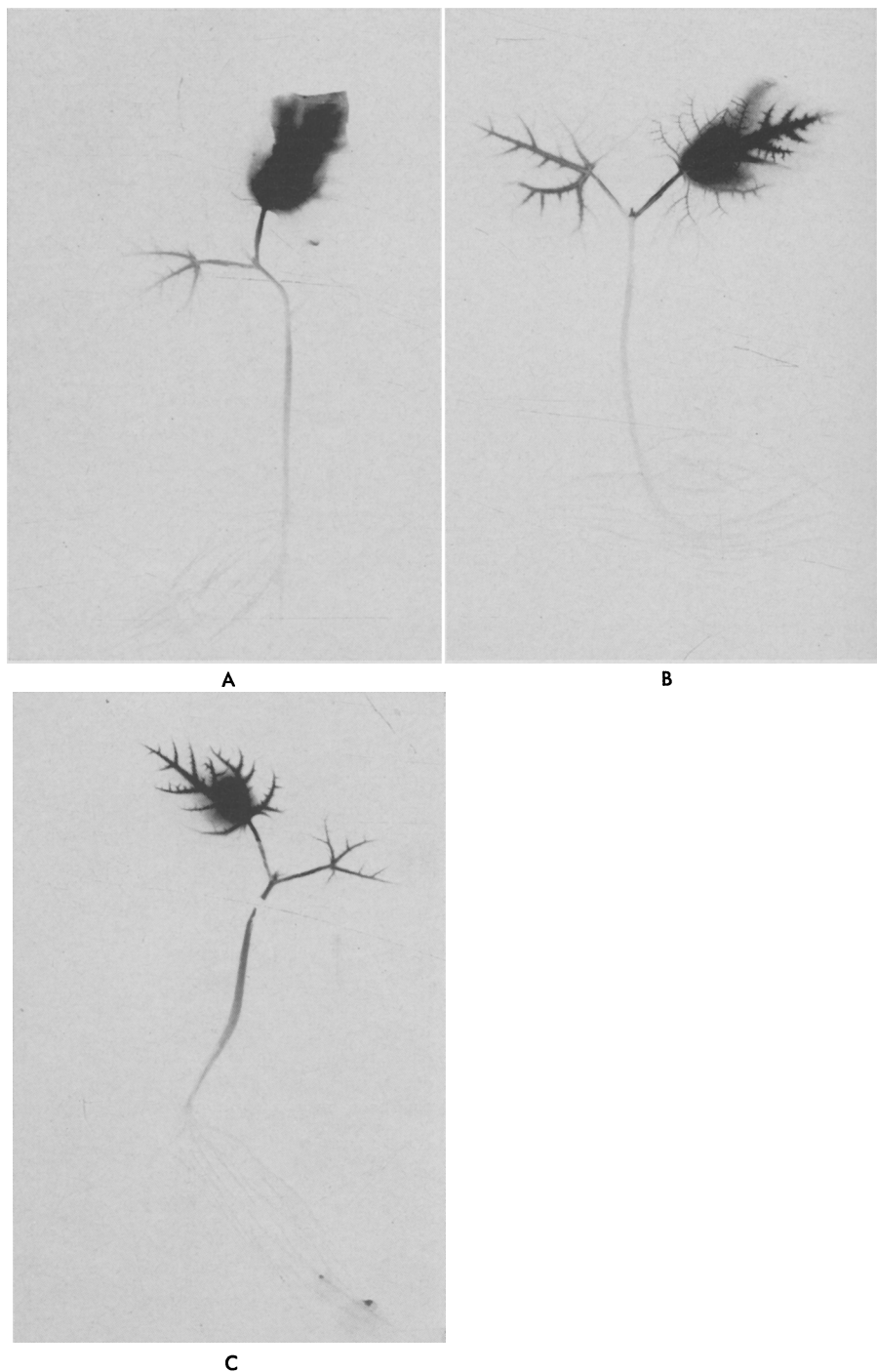
Fig. 19. *A*, radioautograph of bean plant from a humid-chamber treatment. *B*, plant treated on greenhouse bench. *C*, plant from out-of-doors, dry treatment. All plants: dosage 50 µg, treatment period 4 hours, exposure 2 weeks.

A   B
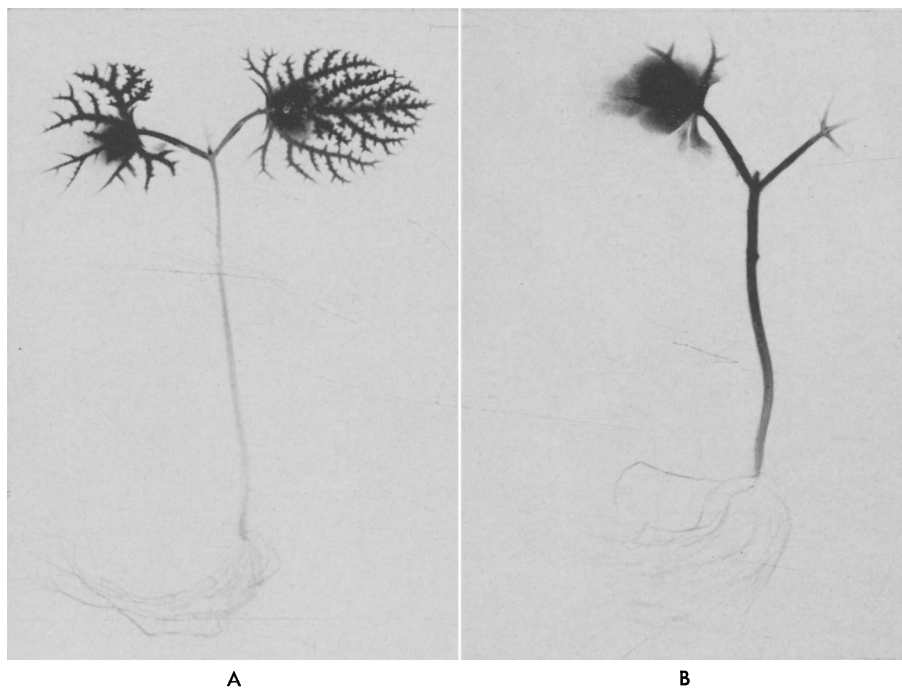
Fig. 20. *A*, radioautograph of a bean plant treated on both leaves. Dosage 50 μg per leaf, treatment period 4 hours, exposure 2 weeks. *B*, radioautograph of a bean plant treated on one leaf. Dosage 50 μg, treatment period 4 hours, exposure 4 weeks.
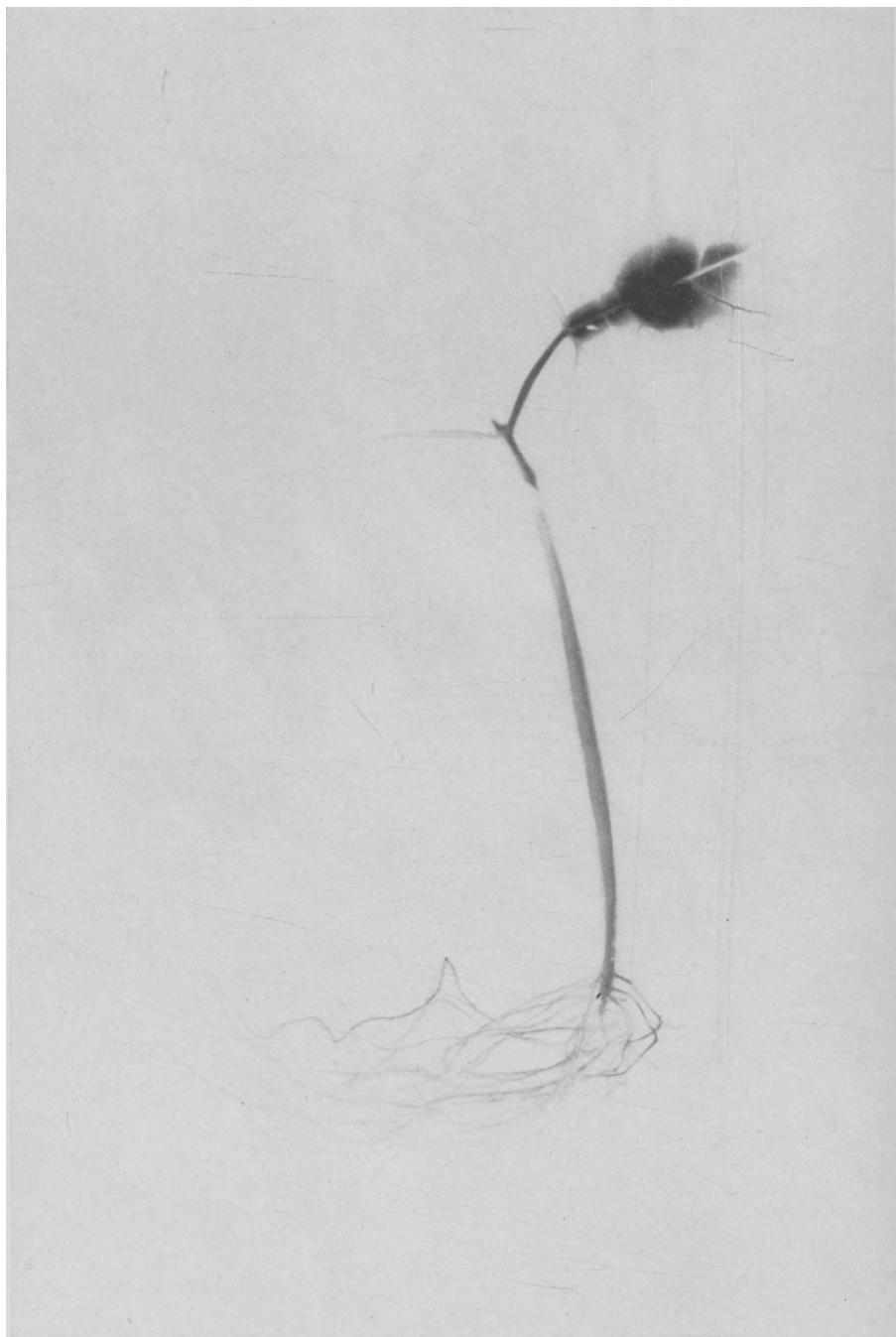
Fig. 21. Radioautograph of a bean plant treated in the center of one leaf over the midrib. Dosage 20 μg, treatment period 4 hours, exposure 4 weeks.

A   B
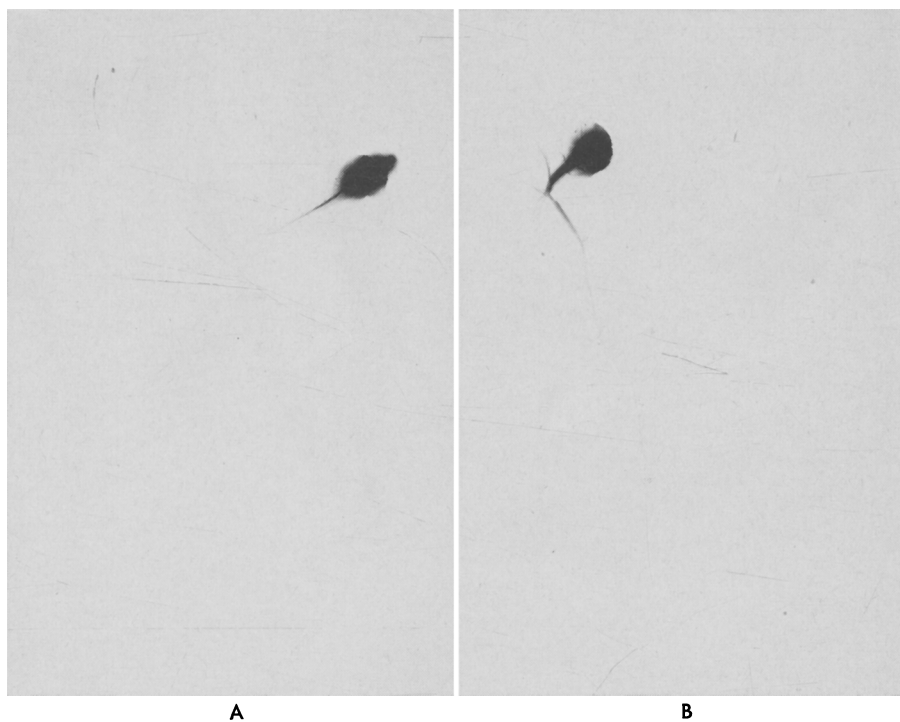
Fig. 22. *A*, radioautograph of a bean plant treated on the tip of one leaf. Dosage 50 μg, treatment period 4 hours, exposure 4 weeks. *B*, similar radioautograph of a plant treated on one lower leaf lobe. Dosage 20 μg, treatment period 4 hours, exposure 4 weeks.
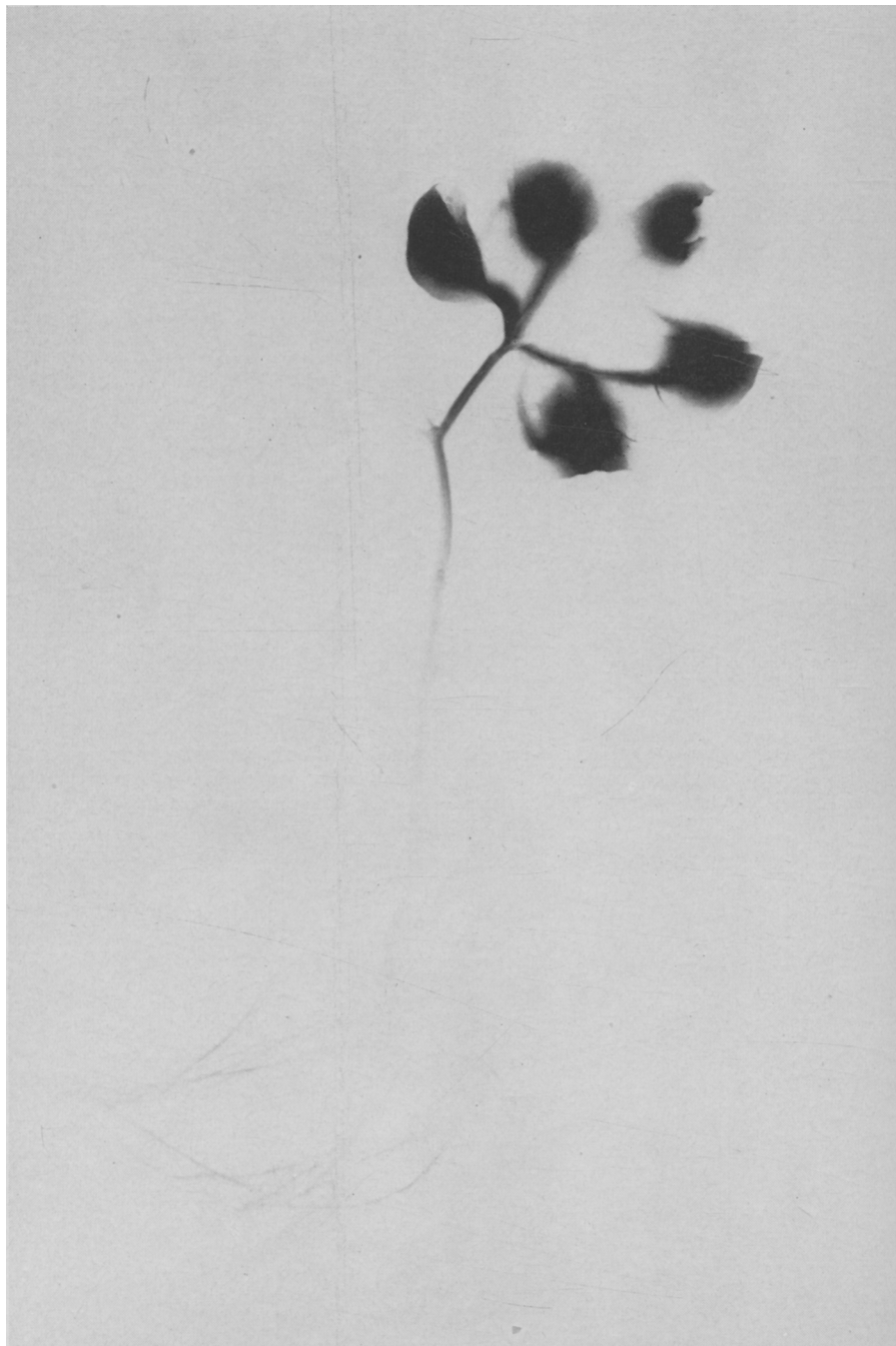
Fig. 23. Radioautograph of a bean plant that received five 20-μg treatments around the edge of one leaf. Dosage 20 μg per treatment (100 μg total), treatment period 4 hours, exposure 4 weeks.
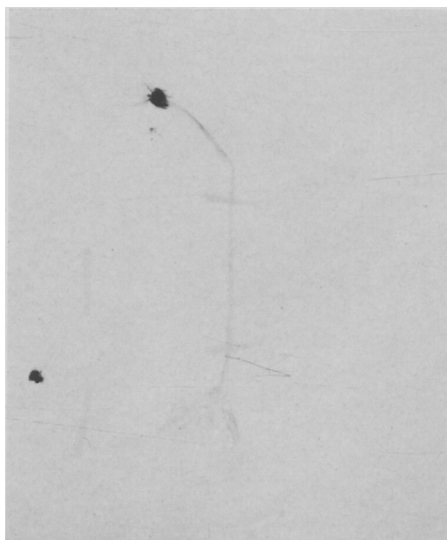
Fig. 24. Radioautograph of a freeze-dried bean plant. Dosage 5 μg, treatment period 1 hour, exposure 4 weeks.
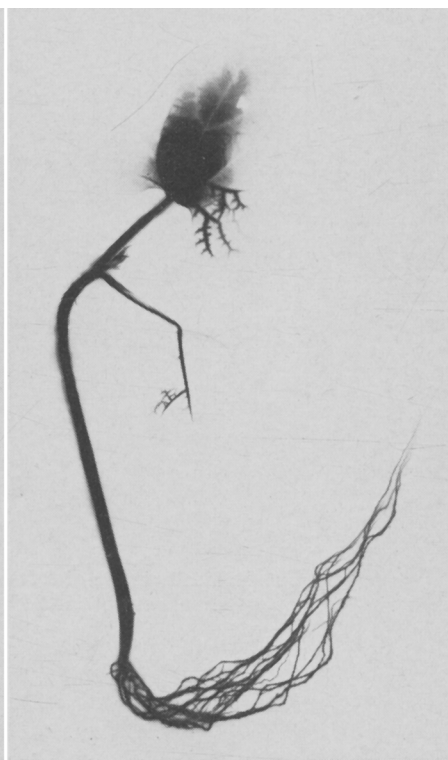
A   B

Fig. 25. *A*, radioautograph of a freeze-dried bean plant. Dosage 5 μg, treatment period 4 hours, exposure 4 weeks. *B*, radioautograph of a dry-ice-killed, blotter-dried bean plant. Same dosage, treatment period, and exposure.
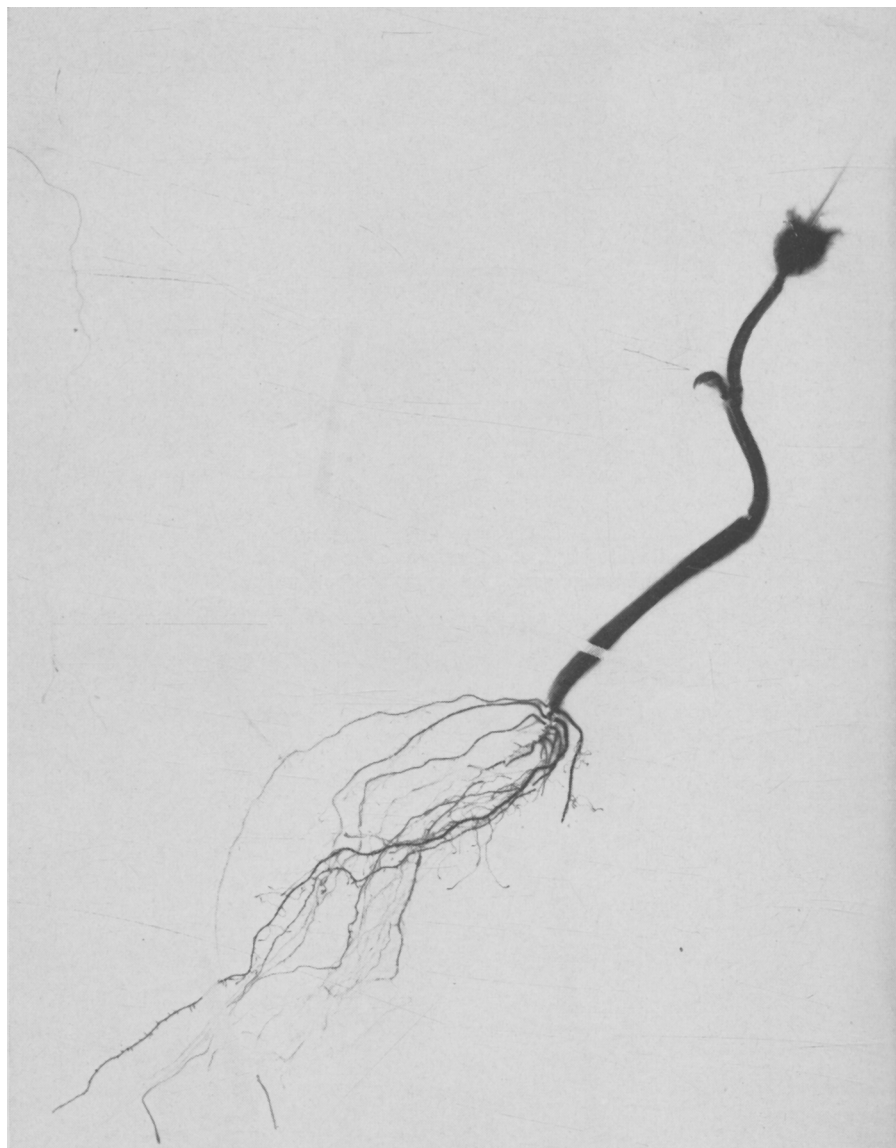
Fig. 26. Radioautograph of a freeze-dried bean plant. Dosage 50 µg, treatment period 24 hours, exposure 4 weeks.

Fig. 27. Radioautograph of a young cotton plant. Dosage 10 μg, treatment period 4 hours, exposure 2 weeks.
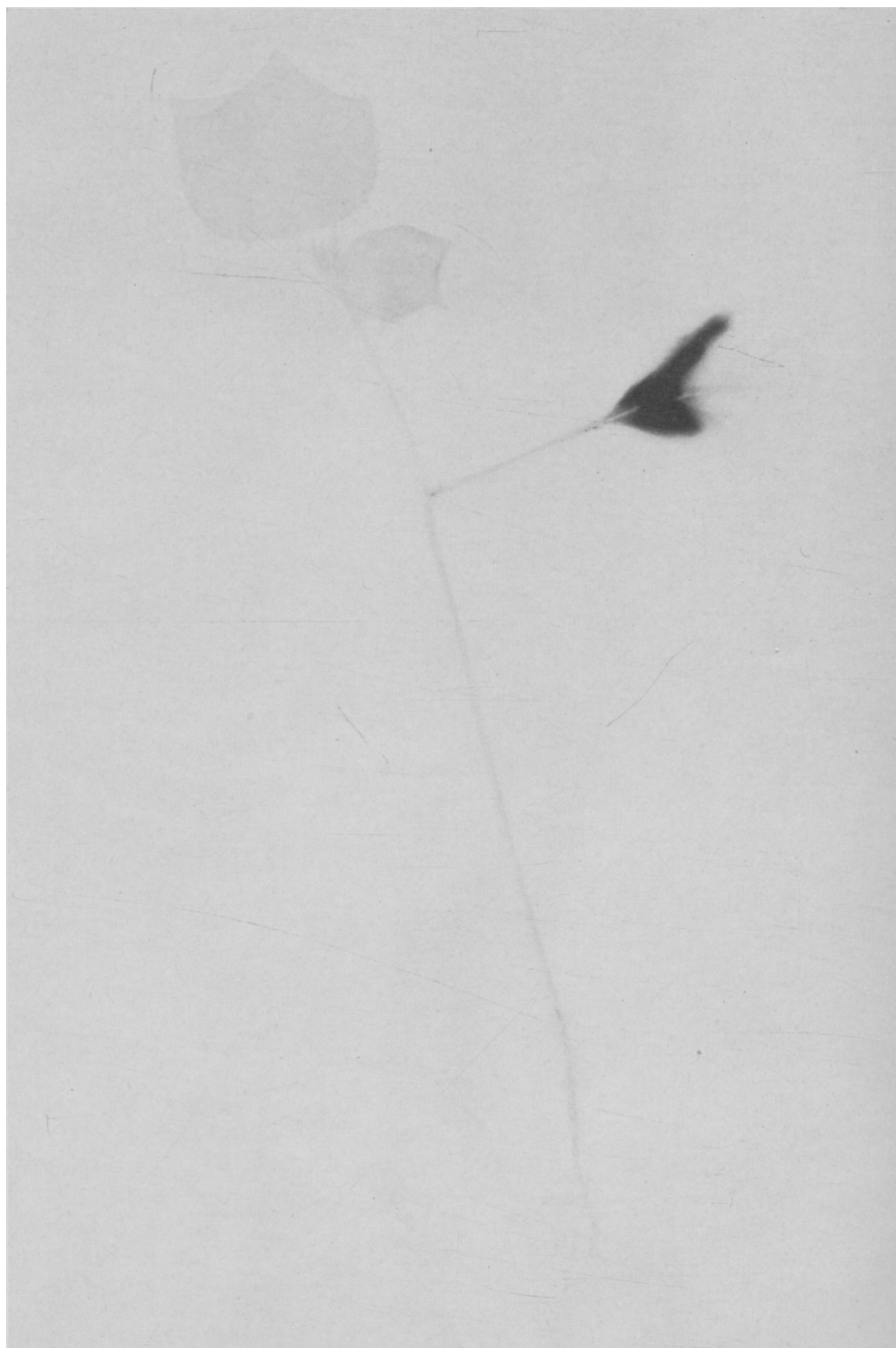
Fig. 28. Radioautograph of a somewhat older cotton plant with true leaves expanding.
Dosage 10 μg, treatment period 2½ days, exposure 2 weeks.
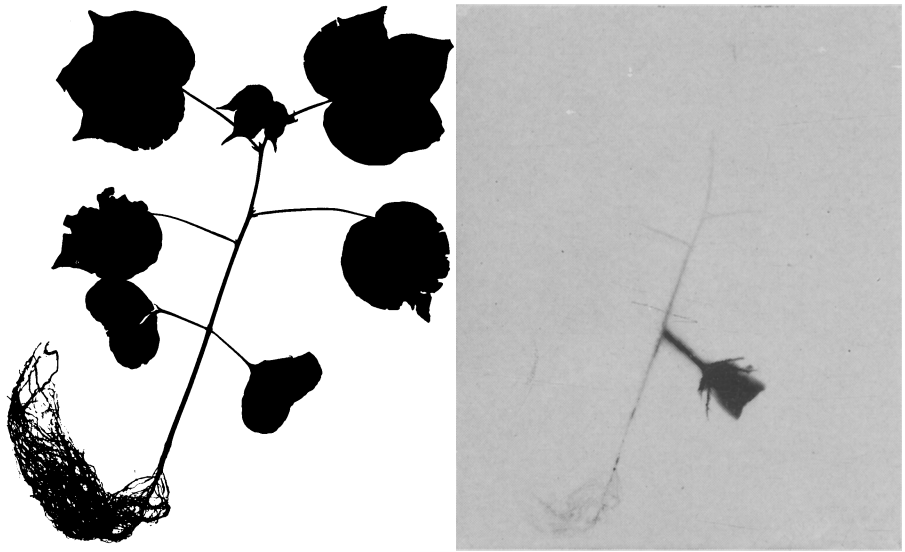
Fig. 29. Radioautograph of a cotton plant with four expanded true leaves. Treatment on one cotyledon. Dosage 50 μg, treatment period 4 hours, exposure 2 weeks.
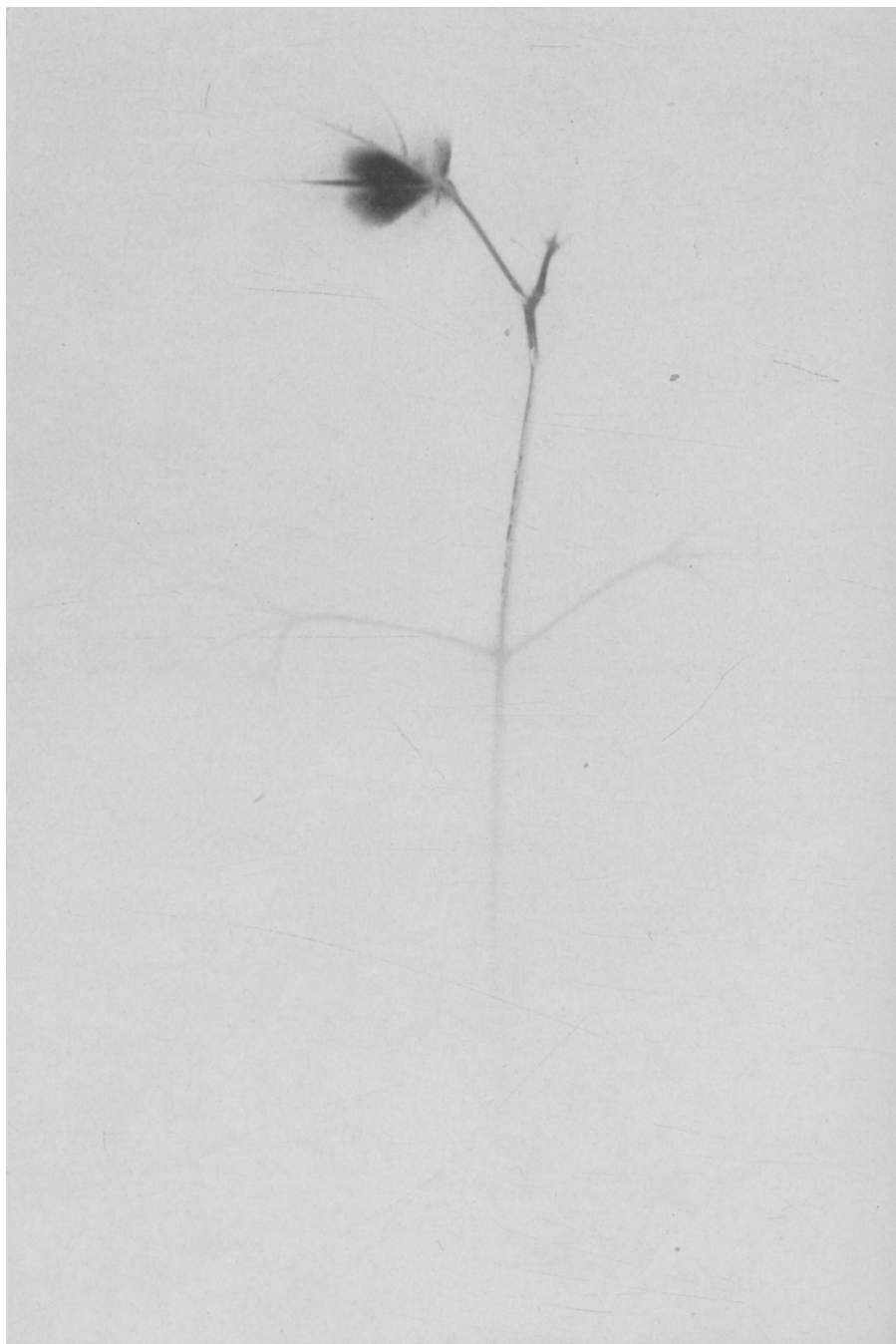
Fig. 30. Treatment on an expanded true leaf. Dosage 10 μg,
treatment period 4 hours, exposure 2 weeks.
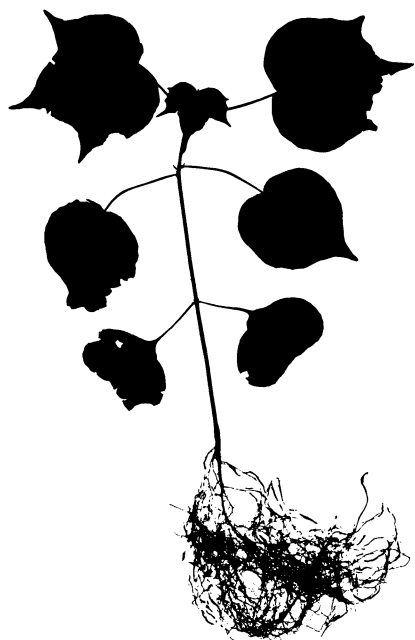
Fig. 31. Treatment on the fourth expanding leaf. Dosage 50 μg,
treatment period 4 hours, exposure 2 weeks.
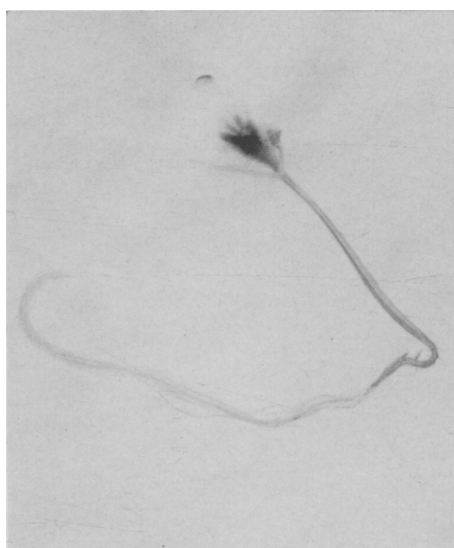
Fig. 32. Radioautograph of a young cucumber plant treated on one cotyledon.
Dosage 5 μg, treatment period 1 hour, exposure 4 weeks.
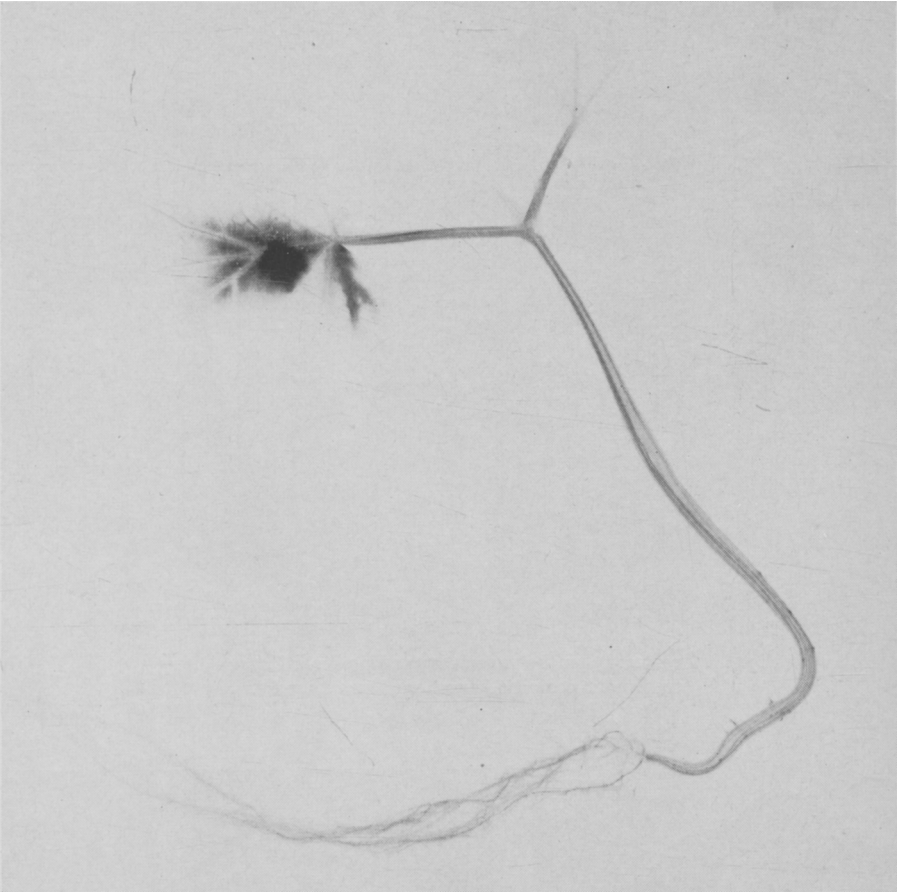
Fig. 33. Similar plant treated on the first expanded leaf.

Fig. 34. Similar plant treated on the second expanding leaf.
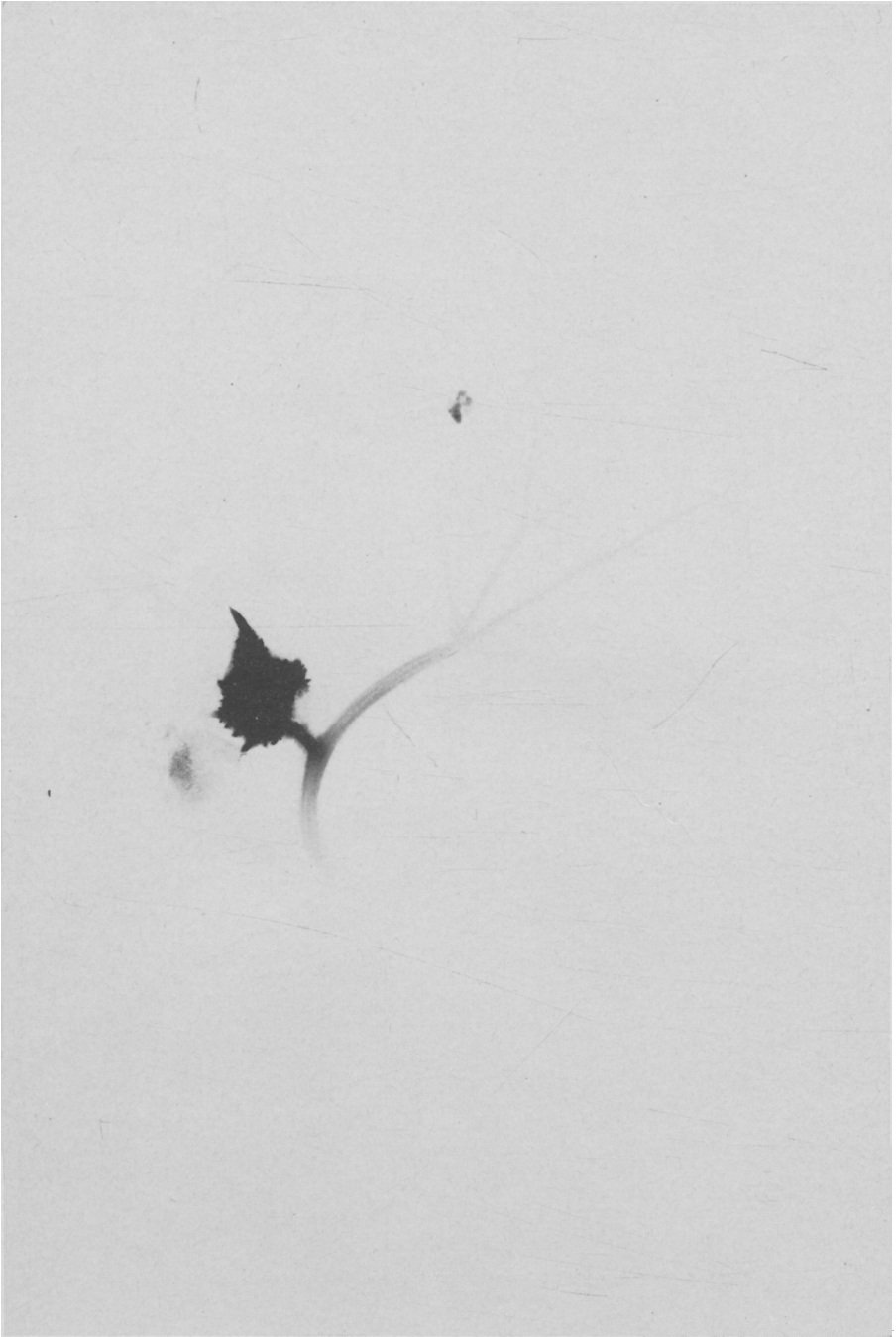
Fig. 35. Similar plant treated on the third expanding leaf. Here the
treating solution seems to have flowed down onto the stem.
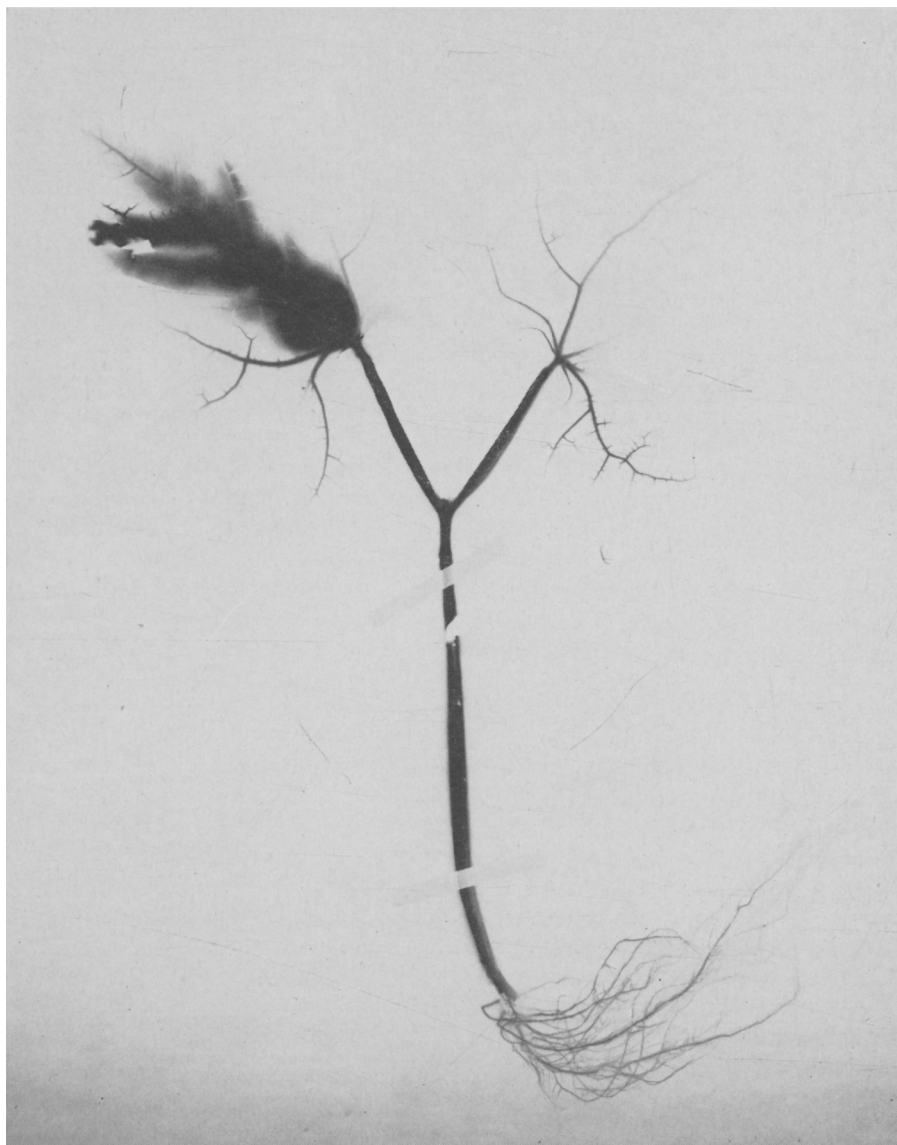
Fig. 36. Radioautograph of a bean plant killed by quick-freezing and dried in filter paper between warm, dry blotters. Dosage 50 μg, treatment period 1 hour, exposure 4 weeks.

Fig. 37. Radioautograph of the filter paper upon which the frozen plant of figure 36 was placed for drying.