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HERBICIDES
Their Absorption and Translocation

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Autographs of bean plants treated with radioactive 2,4-D prove that absorption is rapid, that the chemical migrates to the vascular tissues, and that it translocates at rates up to 100 cm. per hour through the stem and into the roots. The epicotyl of young bean plants bends, owing to the presence in it of 2,4-D. Radioactivity decreases in time in the roots and treated leaf; it accumulates up to 72 hours in hypocotyl, epicotyl, and terminal bud. An adequate water supply is required for bending of the epicotyl from 2,4-D but not for translocation through the vascular system. When radio-2,4-D is applied to spots on the bean leaf, much more 2,4-D is absorbed and translocated from spots over the midrib than from spots on the edge or tip of the leaf.

HERBICIDE SYMPOSIUM

If a herbicide is to kill the cells of leaves after application, it must penetrate the cuticle and move into the mesophyll. To kill stem and root cells at a distance from the foliage to which it is applied, it must migrate across the mesophyll into the vascular system and there move or be carried to the lower portions of the plant. Each of these processes is of both practical and theoretical interest to plant physiologists.

Cuticle

It is common knowledge that the surfaces of leaves are coated with cuticle, a fatty or waxy layer that tends to prevent rapid loss of water vapor.

Figure 1 shows a transverse section of *Photinia* or toyon, a common woody plant of the range lands of the West Coast. The upper epidermis is a distinct layer with well-developed cuticle. The lower epidermis is less strongly cutinized and is punctuated by stomata. Near the center of the section is a small vascular bundle. To enter the phloem which occurs on the lower side of the bundle, a chemical must traverse about 10 cells that constitute epidermis, palisade parenchyma, spongy parenchyma, and bundle sheath.

Figure 2 shows a pear leaf, typical of woody species encountered on the range. This section illustrates the larger veins and here, again, passage of a chemical from the upper epidermis to the phloem involves passage through living cells of several distinct types.

Figure 3 is a section of a wheat leaf, typical of many herbaceous species. Again, movement into the phloem involves passage through the cuticle, migration through cells of several types, and finally release into the phloem tissues.

The lipoid nature of the cuticle is widely recognized and it undoubtedly has played an important role in enabling plants to migrate from water to land and thence to the arctic regions, to the deserts, and to the great array of environments in which plants grow. In a sense, cutinization has enabled plants to take their aquatic environment with them into these various regions. The intercellular air within the stomatal chambers seldom attains a relative humidity much below saturation, for each 1% increment below the 100% level represents a drop of many atmospheres in diffusion pressure deficit of the air, and mesophyll cells cannot tolerate an intercellular atmosphere below about 98% relative humidity.

Relatively impervious to all polar compounds, the cuticle also prevents the ready inward movement of herbicidal chemicals. What is known concerning its detailed structure?

Figure 4 gives two interpretations of the submicroscopic structure of cuticle as described by Frey-Wyssling (*3*). In the upper view solid lines in the horizontal and vertical directions designate cellulose; dashed lines, pectin. The diagonal grid represents the interlinked cutin chains that make up the lipoid phase. There is no attempt here to represent the actual volumetric relations of these substances.

The lower view of Figure 4 shows the intercalation of cutin wax in the cuticle layer. It is apparent that such walls are made of four distinct substances that may vary widely in composition and in distribution: cellulose, pectins, cutin waxes, and cutin.

Cellulose, fibrillar in nature, is responsible for the tensile strength of the walls. Pectins are amorphous and highly hydrophilic; they account in part for the ability of the walls to retain water.

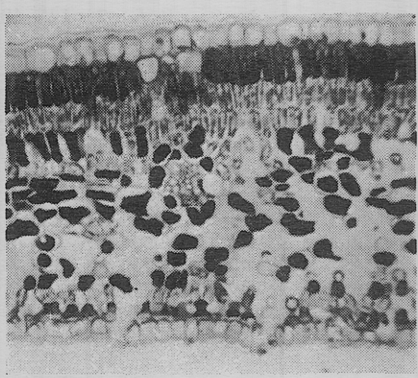


Figure 1. Transverse section of *Photinia* or toyon

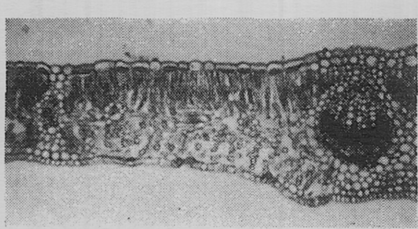


Figure 2. Pear leaf, typical of woody species encountered on the range

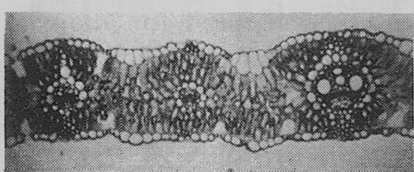


Figure 3. Section of a wheat leaf, typical of many herbaceous species

Cutin waxes are short-chain esters and alcohols of relatively low molecular weight and lack reactive end groups; some are cyclic in structure. Cutins are polymerized acids and alcohols of high molecular weight. They contain reactive end groups capable of forming esters and ethers, they may contain unsaturated linkages, and they dissociate, with the result that the leaf surface has a residual negative charge.

Cellulose and pectins are hydrophyllic compounds permeable to water and polar compounds. The ready movement of sugars, alcohols, and many other organic compounds through parenchyma cell walls in plasmolysis tests is evidence of such permeability.

Cutin and cutin waxes are lipophyllic, and the ready penetration of light aromatic oils of the xylene type is evidence of their permeability to such compounds. Leaves having continuous cuticle are much less permeable to heavy oils and it is common experience that viscous fuel oils may coat leaves and, even though toxic, may cause little or no injury for days.

Young leaves have thin cuticle, which is very permeable, particularly to light oils. As leaves mature, the cuticle becomes thicker and less permeable to ap*plied substances. These relations are* illustrated by the following facts: Dinitro substituted phenols are roughly twice as toxic as equivalent concentrations of their sodium salts; aliphatic *esters of 2,4-D are two to four times as* toxic as the sodium salt, but they do not translocate well; the glycol-type esters of 2,4-D retain the high toxicity of the light esters but they translocate about as well *as the straight acid.* In the control of deep-rooted perennial weeds on agricultural lands and brushy species on ranges, these facts are of paramount importance.

Using 2,4-D as one example, it is evident that its salts in aqueous solution are highly ionized, particularly the salt of a strong baselike sodium. If acid is added to the solution of the sodium salt, ionization is repressed progressively as the pH of the solution is lowered. That this process enhances the absorption and translocation of 2,4-D is shown in Table I, which indicates the role of pH. The data are average curvatures of 10 plants that have been subjected to the "bean test" as illustrated in Figure 5. Similar results have been obtained using 2,4,5-T, and toxicity tests with dinitro substituted phenols confirm this relation.

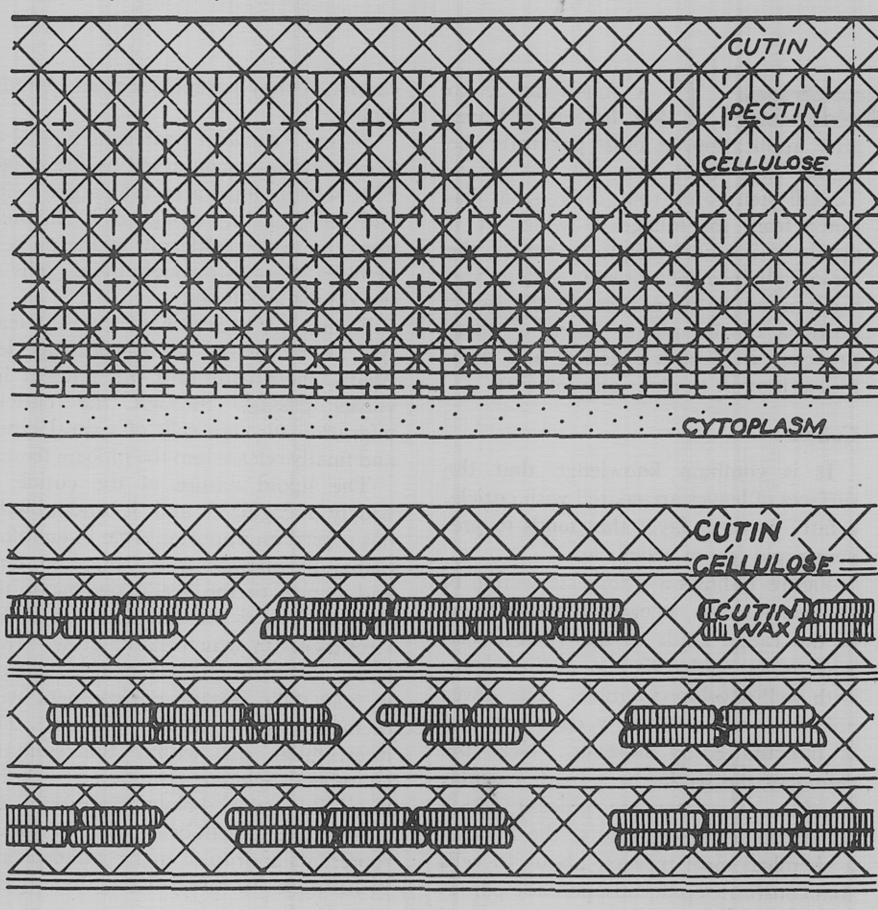
Day (*7*) used 2,4-D acid to study 2,4-D absorption and translocation. Utilizing this same bean test, he showed that movement of the chemical from the cuticle to the phloem required about 1 hour. The rate was 25 to 35 microns per hour. Translocation through the midrib and petiole to the epicotyl occurred at an average rate of about 50 cm. per hour, and somewhat over 40 minutes were required for the initiation of bending. The maximum amount of absorption occurred in the first 4.5 hours. The time required for absorption and translocation was independent of dosage.

To pursue this type of study further, 2,4-D containing carbon-14 in the carboxyl position has been employed. Red kidney bean plants are grown until the unifoliate leaves are fully expanded. Then, under uniform lighting from a combination of fluorescent and incandescent lights they are treated by placing a drop of 2,4-D acid solution in 50% alcohol plus 0.1% Nonic 218 (a surfactant by Sharples Chemicals, Inc.) upon one leaf. At specified times the roots are washed free of soil and the plants are rapidly killed by being placed between blocks of dry ice. They are then dried and autographed on x-ray film. (See pages 53 and 54 for rest of figures.)

Mechanics of Absorption

There are three possibilities for the applied material: It may remain on the outer surface of the cuticle and, upon drying of the carrier, deposit as a liquid or as fine crystals; it may penetrate into the cuticle and remain there in solution

Figure 4. Two interpretations of the submicroscopic structure of cuticle as described by Frey-Wyssling (*3*)



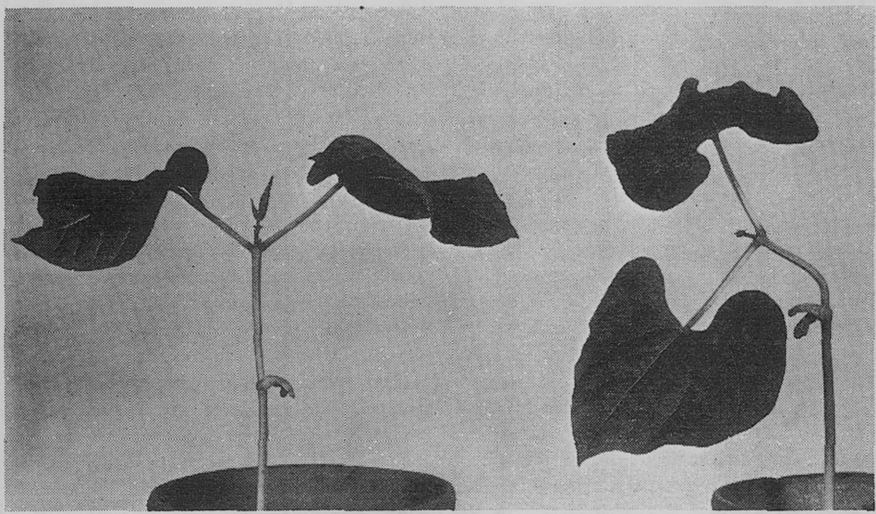


Figure 5. Average plant that has been subjected to the "bean test"

in the fatty medium; or it may penetrate the cuticle and then part into the aqueous phase of the epidermal cells, whence by continued migration it may eventually reach the vascular system.

Ions of the salts of the substituted phenol derivatives probably remain predominantly on the leaf surface, only a small portion actually penetrating to the interior. That portion, being highly polar, may readily part into the aqueous phase.

Light esters and, in fact, heavy esters of a highly aliphatic nature can readily penetrate the cuticle, but field tests and greenhouse results seem to indicate that they have little tendency to part in quantities sufficient to move into the vascular system. Rapid killing of leaves is common, but aliphatic ester forms of 2,4-D do not move into roots in sufficient quantity to be effective. Probably the crystallized cutin waxes are of importance in this effect and the cutin acids are also capable of dissolving and holding such esters. Trials of the octadecyl ester of 2,4-D by the bean test gave no indication whatsoever of movement from the treated leaf.

Heavy esters formed from alcohols having both water and fat solubility are proving more effective as translocated herbicides than either the salts or the aliphatic esters. And 2,4-D acid, if applied in the proper way, has proved highly effective. The original Weedone contained 2,4-D acid in Carbowax as a cosolvent. It was very effective against perennials. The butoxyethanol ester also shows effective translocation, and many formulations on trial have long chain substitutions that are both lipophilic and hydrophilic.

On brushy species, where translocation is essential to the successful use of a herbicide, oil in the formulation seems essential. One of the most effective formulations consists of suspended 2,4-D acid in an emulsion of a nontoxic oil. When this dries on the leaves, the 2,4-D acid is held on the surface of the cuticle by a thin film of oil and apparently it dissolves slowly and moves on into the vascular system at a concentration that causes little immediate damage to the mesophyll but is sufficient to accumulate in the roots and kill them.

Oil has proved beneficial not only in formulations of the acid but also in ester formulations. Here the oil, soaking into the cuticle, tends to saturate it with respect to its lipophilic capacity, and so to free the 2,4-D ester for movement on into the phloem in the aqueous phase.

Transport of 2,4-D in Plants

It seems well established that movement of 2,4-D in plants occurs with food materials, and the autographs indicate that it travels with the assimilated stream by some sort of mass flow. No other mechanism can account for the rapidity of movement, the unidirectional movement so often found, and the fact that dosage applied has no effect on linear rate of movement. The movement indicated in Figure 8 shows an actual transport rate of 25 cm. in the 30-minute exposure period, and some time must elapse between the time of application and arrival of the tracer in the phloem. If only half of the treatment time is allowed for penetration of the cuticle and movement across the mesophyll, the actual rate would be 25 cm. in 15 minutes or 100 cm. per hour. Day recorded one rate measurement within this range and many other rate studies on phloem transport of viruses and foods indicate such values.

Requirements for Control Of Weeds and Brush

If these views of the absorption and transport of 2,4-D have produced a true picture of the mechanics of these processes, then it is possible to describe the requirements for successful use of the material in controlling perennial weeds and brush. First, there must be active synthesis and normal movement of foods in the plant and these must be moving to growing points and cambium tissues—that is, the plants should be in active growth rather than in a condition where storage predominates. This would indicate the budding or early blossoming stage for many plants. Field observations confirm this as the optimum time for treatment.

For the 2,4-D type the acid form seems best, but it is not especially convenient because of its low solubility in both oil and water. Some emulsifiable formulations of 2,4-D acid have given excellent results in field tests. Second to the acid are the heavy esters, several of which have proved excellent. However, this does not prove that all esters of high molecular weight will be effective. In fact, one might visualize many such esters being placed on the market with insufficient testing, and giving disappointing results.

Oils have proved essential as adjuvants in many formulations. Some preliminary work indicates that aromatic oils of high toxicity do more harm than good in this type of spray mixture. Aliphatic oils of low toxicity are being tested and those in the range from Diesel oil to heavier seem best. Such oils would fit the mechanism, in that they do not directly injure the leaf tissues, where ab-

Table I. Absorption and Translocation of 2,4-D

pH	Hours after Application 1	2	3	4	5	6	7	8
2	0	13	78	90	97	96	88	86
3	0	5	50	71	77	80	74	83
3.3	0	0	18	52	72	89	84	75
4	0	0	21	50	71	86	87	85
5	0	0	20	54	78	86	86	84
6	0	0	9	40	62	82	85	92
7	0	0	4	13	21	28	35	37
8	0	0	0	2	10	16	29	38
9	0	0	0	8	15	18	28	37
10	0	0	0	0	0	6	18	30

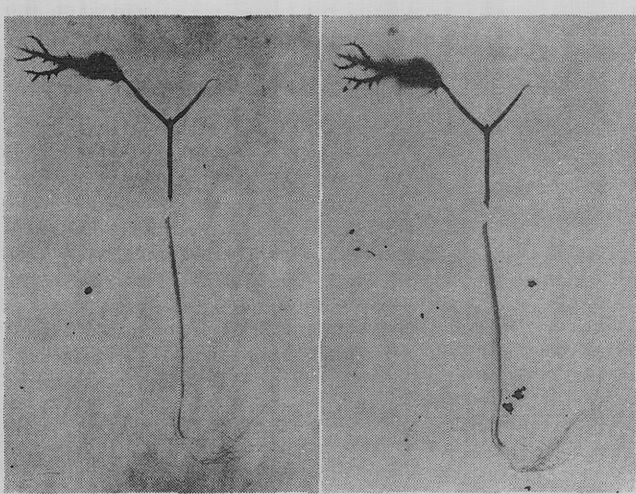


Figure 6. Two autographs made from the same plant, the one on the left having been exposed for 1 week on x-ray film, the one on the right 4 weeks. Exposure of 2 to 4 weeks has proved satisfactory; the longer exposure is best, but it slows the work to have to wait

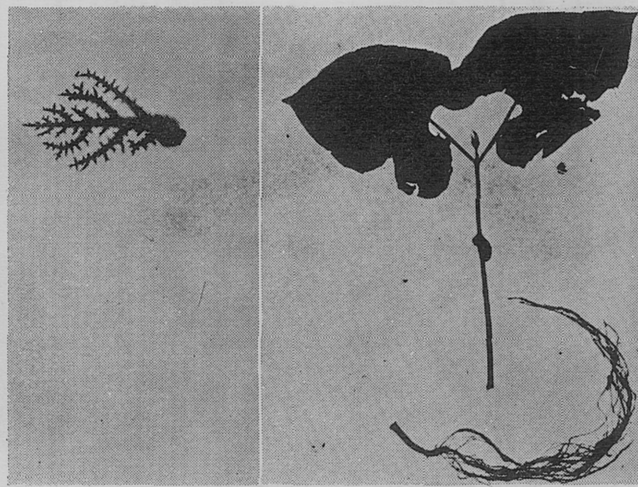


Figure 7. A 30-minute treatment, one of the two distinct types of movement of the radioactive 2,4-D found from several hundred autographs. The figure on the right is an Ozalid print of the autographed plant. In this case there was evidently leakage directly into the xylem and movement from the treated spot to the tip of the leaf in the transpiration stream. A few of the short-time treatments behave in this way

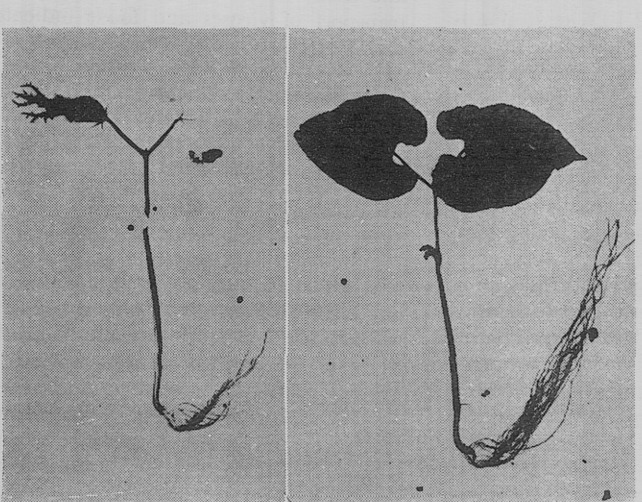


Figure 8. A 30-minute treatment giving what may be called the normal response, shown by the great majority of the autographs. Although there has been some movement toward the leaf tip, the greater part of the transport has been basipetal along the petiole, through the epicotyl and hypocotyl, and on down into the roots. At least half of the length of the roots has been traversed, and longer exposure during the autographing might have shown more extensive movement. There is no bending of the epicotyl. The treatment time was not long enough to allow for the bending response. The fact that extensive translocation has taken place in less time than that which Day found necessary for absorption into the phloem is probably explained by the presence of the surfactant in the applied solution

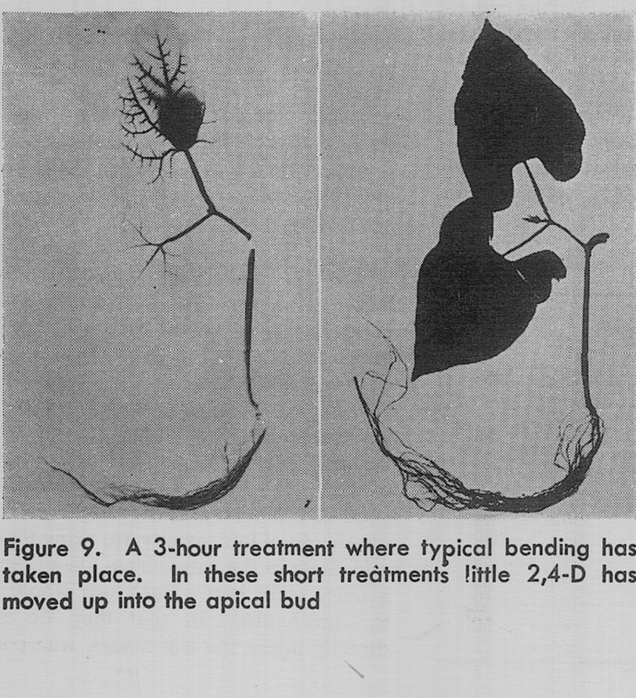


Figure 9. A 3-hour treatment where typical bending has taken place. In these short treatments little 2,4-D has moved up into the apical bud

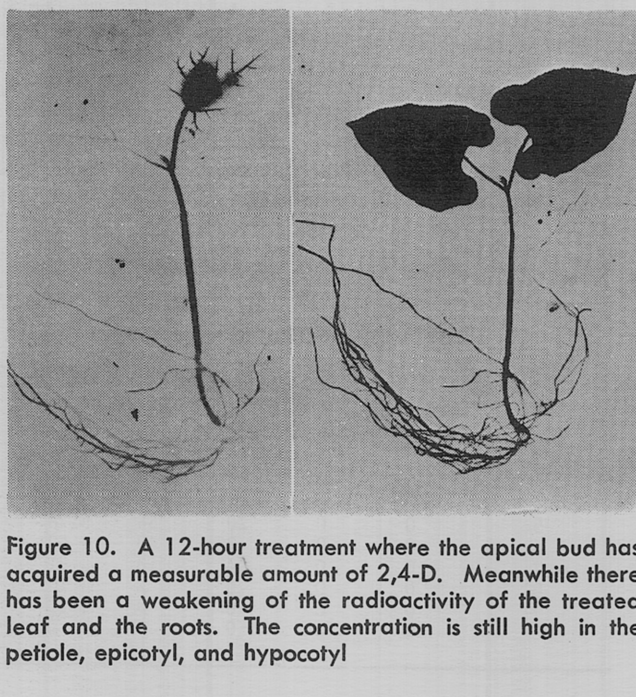


Figure 10. A 12-hour treatment where the apical bud has acquired a measurable amount of 2,4-D. Meanwhile there has been a weakening of the radioactivity of the treated leaf and the roots. The concentration is still high in the petiole, epicotyl, and hypocotyl

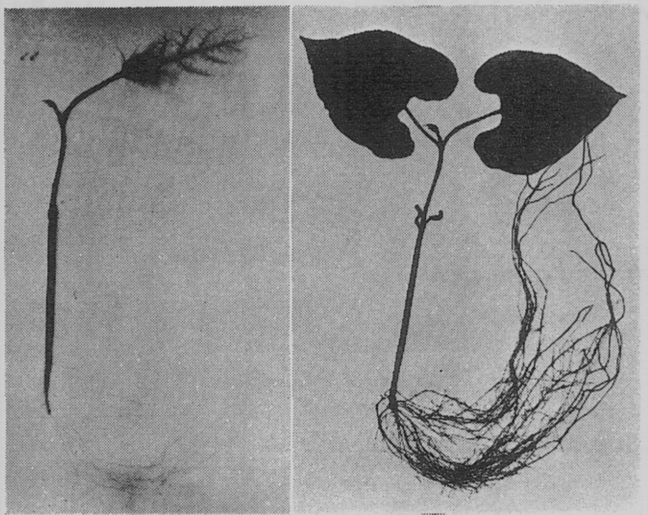


Figure 11. A 72-hour treatment where the apical bud has concentrated the 2,4-D, the petiole, epicotyl, and hypocotyl still retain appreciable quantities, but the roots and the outer part of the treated leaf have lost most of their radioactivity. This is attributed to assimilation of the 2,4-D with evolution of radioactive carbon dioxide. However, this aspect of the problem has not been studied in detail

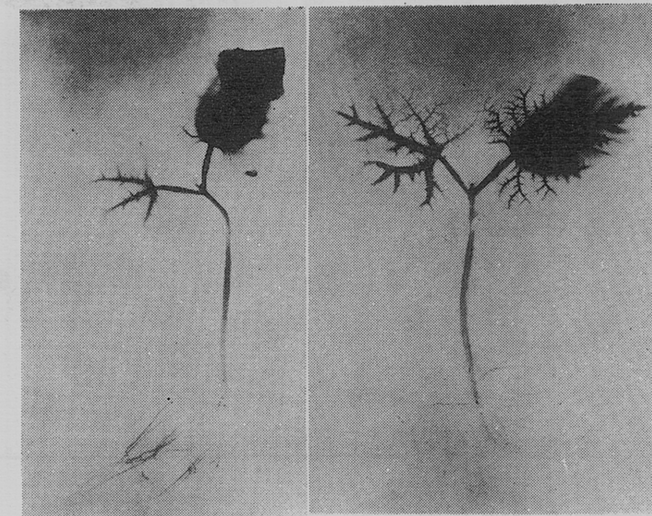


Figure 12. Two plants treated at the same time and autographed for the same period—2 weeks. The plant on the left was given the normal treatment, having been irrigated 1 hour before treating. The one on the right had not been irrigated since the previous day. In the bean bending test it had been found that irrigation before treatment was essential to the bending response. Evidently absorption and translocation are not dependent on irrigation

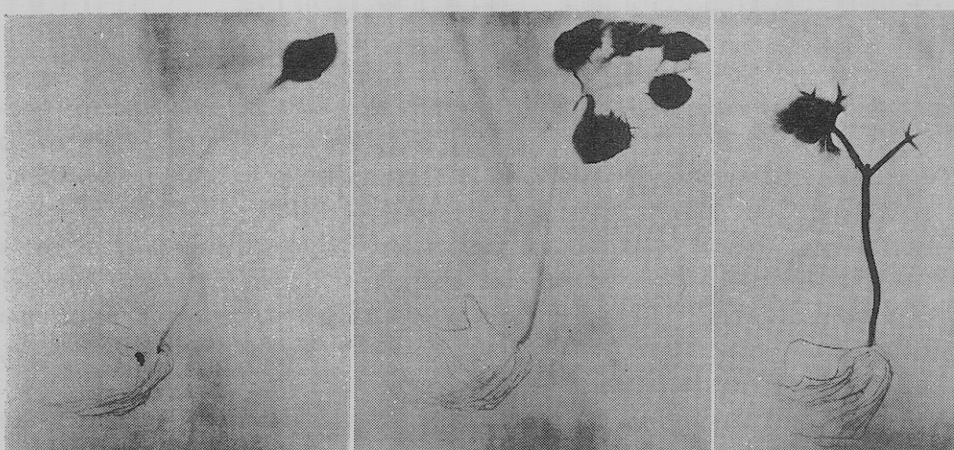


Figure 13. Effect of placement of the droplet of 2,4-D. On the left-hand plant a 50-microgram application was placed on the midrib near the tip of the leaf. In the center is shown a plant that received five 20-microgram droplets around the edge of the leaf. On the right-hand plant the 50-microgram droplet was applied to the midrib near the base of the blade. The autographs confirm Day's results and prove that placement is important in these studies

sorption and translocation are taking place, and being aliphatic they most nearly satisfy the lipoid-holding capacity of the leaf cuticle.

An emulsifier is required to stabilize the type of mixture being indicated. One should be selected that is low in inherent toxicity and well balanced with respect to the ratio of oil to water being used, and one that will foster spreading of and wetting by the mixture.

Little is known of deliquescent agents, penetrants, and sequestering agents from the standpoint of the type of spray mixture being described. Hard water should not affect ester forms of 2,4-D; both the oil and emulsion stabilizer probably foster penetration, and prolonged retention of moisture does not seem necessary. However, much remains to be done before one can say that these materials do or do not benefit the action of a formulation.

Application

As for application, Fisher (*2*) in Texas recommends a spotwise (coarse droplets) treatment as compared to a mist application that completely covers the leaf. Figure 13 indicates the reason for this. When the spray lands in restricted areas, even if these are injured, so long as assimilation proceeds, food movement from the remainder of the leaf will provide for transport of the herbicide.

Evidently all leaves on the plant should be treated, particularly the lower ones that are feeding the roots, and dosage should be regulated to cause a minimum of immediate damage. Probably the greatest drawback in the use of the propyl and butyl esters was the rapid injury to treated foliage.

Applications should be made when weather conditions favor rapid assimilation and rapid growth of the plant. Field observations have long since indicated that this is especially important when perennials are being treated.

Acknowledgment

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