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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.

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II. ABSORPTION AND TRANSLOCATION OF 2,4-D BY WILD MORNING-GLORY[1]

ALDEN S. CRAFTS[2]

INTRODUCTION

THE FIRST REPORT of the use of 2,4-D and 2,4,5-T as weed killers in the United States described trials on wild morning-glory (Hamner and Tukey, 1944).[3] The formulations contained the parent acids in Carbowax diluted with water, a type of mixture commonly used in the administration of plant regulators. The reported results indicated successful killing of the roots by spraying the tops. Obviously, translocation of the chemicals was involved.

Subsequent rapid development of the use of 2,4-D as a weed killer followed the introduction and sale of the sodium and ammonium salts and the alkyl esters of 2,4-D. The phenomenal growth of this practice is a familiar story. Despite the tremendous popularity of these 2,4-D products, on careful examination the results failed to show any consistent evidence for effective translocation of the materials into the roots of perennial plants. In fact, many investigators were led to question the effectiveness of 2,4-D as an herbicide against perennial weeds.

Something was evidently wrong because the early Carbowax preparation had undoubtedly been effective as a translocated herbicide. Occasional observations of killing of the underwater roots of tules and cattails after treatment with 2,4-D, and a few isolated examples of the undoubted killing of perennial weeds following spraying proved that, under certain conditions, the plant regulators were being transported to roots in lethal doses.

Is there any ready explanation for these facts? From the chemistry of the compounds it is known that 2,4-D acid is low in water solubility, though moderately dissociated when in solution. Compared with the salts, the acid is relatively low in polarity, and higher in lipid solubility than are the ions of the salts. It had been postulated that the penetration of the dinitro compounds into plant cells depends on the concentration of undissociated dinitrophenol molecules in the spray solution (Crafts and Reiber, 1945).

Characteristics of 2,4-D

In the use of translocated herbicides, the concern is not alone with penetration of cuticle and entry into the living mesophyll; migration to and transport within the phloem are additional prerequisites to success. And for this to take place, the toxicant must be water soluble. In 1948, the writer made the statement, "for penetration of the cuticle and absorption by foliage, nonpolar compounds should be used" (Crafts, 1948). Considering 2,4-D, the series, ammonium salt, sodium salt, alkylamine salt, alkanolamine salt, is in the direction of increasing polarity, water solubility, and hence conveni-

[1] Received for publication June 10, 1955.

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

ence of formulation. And for contact action against mustards in a cereal crop, the margin of safety with respect to toxicity to the weeds is so great that little harm results from the decreasing tendency to penetrate.

Alkyl Esters. Considering now the alkyl esters of 2,4-D, the series, methyl ester, ethyl ester, propyl ester, butyl ester, amyl ester, is in the direction of decreasing polarity and hence increasing lipoid solubility. This should, and actually does, increase penetration. But for two reasons it works against translocation: (1) the high lipoid solubility makes the ester compatibile with the cuticle, and hence lowers the tendency for partition from it; and (2) the low water solubility lowers the ester's compatibility with the vacuolar sap and the aqueous phase of the protoplasm of mesophyll cells. The alkyl esters are noted for their high contact toxicity as indicated by dosage recommendations that are usually about half those for salts. But the same properties that induce rapid penetration and violent contact action weaken partition to the mesophyll and vascular tissues, and increase toxicity which quickly knocks out the mechanism responsible for translocation.

Heavy Esters. The shift to the heavy esters came about largely because of injury problems resulting from the volatility of the light alkyl esters. But the introduction of one or more oxygen atoms into the alcohol chain of the ester added to the water solubility of these compounds which, because of their balanced solubility, gave better performance with respect to partition and translocation (Crafts, 1951*a*). Several of the heavy ester formulations have proved superior to the alkyl esters and salts as translocated herbicides on perennial weeds. However, while there are almost unnumbered possibilities for combining alkylene groupings and ether linkages into heavy esters of 2,4-D, certain considerations indicate definite limitations: First, proper balance of the water and lipid solubility must be maintained to insure both penetration and partition. Second, the length or weight of the alcohol chain is limited, for it may come to overshadow the active 2,4-D group and hence lower activity on a straight molecular weight basis (see table 12, page 150, Robbins, Crafts, and Raynor, 1952). And, because the evidence from tests indicates that the esters are not hydrolyzed during penetration, the mass of the molecule may make it unwieldy for ready translocation through the phloem. Finally, the diesters of glycols seem less effective than do monoesters in translocation trials (Leonard, 1954), indicating that this type of molecule will not provide a way out of the dilemma.

The Acid. The acid stands at the midpoint between the salts and the alkyl esters with respect to polarity. The molecule is small and mobile, yet it is not highly volatile. Its chief drawback seems to be its low water solubility from the standpoint of formulation and application. However, this is a virtue in disguise, as seen from the discussion above, for it acts as a buffer to regulate both penetration and contact toxicity. Emulsifiable formulations of 2,4-D acid are available, combining ease of application with a balanced solubility that permits orderly penetration, continued migration to the phloem, and optimum pickup and transport in that tissue. Such formulations should be free of compounds having contact toxicity. They should provide stable spray mixtures that dry down to syrupy films from which absorption can continue indefinitely. And they should provide for adequate spreading and wetting. From results being obtained in current tests of emulsifiable acid formulations, it seems possible that all these prop-

erties may be combined in a single formulation, and that such formulations will give superior results in the field. This explains the success of Hamner and Tukey with the Carbowax formulation of 2,4-D acid.

Application of 2,4-D to Wild Morning-glory

Like so many problems in agriculture, success of chemical weed control depends in part on the material used but also in part on the state or condition or physiology of the plants being treated. This is particularly true in the use of translocated herbicides. Because wild morning-glory, a widespread and important weed, is fairly representative of many such weeds, it was chosen for the studies designed to carry the results of tracer work to practical application in the field. For reasons obvious from the above discussion, 2,4-D acid was used as the test material. In most of the tests, the carboxyl-labeled 2,4-D acid in 50 per cent alcohol solution with 0.1 per cent Nonic 218 was used. Single-drop applications were used in most cases; some involved multiple-drop application; one experiment employed spray application of a solution to plants in the field.

EXPERIMENTAL RESULTS

Experiments with 2,4-D

The work on wild morning-glory had as its objectives a more effective use of 2,4-D as a translocated herbicide and an explanation of some of the obvious failures in the field use of this chemical. It had been shown that there is an optimum value for concentration of 2,4-D applied (H. H. Smith, 1946). Above and below this value, the effectiveness of the herbicide drops off.

An experiment was designed (K. M. Smith, 1950) involving five formulations, four applications, and a series of observation dates. The triethanolamine salt of 2,4-D was to be used in one application of 1,200 ppm, two applications of 600 ppm, four applications of 300 ppm, and eight applications of 150 ppm.

The isopropyl ester was to be applied in similar series. A micronized lot of 2,4-D acid was formulated by suspending the acid in a lubricating oil of S.A.E. 20 viscosity and making a 6 per cent emulsion in water with an emulsifier to provide stability. An acid-in-alcohol mixture was made by dissolving 2.4 gm of 2,4-D acid in 30 ml of 95 per cent alcohol and diluting to 2,000 ml with distilled water. Application was started on July 16, 1949, and subsequent applications were made at weekly intervals.

The amine salt was carried through the whole schedule; the ester was applied once at 1,200 ppm, twice at 600 ppm, and four times at 300 ppm. (At 150 ppm, the plants were dead after five treatments, so that all of the chemical could not be used.) The two acid formulations killed the plants so rapidly that only the 1,200-ppm and 600-ppm applications were made; after three applications at 300 ppm the plants were dead. The ppm in all cases were calculated on the acid equivalent basis so that all plants carried through the schedule received the equivalent of 1,200 ppm of 2,4-D acid. The volume rate of spraying was 100 gallons per acre.

Figure 1 shows the depth of kill produced by the five formulations two months after treatments were completed. These are averages for only six

plants per treatment, hence small differences are of doubtful significance. Since all plants received the same amount of 2,4-D, the larger differences probably relate to the formulations and application methods. Examination of results (fig. 1) indicates that either the amine salt was very slow in entering or it had not translocated at the high concentrations. By the end of the four-week application period the 300-ppm treatment had brought about considerable movement within the plants. The ester, on the other hand, entered rapidly, and the optimum occurred at 600 ppm. Possibly the 1,200-ppm treatment was too toxic, and caused injury in the phloem. The acid in oil gave consistently good results. Acid in alcohol was less effective, and Smith (1950) considered that it had so low a surface tension that it ran from the plant at the 100-gallon-per-acre rate of application. It also dried rapidly, and may have failed to continue to move in from the residue formed. The slopes of the two acid curves indicate that even higher dosages could be used before toxicity would reach a point sufficient to reduce effectiveness. This indicates a regulated absorption and migration in contrast to a rapid accumulation in mesophyll cells resulting from the amine and ester forms.

The fact that the optimums for the salt and ester curves are at different points indicates the possibility that different forms of 2,4-D may have different concentration:volume relations. We know from experience that they have different dosage relations—for instance, the esters versus the salts. This difference in the optimum concentration is probably directly related to the relative rates of absorption and migration within the leaf, as these determine accumulation of 2,4-D within living cells.

Smith's (1950) acid-in-oil treatment involved the deposit, on the leaves, of a suspended, micronized 2,4-D acid in an oil film. Absorption in this case should be slow and regular, depending upon the solubility of the acid in the cuticle and its migration into living cells. The American Chemical Paint Company distributed a number of samples of micronized 2,4-D acid under the code designation of LFN472. After much field testing, the company has abandoned this for the emulsifiable acid formulation, Weedone 638. However, the Fruitgrowers Chemical Company, Limited, of New Zealand is still testing suspended acid formulations of both 2,4-D and 2,4,5-T, and the Stauffer Chemical Company is producing 2,4-D and 2,4,5-T acid pastes for use in the suspended form. Excellent results have been obtained with these products under certain conditions, and it seems possible that they may yet find a place among our weed-control chemicals.

Smith (1950) performed one additional experiment that is of interest. Using bean plants, he applied a 40-μg dose of acid to a single unifoliolate leaf in one droplet, two droplets, and four droplets. Figure 2 shows the relation between curvature of the bean plants and area over which the given dose was dispersed. Apparently the 40-μg dose at 1,000 ppm 2,4-D acid was not toxic, and the average bending of six replicates was over 60°. When the dose was placed over a larger area in two drops, the angle was about 30°. With four drops, on an even larger area, the angle was less than 20°. This bears out the conclusions of Loomis (1949), Smith (1946), Fisher (1952), and Crafts (1953, 1956) with respect to the efficiency of coarse versus fine droplets in spray application.

With the above experimental work and wide field experience as a back-

ground, a project was initiated involving the use of labeled 2,4-D as a translocation indicator. In addition to the studies already described, on beans as test plants (Crafts, 1953, 1956), tests were carried out on wild morning-glory plants in the greenhouse and in the field.

Experiments with 2,4-D*[4]

Seedling Studies. The first experiment involved a time series on morning-glory seedlings in the cotyledon stage. Greenhouse-grown plants were treated by applying 10 μg of 2,4-D* in 50 per cent ethyl alcohol on one cotyledon in the form of a droplet of 0.01 ml. The treatment times were ¼, ½, 1, 2, and 4 hours. The plants were killed between blocks of dry ice, dried between warm, dry blotters, and autographed for one week. There were five plants per treatment.

While there was undoubtedly some movement of 2,4-D in the xylem of these seedlings during drying (Crafts, 1956), the consistent differences with time indicate that this was not serious. Average movement in the ¼-hour treatments was less than ½ inch; for the ½-hour treatments, about ¾ inch; for the 1-hour treatment, ¾ inch; for the 2-hour treatment, 1¾ inches; and for the 4-hour treatment, 1¼ inches. The roots of these seedlings that could be washed free of soil and carried through the drying process averaged 2¼ inches in length. Figure 3 shows the autograph of three plants treated for 2 hours. Evidently absorption and translocation were taking place in these seedlings, but the velocity of movement was not high. Included in this experiment were some trials on larger plants, and in these translocation was more extensive. Five plants in the 2-leaf stage averaged downward movement of 2 inches in 4 hours; four of the plants had upward movement from the cotyledon into the growing shoot. Five plants having four true leaves each averaged 4 inches downward movement in 4 hours. Some larger plants having a foot or more of growth showed variable amounts of movement. One showed strong movement both upward and downward from a cotyledon that was still green and healthy; a similar plant showed no movement from a young upper leaf. The treating solution used in this and the following experiment did not contain a surfactant, and this may be one reason for the wide variability among plants.

The second experiment involved a 4-hour treatment time, with 1 μg of 2,4-D* in 50 per cent ethyl alcohol, on plants in the cotyledon, 2-leaf, 5-leaf, and 10-leaf growth stages. Eight plants in the 10-leaf stage, treated on a lower leaf, showed no movement beyond the treated leaf. Results on 11 plants in the 5-leaf stage were variable, five plants showing some transport and six plants none. Eighteen out of 20 plants treated in the cotyledon stage showed some transport. The low dosage, the lack of a surfactant, the short exposure on the film (1 week), and the high light intensity and low humidity prevailing may all have contributed to the general lack of absorption and translocation in these experiments.

The third experiment involved use of a test solution of 2,4-D* in 50 per cent ethyl alcohol containing 1 per cent Nonic 218. The dosage was 1 μg; the treatment time was 8 hours. Twenty plants were treated: five in the 6-leaf stage treated on an upper leaf; five in the same stage treated on a

[4] The symbol 2,4-D* indicates the radioactive form of 2,4-D. The 2,4-D* used had C^{14} in the carboxyl position. It was purchased from Tracerlab.

cotyledon; five in the 12-leaf stage treated on a tip leaf; and five similar plants treated on a cotyledon. The 6-leaf plants treated on a tip leaf averaged 1 inch of movement down the stem. The leaves were from half to full grown. Similar plants treated on the cotyledon had 2,4-D* well distributed throughout the upper root systems. All plants transported 2,4-D*, the average distance being 5½ inches, the greatest, 8 inches, with movement into lateral roots in several instances. In the 12-leaf plants, treatment of tip leaves resulted in even more restricted movement than in the 6-leaf plants. Figure 4 is representative of plants treated on a tip leaf. Cotyledon treatment resulted in extensive distribution in the roots in four of the five plants (fig. 5).

The next experiment involved 30 plants in the 6-leaf stage and 12 plants in the 12-leaf stage. Treatment was for 4 hours with 1 μg of 2,4-D* in 50 per cent alcohol solution containing 1 per cent Nonic 218. The dried plants were exposed on the X-ray film for 2 weeks. Ten 6-leaf plants treated on a small tip leaf showed movement beyond the petiole in only one case and for a distance of less than 1 inch. Treatment of ten 6-leaf plants on a leaf situated near the middle of the plant resulted in extensive movement in the stems of eight out of 10 plants, and in four of the eight there was some 2,4-D* in the roots. Figure 6 shows one such plant. Treatment of ten 6-leaf plants on a basal leaf resulted in movement into the roots of seven plants. In four of these there was some upward movement in the stem. In the 12-leaf set, there was no transport out of tip leaves. Four out of five plants in this set treated on middle leaves showed considerable movement; and four out of five treated on a lower leaf had 2,4-D* in the basal stems and roots. Transport was not extensive in any of these plants, but the dosage was only 1 μg. From the nature of the autographs it seems apparent that some of the tracer is retained in living cells along the route of transport. This brings about a continuous diminution in concentration which, with such small applications, probably limits the extent of movement.

One further experiment was conducted (July 9, 1952) with 2-leaf and 4-leaf seedlings, exploring the possibilities for movement from upper leaves and cotyledons. Vigorous seedlings growing in the greenhouse were used, and some excellent autographs were obtained. The treating solution contained 1 per cent Nonic 218, the dosage was 1 μg, and the test period, 4 hours. The plants again were killed between blocks of dry ice, and dried between warm, dry blotters. The exposure on the X-ray film was two weeks. Of 10 plants in the 2-leaf stage, four treated on young, expanding tip leaves had 2,4-D* in the treated blade and petiole. Of six treated on larger leaves, two had the tracer well down the stems, four had it into the roots. Figure 7 illustrates some of the latter. Of 10 similar plants treated on the cotyledons, all had the tracer in the roots, and in three it was also present in tip leaves. These seedlings illustrated very well the possibility for two-way movement from a leaf that was actively transporting (fig. 8*a* and *b*). Considering now the 4-leaf plants, of 10 treated on tip leaves, five contained 2,4-D* in the roots (fig. 9*a*), three had it in the stems only, and two in petioles and treated leaves (fig. 9*b*). Of 10 plants in the 4-leaf stage, treated on their cotyledons, eight had the tracer well into the roots, two had it only in the treated leaves and petioles.

Because we suspected that some of the variability in response found in

these experiments might have resulted from contact injury by the test solution, a series of applications was made on morning-glory leaves at various positions on the plant. These consisted of alcohol alone and with 1 per cent and 0.1 per cent Nonic 218. It was found that the 50 per cent alcohol being used caused no injury when applied in 0.01-ml droplets. When 1 per cent Nonic 218 was included there was some injury to young leaves at the tips of the plants; 0.1 per cent Nonic 218 caused only slight damage to very young leaves, none to expanded leaves. As a result of this test, the 2,4-D* solution being used was changed to contain 0.1 per cent Nonic 218; the 50 per cent alcohol concentration was retained.

The next experiment was designed to find if there were any significant differences in the distribution of 2,4-D* attributable to the method of killing. Large, greenhouse-grown plants were used. Five were cut into six portions at the end of the 4-hour treatment period and then quick-frozen, thawed, and dried between warm, dry blotters; five were cut while frozen; five were cut after thawing. Ten were killed and dried without being cut into fractions, and of these, five were killed after a 4-hour treatment time and five were killed immediately after treating. Of the 15 plants cut before, during, and after freezing, none showed extraordinary distribution of the 2,4-D*. Evidently, when the treatment time was 4 hours, the artifact of xylem movement upon thawing was not causing abnormal movement. There was no extensive transport into tips or roots in these plants.

In the five plants having zero treatment time, the most extensive movement was for a distance of about 4 inches in each direction from the treated leaf of one plant. Two other plants had less extensive movement, two had none at all. Of the five contrasting plants with 4-hour treatment time, one showed transport for about 22 inches into the roots, two moved the 2,4-D* 20 inches, one transported it 4.5 inches, and one carried it only 1 inch into the petiole.

An experiment using the same solution and dosage, a 4-hour exposure period, and killing with dry ice, tested movement in plants in the bud stage, in the blossoming stage, and in a stage having full-grown seeds. In the plants treated in the bud stage, two out of three had 2,4-D* in the young growing tip, and the third had some upward movement (fig. 10). All three had some transport in the basipetal direction, but only one had 2,4-D* in the roots. Of 10 plants treated in the blossoming and seed stages, none had 2,4-D* in the tips, but seven had some upward movement. Downward movement predominated in these more mature plants, all having the tracer to a distance of 4 inches or more, five having it over 6 inches, and two having it present in the roots.

The final greenhouse experiment with morning-glory attempted a comparison of four surfactants. The results were inconclusive because of lack of uniformity in the plants. Movement was upward in some, downward in others, and completely lacking in several. These were large plants in pots. Probably small seedlings would be better for such testing, with at least 10 replicates used for each material.

Field Experiments. A number of experiments using 2,4-D* as a translocation indicator were conducted in the field.[5] The first experiment was performed on May 19, 1952, and the second on May 30. A third was started

[5] Mrs. Barbara Kean conducted these experiments.

on September 24, and three were carried out in October of the same year. Thus it is apparent that these experiments covered two rather distinct climatic situations and that they represented different stages of development of the plants. These facts, considered in connection with the age of shoots and the location of the treated leaves, give clues for interpreting the results obtained. In all cases, 0.01-ml droplets of the test solution, containing 5 μg of 2,4-D*, applied to individual leaves constituted the testing method. No wetting agent was used in the first two experiments. The plants were dug from the ground, killed with dry ice, and dried between warm, dry blotters. Autograph exposure was for one week.

The first experiment involved some young shoots coming up from old roots in a field that had been disked to destroy annual weed growth. The leafy shoots aboveground were from 4 to 12 inches long; below the soil level they came off the roots at a depth of around 8 inches. No buds or blossoms were present. All shoots were treated on a lower leaf; the treatment periods were 2 and 4 hours. Of 25 shoots treated, only one showed upward movement and that for only 1½ inches. Twelve shoots had some downward movement, their average being 5.5 inches. Evidently these young vegetative shoots were still growing at the expense of the underground storage roots and had scarcely started replenishing the root reserves. The 4-hour period resulted in greater movement, with 10 plants out of 15 averaging 5.9 inches. The 2-hour period resulted in transport in three out of 10 plants, with an average distance of 4 inches.

In the second field experiment, 20 plants from the same location were treated on May 30. Ten of those were in the blossoming condition, and all such were treated on a lower leaf. Ten were treated in the preblossoming stage, five on an upper leaf and five on a lower leaf (fig. 11). The treatment period was 8 hours. Here the physiological condition of the plants had a profound effect on the results, and the effects bear a definite relation to field observation of commercial spraying. Of the 10 blossoming plants, only one showed any movement, and that for only 1½ inches in a basipetal direction. Of the preblossoming plants, all displayed some movement—those treated on an upper leaf averaged 4 inches downward and 1.2 inches upward; those treated on a lower leaf averaged 13.2 inches downward and 1.6 inches upward. These results agree with the common recommendation to spray wild morning-glory with 2,4-D in the prebloom stage. Later treatment often fails to kill the roots to any appreciable depth. Apparently, during blossoming, food materials are largely used locally in the production of flowers and seeds. Only later, when the seeds are mature, does downward transport resume, and at that time if the roots are mature they fail to react to 2,4-D.

By the time field testing was resumed in the fall, a test solution had been standardized—500 ppm of 2,4-D* in 50 per cent alcohol, so that 0.01 ml gave 5 μg. Nonic 218 was used at a concentration of 0.1 per cent. Dry-ice killing was continued as it apparently gave no artifact following the 4-hour treatment time. Exposure on the X-ray film was 4 weeks.

The first experiment in the fall used 14 plants, eight growing in a dry field and six on an irrigation ditch where there was ample moisture. All shoots were treated in a prebloom stage on a middle leaf. All plants in the dry condition moved the 2,4-D* downward for an average distance of 7.4 inches (fig. 12*a, b*); two moved it upward, one for 6 inches (fig. 13) and

one for 1 inch. All plants in the moist situation moved the 2,4-D* downward for an average of 10 inches; five out of six moved it upward for an average of 1.6 inches (fig. 14). From these results it seems that available moisture promotes the translocation of 2,4-D*. However, plants in a dry situation move the chemical downward, and lack of response in such cases probably reflects lack of response at the site of ultimate action rather than lack of transport into the roots. This same conclusion is borne out by further work.

An experiment performed on October 1, 1952, utilized 15 plants, five in the bud stage, five in bloom, and five with seeds forming. Treatments were near the tops of shoots. All plants but one in the blossoming stage transported the tracer in a basipetal direction; seven moved it upward. Movement was most rapid and prominent in the stems of the plants in the seeding stage.

A similar experiment on October 8, treating in the middle of shoots, gave an average transport downward of 7.4 inches for the plants in bud, 5 inches for the plants in bloom, and 10.4 inches for those forming seeds. One plant in bloom moved the tracer 1 inch upward; one in seed moved it 4 inches upward.

A third test on October 9, treating at the base of shoots in bud, in bloom, and in seed, gave the following figures: shoots in bud, downward 8.6 inches, upward 0; shoots in blossom, downward 5.4 inches, upward 0.8 inch; shoots in seed, downward 3.6 inches, upward 0.2 inch.

It is apparent that these morning-glory shoots were actively transporting foods and that 2,4-D* moved where the foods were going. Movement was predominantly downward; it was more active in plants in the bud and seed stages than in those in blossom; it took place in plants in dry as well as moist situations.

A study of 2,4-D* translocation in morning-glory growing in the field was begun in August, 1953.[6] A site on Yolo sandy loam was chosen, to facilitate digging of the roots. One patch of morning-glory was irrigated with a sprinkler until the soil was moist to a depth of 2 feet. A similar unirrigated plot was used where testing proved that there was available water at a depth of 5 feet. A trench 4 feet deep was dug, and a trowel and ice pick were used to remove the individual roots. In this way, roots were excavated to depths of 5 feet or more.

On August 3 and 4, plants in the two situations (moist and dry) were treated with 5 μg of 2,4-D* per application. The solution was in 50 per cent alcohol, and included 0.1 per cent Nonic 218. This test solution was made up from a batch of 2,4-D* containing 1.27 millicuries of activity per millimol. A preliminary run was made on August 3, a comprehensive experiment was started August 4, and collections continued until August 6. The plants were killed with dry ice, dried between blotters, and left on the X-ray film for 4 weeks. The preliminary test gave variable results—one plant transported 2,4-D* to a depth of 51 inches in the root system during a 75-hour period of treatment. This was the total depth to which the root was removed; the tracer probably extended somewhat farther down.

The test started on August 4 involved 18 plants, nine in the wet and nine in the dry situation. One in each situation was treated on a tip leaf and given a treatment period of 4 hours, another was treated for 27 hours,

[6] Under the direction of J. E. Pallas, Jr.

and a third for 48 hours. Similar groups of three were treated on a leaf in the center of the plant and on a basal leaf.

The most significant difference related to the period of treatment. The average distance of transport in the 4-hour period was 11 inches, for the 27-hour period, 30 inches, and for the 48-hour period, 39 inches. A number of roots in the longer period treatments had 2,4-D* to their lower extremities; 68 inches was the greatest length of root excavated, and it autographed to the end.

The average distance of transport for all time groups in all the treatments in wet soil was 32.5 inches; in dry soil, 23.9 inches. However, variation among the plants in wet soil was from 0 to 68 inches, among those in dry soil, from 0 to 54 inches. Differences between treatments on tip, middle, and basal leaves were not significant. Considering that these were all fairly mature plants with available soil moisture, even in the dry situation, it seems logical that they should have behaved alike. Transport evidently continued for more than 4 hours, and in some instances extended to the full depth of excavation. One observation that was evident from the previous experiments was repeated here several times: 2,4-D* transport is not limited to vertical roots. In several instances, as noted in figures 15 and 16, the tracer moved down a rhizome until it merged with a horizontal rhizome or root. In nearly every case of this type, movement continued along the horizontal structure. This contradicts interpretation of field tests to the effect that 2,4-D moves by polar transport and does not enter horizontal laterals.

One additional experiment on morning-glory was conducted in the field during the 1953 season. In this, 0.5 ml of the 500-ppm stock solution of 2,4-D* (1.27 mc per mM) was mixed with 0.145 ml of Weedone 638[7] and made up to 77.7 ml. This was applied to 1 square yard of a small patch of morning-glory foliage in the field. The application was designed to deliver ¾ pound of 2,4-D per acre with 5 μg of 2,4-D* per average plant. Figure 17 shows one of the treated plants. Square-yard areas in the wet and dry situations mentioned above were treated, and plants were collected, killed, and dried after periods of 4 hours, 24 hours, 27 hours, 48 hours, 72 hours, 8 days, 2 weeks, 3 weeks. Exposure on the X-ray film was 4 weeks.

Careful evaluation of the results of this study shows that the only factor that produced significant differences was treatment time. The 4-hour treatment averaged 16.5 inches of transport; the 24- and 27-hour treatments combined gave a value of 32.4 inches; the 48-hour period gave 39 inches; and the 72-hour period, 45.2 inches. The 8-day, 2-week, and 3-week treatments gave transport values that totaled the entire lengths of the excavated roots and were therefore not averaged (fig. 18). The dry treatments gave the more rapid transport in the 4-hour period, but with longer treatment the plants from the irrigated plot had the more extensive movement. However, the plants per treatment were too few to give significant differences. The important point is that roots were impregnated with 2,4-D* to the depth of 5 feet or more by 72-hour treatments and all longer periods of treatments. Again, several plants used in these tests produced evidence for movement into horizontal lateral roots (fig. 19).

[7] An emulsifiable acid formulation of 2,4-D manufactured by the American Chemical Paint Company, Ambler, Pennsylvania.

DISCUSSION AND CONCLUSIONS

While these studies on the use of radioactive 2,4-D have not produced new and startling information, they give a fairly comprehensive view of 2,4-D transport in plants and they have confirmed a number of field observations on 2,4-D response of wild morning-glory, thus strengthening the convictions behind our current recommendations.

Interpretation of much experimentation suggests that 2,4-D moves with foods in plants (Crafts, 1951*b*). If this is true, then certain relationships must be apparent: (1) 2,4-D transport must be preceded or accompanied by photosynthesis or hydrolysis and movement of reserve foods. (2) 2,4-D should move out of mature leaves, but should remain in young leaves that are importing foods. (3) 2,4-D should move from lower leaves to roots, from upper leaves to growing shoots or fruits. And as a corollary it may move in both directions from median leaves. (4) Depending on the growth of the plant, movement of 2,4-D at times should be predominantly downward into roots, at other times it should be predominantly into growing shoots, and sometimes it should be predominantly into flowers, fruits, and seeds. (5) If 2,4-D movement accompanies the movement of foods, there should be no polarized movement in vascular channels in the sense of the polar basipetal movement of IAA in the oat coleoptile. Having set up this series of generalizations, it should be of interest to check the present results to see how well they agree.

Although no specific tests on starved plants have been run on wild morning-glory (see, however, experiments on beans reviewed [Crafts, 1951*b*] or described [Crafts, 1956]), certain preliminary work with seedlings indicated that favorable illumination is required to bring about translocation of 2,4-D*, and even then some plants may not respond within a given short transport period.

Many autographs prove that 2,4-D* will not move out of young tip leaves of well-developed plants. It moves readily from mature, green cotyledons and from full-grown leaves. Movement to roots in well-developed plants is greater from basal than from tip leaves. Movement to growing tips, flowers, and fruits may take place from any well-developed leaves. Two-way movement was observed in a number of plants. In some, movement to growing tips took place at such low concentration that only the tip would autograph; the intervening stem was too low in activity to expose the film.

Movement from cotyledons is initially into roots; after the shoot has grown a few inches, movement may take place both downward and upward. From cotyledons, it is always predominantly downward. Considering transport in shoots growing from old established roots, movement fails from the early, rapidly expanding leaves; at the bud stage it is predominantly downward into the roots; at the flowering and early fruiting stages it is into the flowers and fruits, and much less strongly downward; in the ripe seed stage after end growth has ceased it is again predominantly downward. Failure of spray treatment at this stage is apparently not the result of failure of transport.

Every test involving movement from a vigorous shoot down the stem and rhizome to a horizontal lateral resulted in lateral movement. Apparently, polar movement of 2,4-D* in a directional sense is nonexistent in morning-

glory. The only directional effect is from regions of food synthesis to regions of rapid food utilization.

If transport is active in mature morning-glory in the ripe seed stage, and also in horizontal laterals, why does late summer spraying fail and why do lateral roots so often survive the spray treatment? The answer seems to be in the final response of the root tissues to 2,4-D. Dr. van Overbeek pointed out (1947) that 2,4-D was most active against meristematic tissues. While morning-glory roots are young, growing, and meristematically active they respond. After they have matured and stopped growing, starch is being stored in mature cortical cells, and the roots do not respond. Hence, although absorption and translocation take place normally at those stages, spray treatment fails.

In considering the development of morning-glory from old established roots, it is apparent why lateral roots survive. In the spring, all roots have plenty of available soil moisture, and root growth is very active. However, the tops are importing foods from the roots, and the main stream of assimilates is flowing in the wrong direction. In the bud stage the roots are still active and the tops are re-storing foods in the roots; treatment is successful and all roots respond. Soon, however, moisture in the topsoil begins to become deficient; lateral roots mature and cease end growth; starch storage utilizes the foods. Lateral roots fail to respond, but vertical roots and deeper laterals are killed. As soil moisture limits root growth to greater depths, killing of roots fails and treatment is ineffective. If water is supplied by irrigation, root growth may start again and treatment will be successful provided the top growth is still green and healthy.

These growth and soil moisture relations seem to explain why morning-glory may be killed throughout the summer in the moist coastal belt of California, whereas in the dry interior valleys it responds best during the bud stage in early summer and again in the autumn only if top growth is vigorous and soil moisture is available. Failure in the case of green, vigorous plants growing in irrigated soil in summer may result from too rapid drying of the spray or too rapid killing of tops by contact action of poor formulations. In such situations, use of the emulsifiable acid is indicated, and tests have proved its superiority.

In some of the seedling treatments using 1 μg of 2,4-D, the concentration of the tracer seems to be fully as high in the stem as on the treated leaf (figs. 6, 7, p. 353). This indicates that there is an active process involved in the absorption; a diffusional process would result in a gradient. Reinhold (1954) has shown that IAA absorption involves an active process, and possibly 2,4-D at low concentrations is taken in by the same mechanism.

If an active process is responsible for 2,4-D uptake by leaves, it seems possible that prolonged absorption of the material at low concentration might result in much greater accumulation than would absorption from a toxic concentration that soon kills the leaf. A good many observations from the field bear out this deduction, and it would seem advisable to give the proposition more thorough testing. Smith's (1950) tests reported in this paper involved too long time intervals (one week). From our work with 2,4-D* it seems possible that 8- or 12-hour intervals would be better. Such slow absorption was the thought behind the suggestion of using suspended 2,4-D acid formulations. These have not proved entirely satisfactory, but

as noted (page 338), they are still undergoing test. Incorporation of a heavy white oil has aided in the absorption of micronized 2,4-D acid so that it approaches the esters in effectiveness. Possibly by altering particle size, and including proper surfactants and filming agents, the slow, regulated absorption of 2,4-D acid from a solid phase deposit may yet prove to be a superior method for handling some of the more refractive perennial species.

From the above considerations it should be apparent that formulation of 2,4-D herbicides for use on perennial weeds is important and that, even with the best formulations, knowledge of the physiology of the plants is required. This is the story that evolves from the use of radioactive 2,4-D as a translocation tracer in such plants.

SUMMARY

In the use of 2,4-D against perennial weeds, formulation is important. Emulsifiable acid and heavy ester formulations are proving superior to the older salts and light esters.

Droplet treatments with 2,4-D* on greenhouse-grown seedling morning-glory plants produced satisfactory radioautographs. Surfactant in the formulation increased absorption and translocation. From cotyledons, movement was downward into roots; from middle leaves, it was downward or both upward and downward; there was little or no movement out of very young, expanding tip leaves. Some tracer was retained in living cells along the route of transport.

Tests on field-grown plants proved that 2,4-D* can be used as a tracer in the field. Young vegetative shoots from old roots treated in May were not highly active; only 12 out of 25 treated shoots moved the tracer downward; the distance averaged 5.5 inches. In a later test, 10 preblossoming plants and 10 blossoming plants were treated. Five preblossoming plants treated on an upper leaf moved the tracer down an average of 4 inches; on a lower leaf, 13.2 inches. Of 10 blossoming plants, only one moved the tracer—downward 1.5 inches.

Tests in September and October proved that 2,4-D* was moved most actively in plants growing in moist soil. Movement was most rapid and extensive in plants in the seeding stage. These tests indicate that 2,4-D* moves where foods are moving in morning-glory plants.

A group of large plants growing in a Yolo sandy loam was used in August, 1953, for tracer tests. After treatment, roots were dug to depths of 5 feet or more. Treatment time was the predominant factor determining depth of transport. After a 4-hour period, movement was 11 inches; after 27 hours, 30 inches; after 48 hours, 39 inches. In some of the latter plants, 2,4-D* was found in the lower extremities—in one, to a depth of 68 inches. Transport was not limited to vertical roots. Plants sprayed with an emulsifiable acid formulation, with 2,4-D* added, carried the tracer to the full depth of excavation in 8-day, 2-week, and 3-week periods.

ACKNOWLEDGMENTS

Several people were responsible for the experiments described in this paper. Mrs. Barbara Kean, Charles J. McCarthy, and James E. Pallas, Jr. planned the work, treated the plants, and prepared the autographs. Some early work

by Kenneth M. Smith (1950) in partial fulfillment of the requirements for the Master of Science degree is cited as background material.

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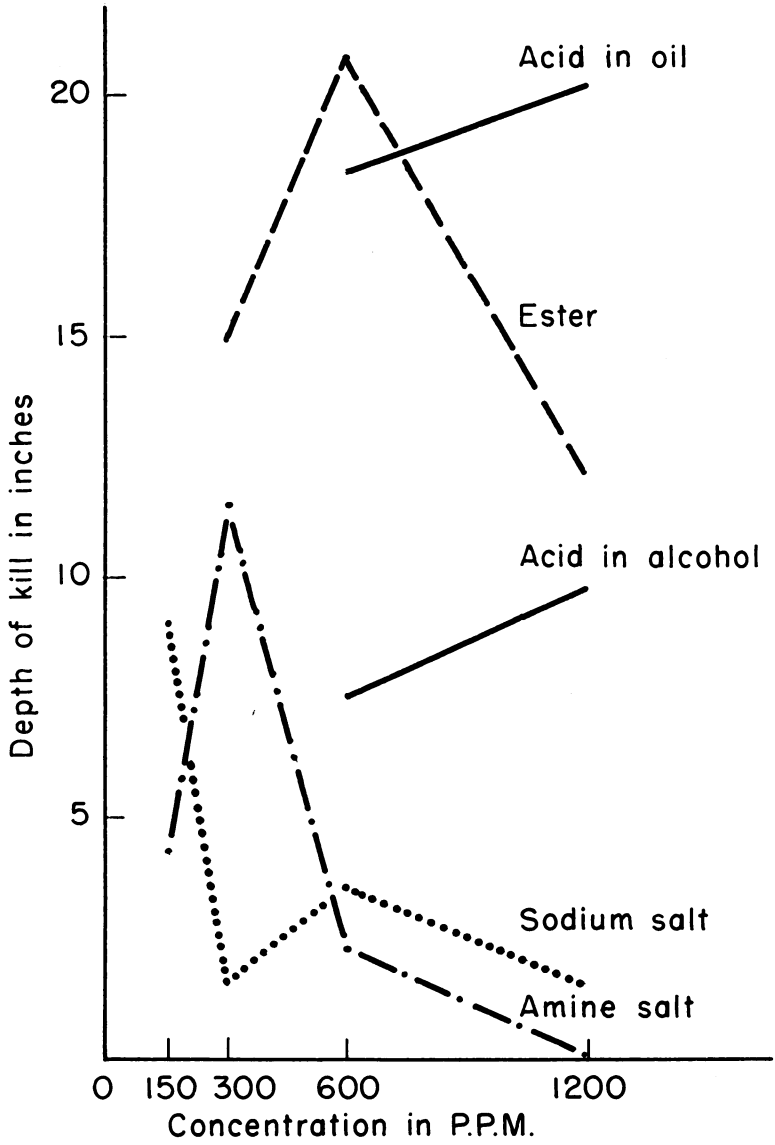

Fig. 1. Depth of kill produced by five different 2,4-D formulations, shown in inches as an average of six plants. Concentrations are shown for the individual applications. With the 150-ppm plots excepted, the total amount applied was, in all cases, 1,200 ppm.

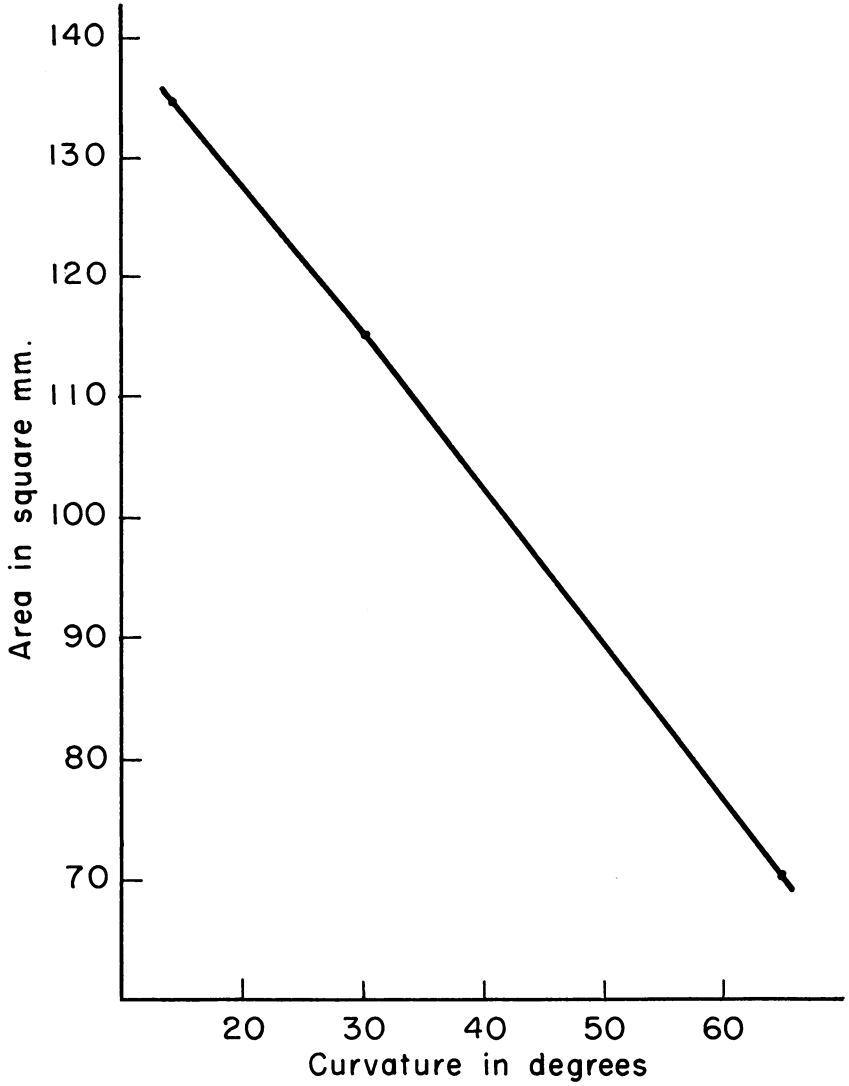

Fig. 2. Effect of area exposed to the herbicide on the intensity of plant response. Total amount of herbicide was, in all cases, the same; concentration was, in all cases, 1,000 ppm. Area covered was altered by applying in one drop, two drops, and four drops. Response is indicated by degree of curvature.

Fig. 3. Radioautograph of wild morning-glory seedlings, each treated on one cotyledon for a period of 2 hours. Dosage, 10 μg of 2,4-D*. Exposure, 1 week.

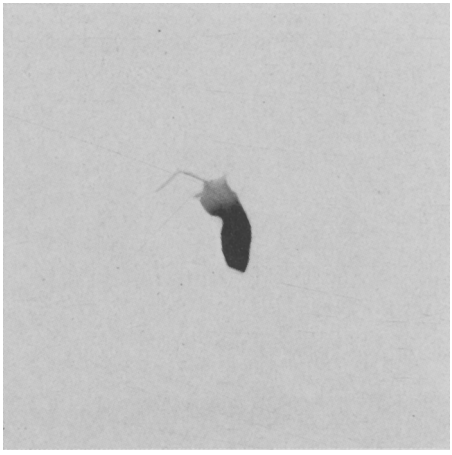

Fig. 4. Radioautograph of plant treated on a tip leaf that was actively growing. Evidently this leaf was still importing foods. Dosage, 1 μg with 1 per cent Nonic 218. Exposure, 1 week. Treatment period, 8 hours.

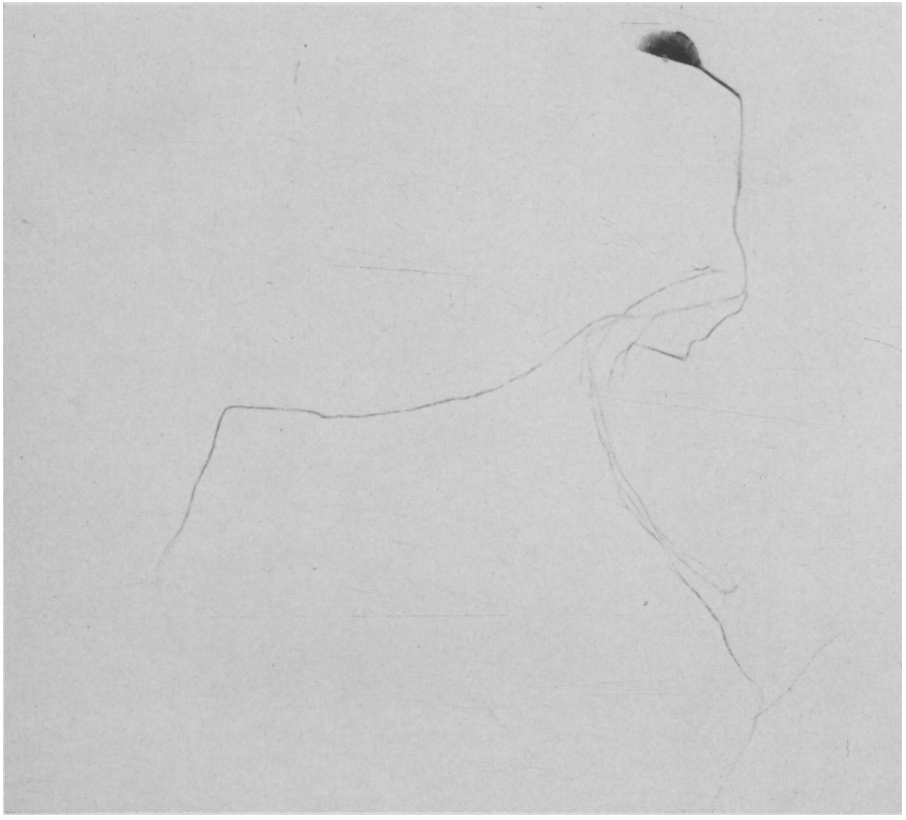

Fig. 5. Radioautograph of a plant treated on one cotyledon. Dosage, 1 μg of 2,4-D* with 1 per cent Nonic 218. Exposure, 1 week. Treatment period, 8 hours.

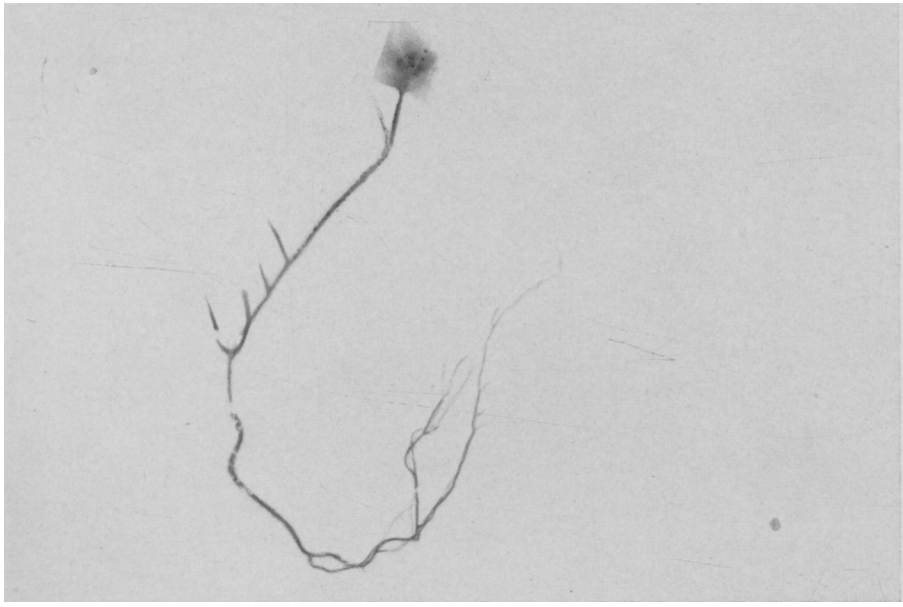

Fig. 6. Radioautograph of a 6-leaf plant, treated on one middle leaf, showing extensive movement of 2,4-D* into the roots. Note uniform distribution of 2,4-D*. Dosage, 1 μg with 1 per cent Nonic 218. Exposure, 2 weeks. Treatment period, 4 hours.

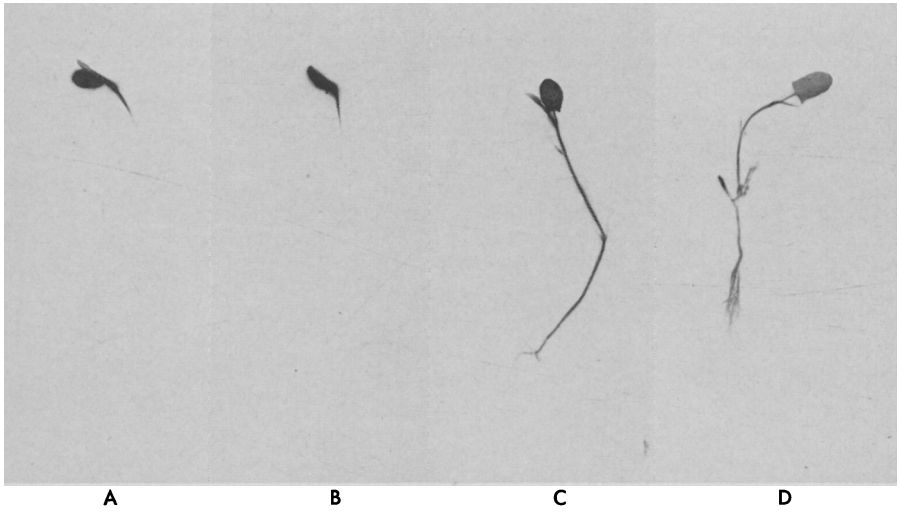

Fig. 7 *A*, *B*, *C*, and *D*. Radioautographs of young morning-glory plants treated on young leaves. Plants *A* and *B* illustrate lack of transport from very young leaves; plants *C* and *D* show transport from more developed leaves. Note, in the latter, the uniform distribution of 2,4-D* within the treated plants, indicating active transport rather than a purely diffusional movement. Dosage, 1 μg with 1 per cent Nonic 218. Exposure, 2 weeks. Treatment period, 4 hours.

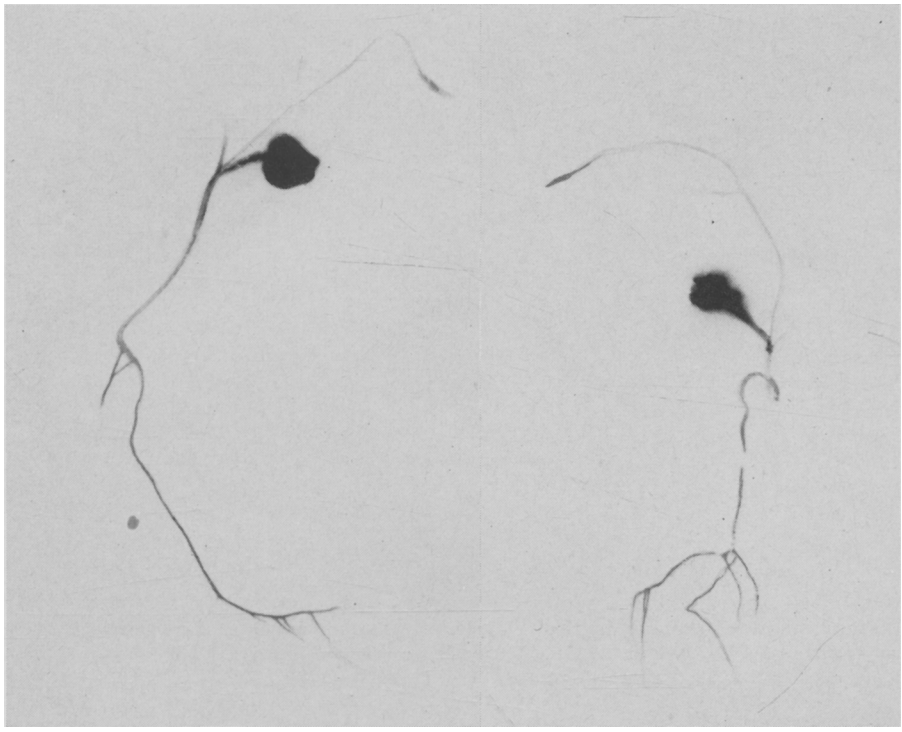

Fig. 8 *A* and *B*. Radioautographs of young morning-glory plants treated on one cotyledon. Movement in these has been two-directional. Dosage, 1 μg with 1 per cent Nonic 218. Exposure, 2 weeks. Treatment period, 4 hours.

Fig. 9 *A* and *B*. Radioautographs of young morning-glory plants treated on a tip leaf. *A*, movement from a fully expanded leaf into the roots; *B*, retention of 2,4-D* in the treated leaf. Dosage, exposure, and treatment as in figure 6.

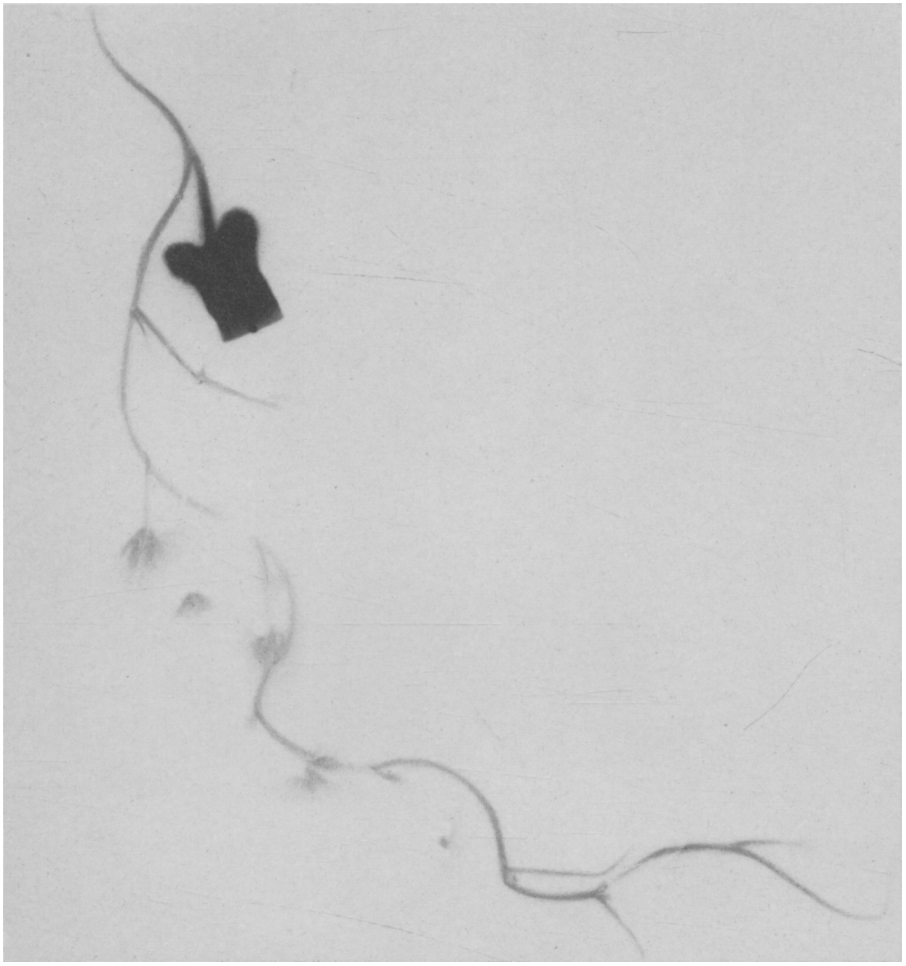

Fig. 10. Radioautograph of wild morning-glory plant treated on a middle leaf in the bud stage. Movement of 2,4-D* was predominantly upward, and a slight activity can be seen in the young tip of the stem; the image is too light, however, to reproduce. Dosage, 5 μg with 0.1 per cent Nonic 218. Exposure, 4 weeks. Treatment period, 4 hours.

Fig. 11. Radioautograph of field-grown morning-glory plant treated on May 30, 1952. Plant was in the preblossoming stage, and treatment was on a lower leaf. Dosage, 5 μg with no surfactant. Exposure, 4 weeks. Treatment period, 8 hours.

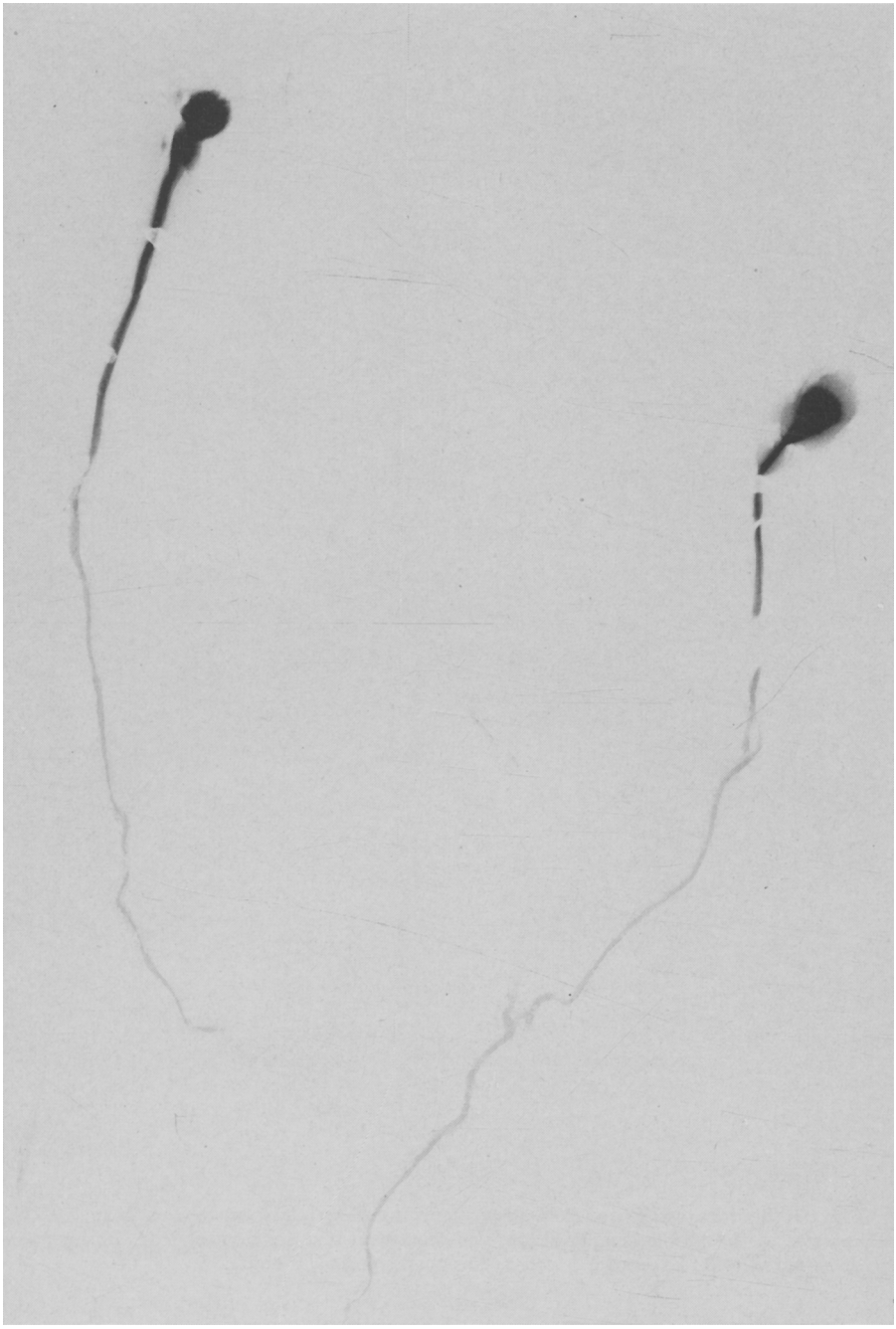

Fig. 12 *A* and *B*. Radioautographs of two field-grown plants from a dry location. Date of treatment, September 24, 1952. Treatments were on middle leaves in the preblossom stage. Dosage, 5 μg with 0.1 per cent Nonic 218. Exposure, 4 weeks. Treatment period, 4 hours.

Fig. 13. Plant treated as in figure 10. Movement here is both upward and downward.

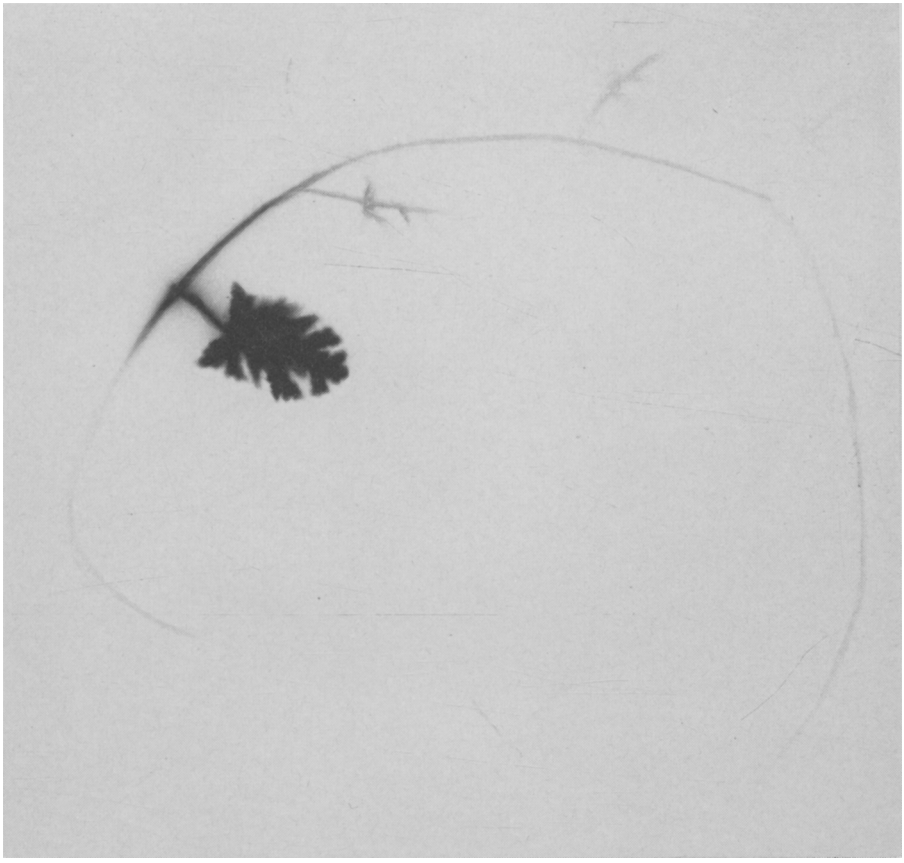

Fig. 14. Radioautograph of a plant from an irrigation ditchbank. Movement here is both upward and downward.

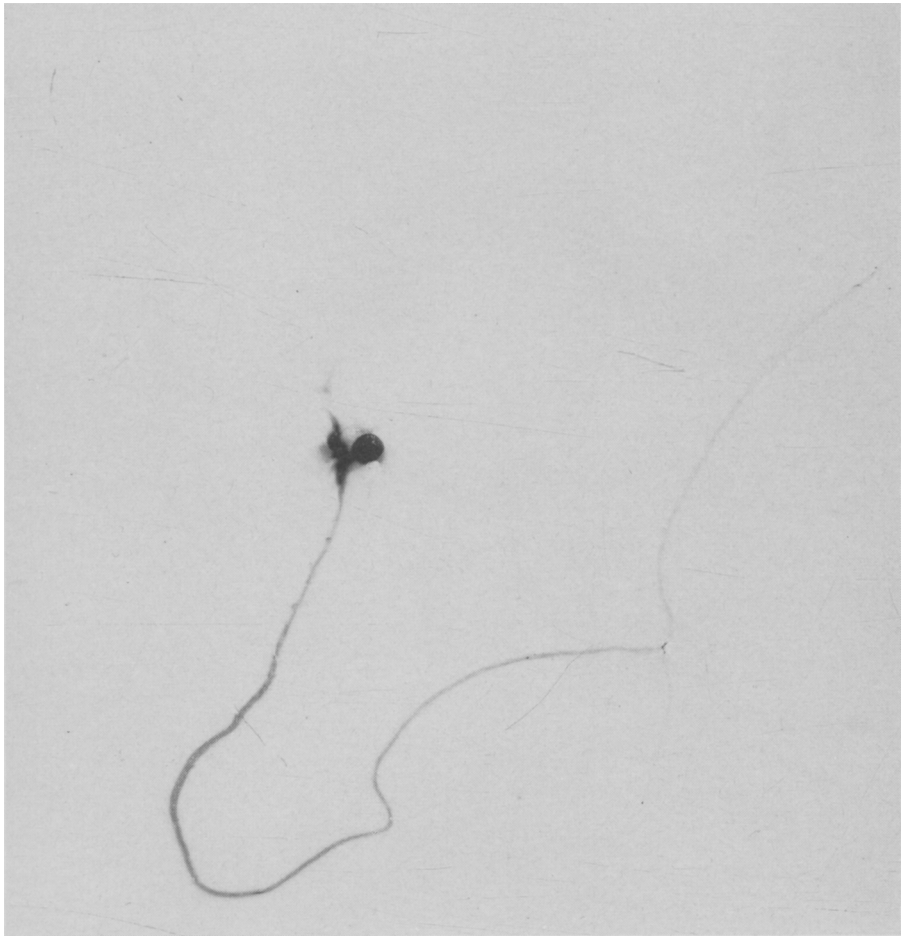

Fig. 15. Radioautograph of plant treated on a lower leaf, August 4, 1952. Note the two-way movement along the horizontal root. Dosage, 5 μg with 0.1 per cent Nonic 218. Exposure, 4 weeks. Treatment period, 27 hours.

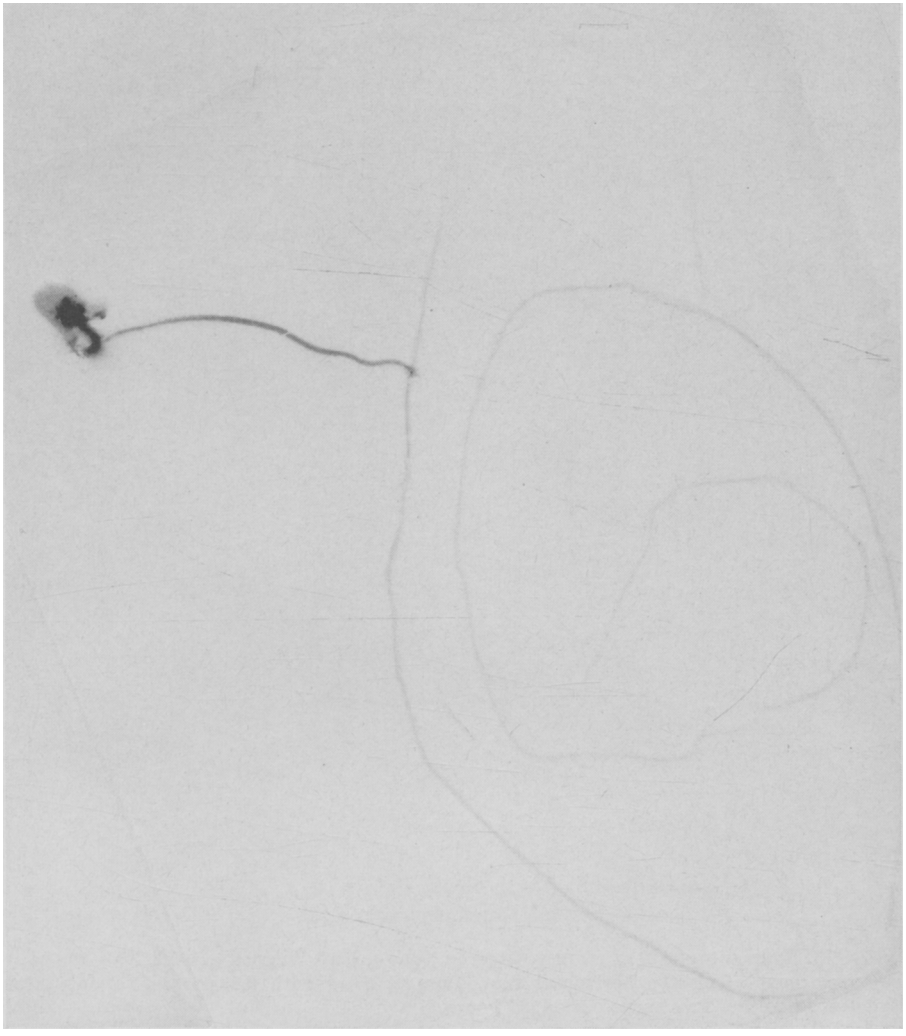

Fig. 16. Plant treated as in figure 13 except that the treatment period was 48 hours.

Fig. 17. Radioautograph of a sprayed plant. Dosage, approximately 5 μg. Exposure, 4 weeks. Treatment period, 4 hours.

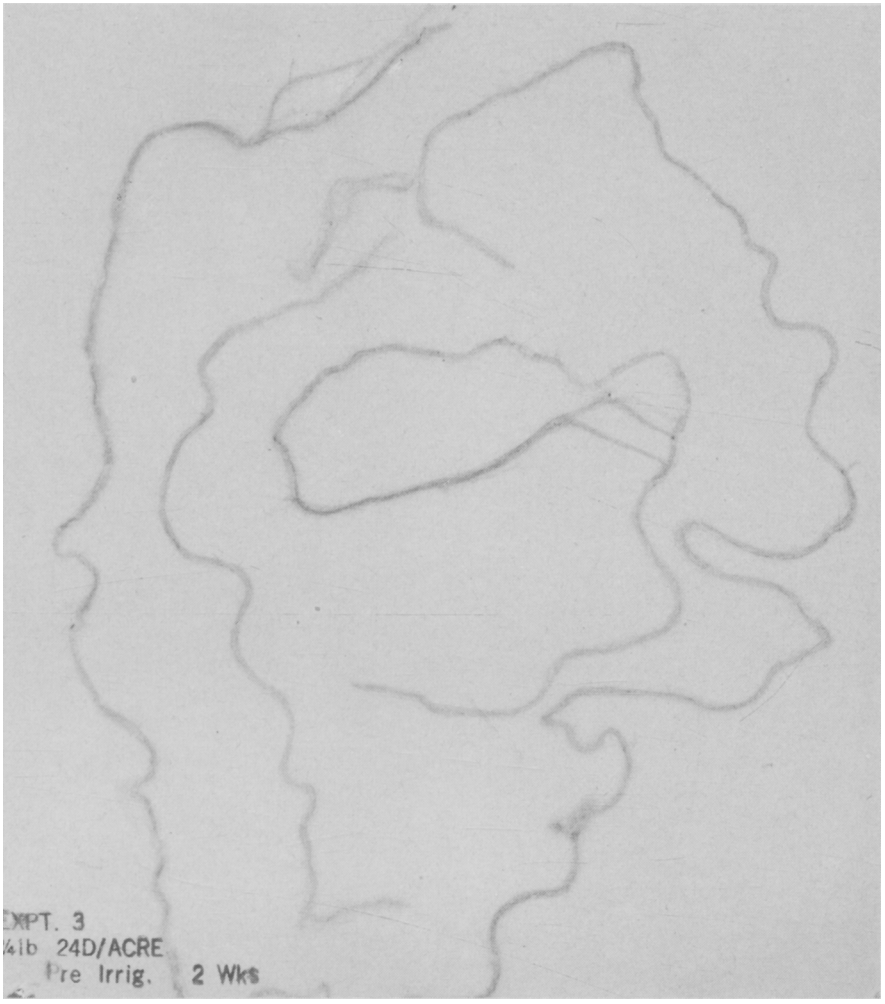

Fig. 18. Radioautograph of root of a sprayed plant. Tracer had moved to the total depth excavated following a treatment period of 2 weeks. Dosage, approximately 5 μg. Exposure, 4 weeks.

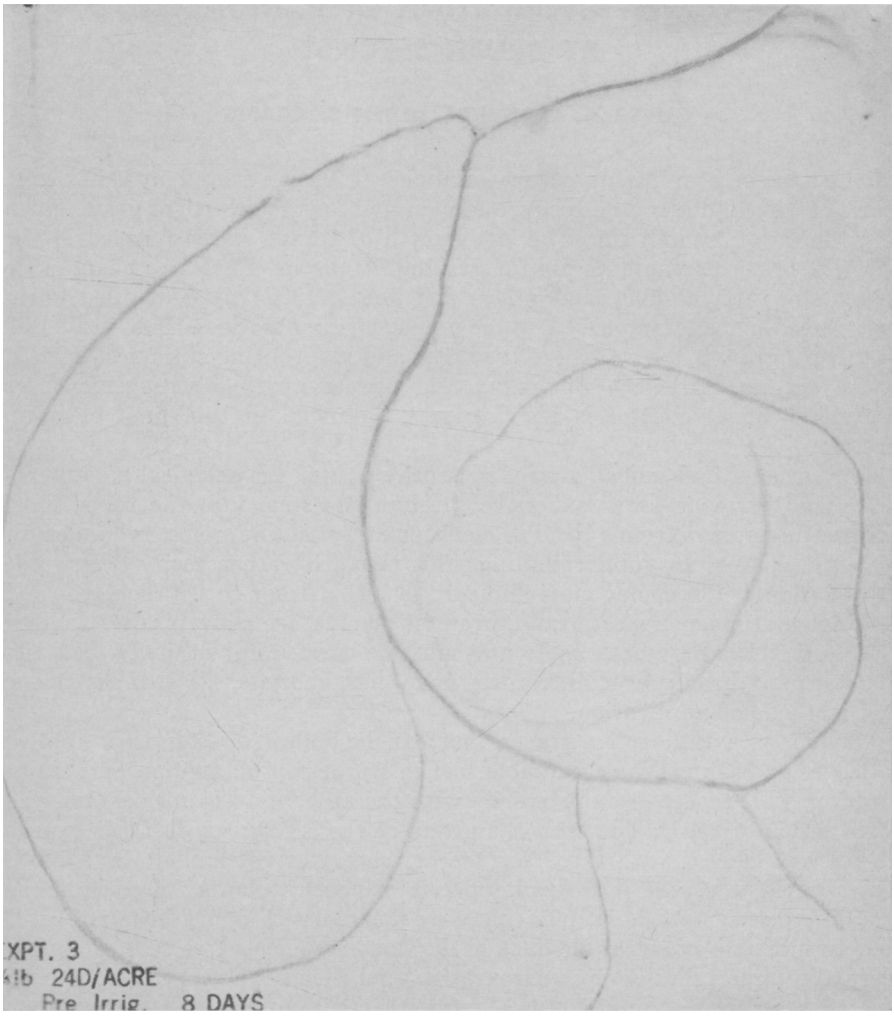

Fig. 19. Plant like that of figure 16 except that the treatment period was 8 days. Note movement of the tracer into a horizontal branch (left) as well as into the vertical root.

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