HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOLUME 28 NOVEMBER, 1958 NUMBER 5

STUDIES ON THE ABSORPTION AND TRANSLOCATION OF 2,4-D IN BEAN PLANTS

O. A. LEONARD

UNIVERSITY OF CALIFORNIA · BERKELEY, CALIFORNIA

Relationships between the composition of 2,4-D solutions and their effectiveness as herbicides were investigated. The measure of effectiveness was the reduction in fresh weight of the growth above the primary leaves of bean plants. Two methods of application were used: the drop method (0.01 ml of solution) and the spray method. Variables studied were: diluents, humectants, surfactants, types of amines and esters, concentration, and dosage. Other studies included the effect of point of application on effectiveness and on the distribution of radioactive 2,4-D in the treated leaf and in the plant. For comparative purposes, some similar studies were conducted with radioactive aminotriazole and maleic hydrazide.

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STUDIES ON THE ABSORPTION AND TRANSLOCATION OF 2,4-D IN BEAN PLANTS[1]

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INTRODUCTION

NUMEROUS FORMS and formulations of 2,4-D (and other phenoxy herbicides) are possible, and methods of application may vary widely. Perhaps this is why most investigators have chosen to study such chemicals as auxins (that is, at dosages far below those used to kill plants) rather than as herbicides. The interrelationships between time of application and formulation (including additives), concentration, and methods of application are certainly important aspects to be considered in any basic approach to the study of 2,4-D as a herbicide.

With the development of radioactive compounds, problems of absorption and translocation of foliage-applied herbicides have been considerably clarified. Recently Crafts and Yamaguchi (1958) reported on some comparative studies with several radioactive herbicides. Although the studies with radioactive materials reported herein are primarily with 2,4-D (2,4-dichlorophenoxyacetic acid), their results can be better evaluated by comparison with a few studies with labeled ATA (3-amino-1,2,4-triazole) and MH (maleic hydrazide)[3] and with the results published by Crafts and Yamaguchi.

The purpose of the studies reported herein was to discover preparations that might be useful under field conditions and to arrive at a better understanding of the behavior of plants treated in a variety of ways. So far these goals have been only partially met; until a marked improvement in the use of 2,4-D is secured, they will continue to be major research objectives. At present the efficiency of 2,4-D is low to extremely low, as has been indicated by the data of Leonard and Crafts (1956) and Hay (1956). The original objective was to find superior formulations that might be tested under field conditions on woody plants; this has been the primary research of the writer.

[1] Received for publication August 1, 1957.

[2] Associate Botanist in the Experiment Station, Botany Department, Davis.

[3] Radioactive 2,4-D was purchased from Tracerlab, Inc; ATA was supplied by the American Chemical and Paint Co., and MH by the Naugatuck Chemical Division of the U. S. Rubber Co.

Absorption is considered to consist of two phases—penetration and cellular absorption. Penetration is mainly a physical process involving cuticular penetration or passage through cuticular cracks or stomata. Cellular absorption is a physiological process, but it may certainly be nonspecific and influenced by diffusion; however, energy changes may be involved. Translocation involves the movement of substances in plants. Both intrafascicular and extrafascicular translocation (that is, movement of substances inside and outside the vascular bundles) are considered in this paper.

Important aspects of this study are concentration of 2,4-D and method of application. These are important considerations from both practical and theoretical standpoints. It is indicated that there can be no such thing as a "best" mixture or preparation, but that the most effective formulation will vary with the method of application, the physiological condition of the plant, and the species involved. Day (1952) and Crafts (1956) have reported on some effects of 2,4-D on bean plants, as indicated by bending of the hypocotyl and epicotyl. The studies described herein expand the work of Day and Crafts, presenting the data in terms of weight reduction or, in some cases, plant kill.

METHODS

Seed of the red kidney bean (*Phaseolus vulgaris*) was planted in 4-inch clay pots containing Yolo fine sandy-loam soil with some compost to improve its texture. After emergence, the plants were selected for uniformity and thinned to two plants per pot. In the earlier tests 24 plants in 12 pots were used per treatment, but this was later reduced to 20 plants in 10 pots. The treatments were given 11 or 12 days after planting, and the plants were harvested 14 days later.

The drop method. One one-hundredth (0.01) ml of solution was supplied to the midrib on the upper surface of one of the primary leaves, generally within one-half inch of the base of the blade. At the time of treatment, the central bud or shoot above the primary leaves was very small, weighing not over 0.1 to 0.2 gm. The influence of the treatment was determined by removing and weighing all of the growth above the primary leaves. This method of evaluating effectiveness was used in all of the experiments described herein.

The spray method. Sprays were applied uniformly over a surface by using a U.S.D.A. conveyor-belt sprayer. The pots were placed in flats and the flats were placed on a conveyor belt which traveled at a known speed. The sprayer nozzle was mounted at a given height above the primary leaves. The type of nozzle used was designed to apply a uniform band of spray across most of its 80° angle; these nozzles were 8001E and 8002E Teejet tips (fan-type). The applications were made at 30 psi, and the acre-rates of application amounted to approximately 9 and 18 gallons for different tips.

To obtain some basis of comparison with the quantities applied by the drop method, approximate dosages per plant and per square inch were calculated from the total quantities of 2,4-D applied in the various spray treatments (table 1). The bean plants used in these studies had a leaf surface totaling about 16 square inches (one surface); because the blades were tilted, the actual surface exposed to the spray appeared to be about 11 square inches. It will be noted that the lowest concentration listed in table 1 corresponds to the lower limits of a herbicidal spray, although the volume of application per acre is very greatly increased. On the other hand, the highest dosage listed approximately corresponds to the quantities of 2,4-D applied by aircraft to kill weeds. It will be interesting to make certain comparisons between the effects of given dosages per plant and per unit of leaf surface applied by the drop and the spray methods.

TABLE 1

DOSAGE OF 2,4-D (OR OTHER CHEMICAL) PER PLANT AND PER SQUARE INCH OF PLANT SURFACE RESULTING FROM A SPRAY APPLICATION OF 9 GALLONS PER ACRE (8001E TIPS)

Concentration of 2,4-D (ppm)	gm/acre	µg/plant	µg/square inch (plant)
100	3.4	5.9	0.37
1,000	34.0	59.0	3.7
10,000	340.0	590.0	37.0

Lanolin rings were not generally used in these studies because many of the additives would have dissolved the lanolin to some extent, and this would have resulted in an unknown mixture of substances. Lanolin rings were used in a few experiments with radioactive 2,4-D.

Bending of the hypocotyls was measured following methods described by Crafts (1956) and Day (1952).

Radioactive isotopes.[4] 2,4-D* (labeled in the carboxyl C) was dissolved in 50 per cent ethyl alcohol and adjusted to a concentration of 5,000 ppm, with an activity equal to 0.25 µc (microcuries) per 0.01 ml or 10 µl (microliters). The solutions were prepared as the triethyl- and triethanolamine salts. These solutions were divided into three fractions; Tween 20 and Vatsol OT were added to two of these fractions, and the third contained no wetting agent. Dosages of 0.01 ml of solution were applied to the base and the apex of one of the primary leaf blades (upper surface), with and without the use of lanolin rings. Three days after treatment the blades were removed, freeze-dried, and autographed, using Kodak No-Screen X-ray film, for 30 days (fig. 5). The lanolin was removed with cotton before autographing, and the opposite side of the leaf was exposed to the X-ray film.

In another experiment 2,4-D* and ATA* were both used (fig. 6 and 7). In this case the 2,4-D* was dissolved in 95 per cent ethyl alcohol; the ATA* was dissolved in water. Both were adjusted to a concentration of 25 millimols per liter, to which was added 1 per cent Tween 20. The dosage was 0.01 ml, which contained 0.25 µc of radioactivity, and was applied to the bases and tips of the primary bean leaf blades. The application was made within a viscous lanolin-starch ring in which a plastic ring was set. Three days after application, the lanolin-starch ring was removed with cotton and the spot

[4] For convenience, the radioactive forms of these compounds are designated as 2,4-D*, ATA*, and MH*.

covered with masking tape. The plants were sectioned at once and the sections mounted on paper with starch paste; the mounts were freeze-dried immediately and then autographed for one month. Further details relevant to freeze-drying may be found in a paper by Yamaguchi and Crafts (1958).

A similar test was conducted with ATA* and MH*. The details that were different are described under Results in conjunction with the experiment.

Materials used. Many of the materials used are mentioned by their code or trade names. Some information concerning the chemical nature of these materials may be found in table 2. Substances that are referred to by their chemical names are not listed in this table. Much of the information given is not complete; however, these were the descriptions that were available to the writer.

TABLE 2

CHEMICAL MAKEUP OF THE COMPOUNDS WITH COMMERCIAL NAMES AND CODE NUMBERS REFERRED TO IN THIS PAPER

Commercial name	Chemical makeup
Tween 20	Polyoxyethylene sorbitan monolaurate
Span 20	Sorbitan monolaurate
Span 85	Sorbitan trioleate
G-7596 J	Polyoxyethylene sorbitan monolaurate
Myrj 53	Polyoxyethylene stearate
G-1069	Polyoxyethylene sorbitol oleate stearate
G-1006	Polyoxyethylene sorbitol hexalaurate
G-1036	Polyoxyethylene sorbitol hexalaurate
Tween 21	Polyoxyethylene sorbitan monolaurate
Nonic 218	Polyethylene glycol tertdodecyl thioether
Vatsol OT	Dioctyl ester of Na sulfosuccinate
Amine 220	1-hydroxyethyl-2-heptadecenylglyoxalidine
Tergitol penetrant 4	Na tetradecyl sulphate
Trem 619	Polyhydric alcohol ester
Vatsol K	Similar to Vatsol OT
Multimine	Fatty acid salts of an organic base
Sovaspray 100	Synthetic branched chain aliphatic oil
Bayol D	99% UR oil with viscosity 32 SUS*
Mentor 28	91% UR oil with viscosity 40 SUS*
RDA 228	95% UR oil with viscosity 57 SUS*
Solvesso 150	Aromatic oil
Dow-Corning 200 silicones	Dimethyl silicones
Dow-Corning 5191-35A	Emulsion containing 35% dimethyl silicone
Dow-Corning 5191-35B	Emulsion containing 35% dimethyl silicone
Dow-Corning 550	Phenyl methyl silicone
Dow-Corning 555	Methyl phenyl polysiloxane, vis. 10–30 cstks.
Dow-Corning 701	Phenyl fluid
Dow-Corning RXF 497	Silicone organic copolymer
Dow-Corning RXF 511	Silicone fluids compatible with 2,4-D ester
Dow-Corning RXF 512	Silicone fluids compatible with 2,4-D ester
Multifilm L	Mixture of fatty acids, petroleum sulfonates, and odorless kerosene

* UR, unsulfonatable residue; SUS, Saybolt universal seconds viscosity at 100° F.

RESULTS

The Drop Method

Water and oil as diluents for esters. The effectiveness of water, oil emulsions, and oil as diluents for esters of 2,4-D and 2,4,5-T were studied in preliminary tests. Actual kill was used as a measure of effectiveness. Kill was greatest with oil, intermediate with the oil emulsion, and least with water alone as the diluent. In these tests, the dosages ranged from 50 to 100 μg per plant. It is thought that the decided advantage of oil over the oil emulsion and water was due to its capacity to penetrate quickly into the interior of the leaf and to spread, both internally and externally. The almost instantaneous penetration of the oil into the leaf is readily observed by the rapid development of an oil-soaked appearance. In some tests on oleander leaves it was observed that some silicones and silicone-like compounds appeared to be superior to oils as penetrating agents. The best penetrant appeared to be tetraethoxysilane.

Comparison of hydrocarbons, oils, and silicones. Many experiments were conducted in order to learn more about the most desirable type of diluent to use with 2,4-D esters. Most of these tests were made with the propyleneglycolbutylether (PGBE) ester (pure ester); however, some difficulties were encountered with reference to the solubility of this ester in the pure hydrocarbons, in oils high in unsulfonatable residues (UR), and with many of the silicones. In later tests, it was observed that these difficulties were considerably less with the n-hexyl and n-octyl esters. It is felt that the greater compatibility of these esters with the solvents studied should present a more reliable relationship between the nature of the solvent and effectiveness than would exist if the PGBE ester were used. However, the general conclusions drawn were not appreciably affected by the type of ester used.

The data in table 3 show the results of various oil-like diluents on the effectiveness of the n-hexyl ester of 2,4-D. To prevent the solution from running down the petiole, the drop (0.01 ml containing 12 μg 2,4-D) was applied about three-fourths of an inch from the base of the blade, and the application was made to the leaf that was most horizontal. As stated previously, all of the solutions tested (except the very viscous ones) were observed to penetrate the blade tissue quickly; there was therefore insufficient external solvent left to create any serious movement externally along the midrib or petiole. A general description of the materials used in these studies may be found in table 2.

Some of the solvents were quite toxic to the leaves. Injury that is quickly produced should result in a reduction in the uptake and translocation of the 2,4-D, and this certainly was the case. The influence of slow-developing injury is less certain and probably varies with different plant species. Most of the oils resulted in some symptoms of injury after ten days, but no evidence of injury was visible with some of the silicones, including the dimethyl silicones and Dow-Corning 555 silicone.

Since most of the translocation of 2,4-D out of a bean leaf occurs within 24 hours (Butts and Fang, 1957), visible injury appearing a week or more after treatment may have no influence on the effectiveness of this compound on the bean plant. With plants that translocate 2,4-D over a longer period of time, the slow development of injury may be critical and quite important. Although n-hexane and n-dodecane are saturated aliphatic hydrocarbons, they produced contact injury to the leaves, which appeared to reduce the effectiveness of the 2,4-D. Contact injury by these hydrocarbons has previously been reported (Leonard and Harris, 1952). The two other pure hydrocarbons tested were less injurious to the leaves, and the effect of 2,4-D was greater than with the first two compounds mentioned.

TABLE 3

THE INFLUENCE OF DIFFERENT HYDROCARBONS, OILS, AND SILICONES ON THE EFFECTIVENESS OF THE N-HEXYL ESTER OF 2,4-D ON THE BEAN PLANT. THE APPLICATION CONSISTED OF 0.01 ML. OF SOLUTION CONTAINING 12 μG 2,4-D

Solvent	Injury to treated area			Weight as per cent of untreated plants*
	30 min.	3 hrs.	24 hrs.	
N-hexane	Gray	Wilt	Large dead spot	36 ± 3
N-dodecane	Brownish	Wilt	Large dead spot	40 ± 4
Hydro-tri-isobutylene	Gray	Oil soak	Small dead spot	27 ± 3
N-tetradecane	Grayish	Grayish	No injury	27 ± 4
Solvaspray 100	None	Oil soak	Oil soak	17 ± 2
Bayol D	None	Oil soak	No injury	16 ± 3
RDA 228	None	Oil soak	No injury	13 ± 3
Mentor 28	None	Oil soak	No injury	12 ± 2
Solvesso 150	Wilt	Wilt	Large dead spot	57 ± 5
D-C. 200 5 cstks.	None	None	None	12 ± 2
D-C. 200 100 cstks.	None	None	None	24 ± 3
D-C. 35A	Gray	Wilt	Large dead spot	49 ± 6
D-C. 35B	None	None	None	15 ± 3
D-C. RXF 497	Wilt	Wilt	Large dead spot	64 ± 8
D-C. RXF 511	Gray	Wilt	Dead spot	44 ± 6
D-C. RXF 512	Wilt	Wilt	Large dead spot	75 ± 6
D-C. 550	None	None	None	15 ± 2
D-C. 555	None	None	None	10 ± 2
D-C. 701	None	None	None	12 ± 2
Tetraethoxysilane	Wilt	Wilt	Wilt	50 ± 6

* Mean and standard error.

All of the high-UR oils appeared to be superior to the pure aliphatic hydrocarbons tested. The aromatic oil that was studied (Solvesso 150) produced quick injury to the blades and markedly reduced the effect of 2,4-D. The dimenthyl silicones were completely noninjurious, but these are rather poor solvents for even the n-hexyl ester of 2,4-D. The dimethyl silicone with a viscosity of 5 centistokes appeared to be superior to the one with a viscosity of 100 centistokes. Compounds No. 35A and No. 35B are listed as emulsions of the dimethyl silicones, but there was obviously a great difference between them; the one that resulted in injury to the leaf was much less effective. The best silicone and solvent used in these tests was No. 555, a methyl phenyl polysiloxane with a viscosity of 10–30 centistokes, which was compatible with the n-hexyl ester of 2,4-D. It also seemed very good with the PGBE ester.

Nos. 511 and 512 were prepared for compatibility with 2,4-D but caused so much contact toxicity that their effectiveness was quite low. Tetraethoxysilane was quickly toxic to the blades and likewise decreased the effectiveness of 2,4-D.

Influence of wetting agents on the effect of amines. Seventy or more wetting agents were studied, although not all of them by the drop method of application. The results to be presented below are for the drop method; results with the spray method are given in the following section. The data presented are representative of many tests. Special emphasis has been given to the results obtained with Vatsol OT and Tween 20 because Vatsol OT was consistently the best wetting agent in the drop tests (where 25 μg or more of 2,4-D was applied), whereas Tween 20 was the best wetting agent in tests with the spray method (using concentrations of 1,000 ppm or less; see figure 15). Some other wetting agents are included in the table for comparison.

The results in table 4 show the influence of several wetting agents on the effectiveness of the triethylamine formulation of 2,4-D. The effect of these wetting agents varied considerably, with Tween 20 and furfurylamine having the least effect and Vatsol OT and Vatsol K having the greatest effect; in most tests Vatsol K was slightly less effective than Vatsol OT. One quite noticeable influence of Vatsol was that it caused the drop to spread, especially along the veins. With Tween 20 the drop did not seem to spread. The results achieved with both 50 μg and 100 μg appear to be quite similar with respect to Vatsol OT and Tween 20.

In many experiments it has been observed that some oil added to the amines increased effectiveness, especially if the oil was emulsified. Several wetting agents were added to a nontoxic oil (Bayol D) and the effectiveness was compared, using three amines of 2,4-D (table 5). The oil and the wetting agents apparently reacted differently with the different amines. Vatsol OT with the 2-ethylhexylamine formed a poor emulsion which adhered to the sides of the container and to the pipette, thus probably reducing its effectiveness. Without a wetting agent, the triethanolamine was the least effective, the triethylamine intermediate, and the 2-ethylhexylamine the most effective.

In one experiment different wetting agents were added to an aqueous solution of the sodium salt of 2,4-D. Each plant received 50 μg. The sodium salt without a wetting agent had almost no effect on the bean plant, while all wetting agents contributed to the effectiveness of 2,4-D. The effect produced by Amine 220 was greater than that of any other material, probably because it resulted in the formation of an Amine 220 salt of 2,4-D; it was appreciably superior to Vatsol OT.

Effect of dosage. This experiment was conducted to determine some relationships between dosage and the relative effectiveness of Vatsol OT and Tween 20, used with the triethylamine salt of 2,4-D, in inhibiting the growth of the bean plant. The n-octyl and PGBE esters (in a nontoxic oil) were included in this study for comparison. Some solubility difficulties were experienced with the PGBE ester, especially at concentrations of 5,000 ppm and higher. Butyl cellosolve was added to keep the ester in solution.

TABLE 4

THE INFLUENCE OF WETTING AGENTS ON THE EFFECT OF THE TRI ETHYLAMINE SALT OF 2,4-D ON THE BEAN PLANT. CONCENTRATION OF WETTING AGENT, 0.5 PER CENT

Wetting agent	Weight of bean shoots above primary leaves as per cent of 2,4-D treatment without a wetting agent*
100 μg 2,4-D per plant	
Tween 20	93 ± 10
Amine 220	60 ± 10
Furfurylamine	115 ± 8
Polyethylene glycol 600 dioleate	54 ± 7
Methoxy polyethylene glycol 350 laurate	64 ± 11
Vatsol K	33 ± 5
Vatsol OT	33 ± 7
Nonic 218	73 ± 5
Trem 619	56 ± 5
Propylene glycol	94 ± 6
50 μg 2,4-D per plant	
Tween 20	105 ± 15
Tergitol penetrant No. 4	52 ± 10
Multimine	80 ± 16
Triton X-45	105 ± 17
Trem 619	61 ± 12
Vatsol OT	30 ± 4
Vatsol K	35 ± 8
Polyethylene glycol 600 dioleate	62 ± 10
Methoxy polyethylene glycol 350 laurate	77 ± 13

* Mean and standard error.

TABLE 5

THE INFLUENCE OF DIFFERENT OIL EMULSIONS ON THE EFFECT OF THREE AMINE SALT FORMULATIONS OF 2,4-D ON THE BEAN PLANT. THE EMULSIFIER CONCENTRATION WAS 0.5 PER CENT AND THE OIL (BAYOL D) WAS 2 PER CENT. THE DOSAGE WAS 25 μG OF 2,4-D PER PLANT

Additives		Weight of bean shoot as per cent of untreated plants*		
Oil	Wetting agent	Triethanol amine	Triethyl amine	2-ethyl-hexyl amine
		per cent	per cent	per cent
None	None	68 ± 6	48 ± 5	43 ± 5
Bayol D	Trem 619	38 ± 5	37 ± 5	20 ± 4
Bayol D	Vatsol OT	19 ± 3	20 ± 3	48 ± 5
Bayol D	Methoxy polyethylene glycol 350 laurate	44 ± 6	38 ± 5	25 ± 4
Bayol D	Polyethylene glycol 600 dioleate	40 ± 6	31 ± 5	21 ± 4
Bayol D	Amine 220	22 ± 4	31 ± 5	33 ± 5

* Mean and standard error.

Bending data, obtained one day after the plants were treated, are not included in this report, but the following results were observed. With the triethylamine formulation and no wetting agent, bending was less than for any other treatment, with Vatsol OT next, then the ester, and with Tween 20 having the greatest effect (with dosages of 0.7 to 12 μg per plant). Bending was not greatly increased by dosages above 12 μg per plant.

Results showing the percentage reduction in fresh weight of the bean plants after treatment are shown in figure 1. The amine formulation of 2,4-D with Tween 20 added was by far the most effective formulation with dosages of 0.7 to 3.0 μg, but the benefit due to Tween 20 largely disappeared with applications of 50 μg or more. Vatsol OT appeared to be of only slight benefit at the lower dosages used, but became more effective as the dosages were increased; however, Vatsol was not as effective in this experiment as it was in those summarized in table 4.

At dosages of 0.7 to 3.0 μg the esters were less effective than the best amine treatment. Evidently the base level necessary for activity is higher with the esters than with the amines; furthermore, the solubility or partition of the esters between the oil and the water in the plant was probably also partly responsible for the reduced effectiveness of the esters at the lower dosages. With dosages of 12 μg or more, the esters were more effective than any of the amine treatments. There did not seem to be any difference in the effectiveness of either of the esters that were tested.

Inhibition of growth of the bean plant was related to dosage, regardless of formulation and wetting agents. The capacity of a leaf to absorb and translocate 2,4-D was not impaired within the dosage range that was used. These observations refer only to the specific type of application made in this experiment.

Type of amine. Approximately 25 different amines[5] of 2,4-D were studied, although only 16 were studied by the drop method. Some results with three different amines are shown in table 5. Additives also appeared to influence the relative effectiveness of the different amines. The results presented below are for the amines in water without wetting agents.

The data in table 6 are the results from two different experiments and represent the type of results that were commonly obtained. In considering these data, it should be remembered that the concentration of 2,4-D was 0.5 per cent, a concentration too low to be useful in killing brush such as coyote brush (*Baccharis pilularis*) by aircraft application. For applications of 10 gallons of solution per acre, a concentration of over 3.5 per cent would be needed to result in an application of 3 pounds of acid equivalent per acre. Even though the concentration used was considerably lower than would be used in aircraft application, it was thought that the results might simulate the influence of the various amines when used in high concentrations and applied in low volume.

In comparing the ethanol and ethyl series of amines, it is evident that the latter was consistently more effective than the former. The ethyl- and ethanolamines were less effective than the secondary and tertiary amines;

[5] The amines used in these investigations were supplied by the Carbide and Carbon Chemicals Co.

however, the lack of effectiveness of the ethylamine was less evident than for the ethanolamine, since the latter crystallized out on the surface of the leaf and was not absorbed. The isopropylamine was more effective than the isopropanolamine; again, the low activity of the isopropanolamine appears to have been due to its crystallizing out. The di- and triisopropanolamines did not crystallize out and were considerably more effective than the monoisopropanolamine. Neither the n-butyl nor diisopropylamines were very effective; these amines were not very soluble in water and some of the amine may have adhered to the container. When applied to leaves, both of these materials seemed to form crystals on the leaf surface. Perhaps for these reasons, these amines were not effective. The 2-ethylhexylamine was the most effective amine studied while the di-2-ethylhexylamine was slightly less effective; however, the latter amine could not be used without emulsifying it, so the results are not strictly comparable.

TABLE 6

THE INFLUENCE OF DIFFERENT AMINES ON THE EFFECTIVENESS OF 2,4-D IN SUPPRESSING THE GROWTH OF THE BEAN PLANT. THE APPLICATIONS CONSISTED OF 50 μG OF 2,4-D APPLIED IN 0.01 ML; THE DILUENT WAS WATER

Amine type	Appearance of spot where application was made	Fresh weight as per cent of check (untreated)*	
		Test A	Test B
Triethanol	Small dead spot	54 ± 5	62 ± 5
Diethanol	Small dead spot	56 ± 7	60 ± 4
Ethanol	White crystals, no kill	98 ± 7	90 ± 4
Triisopropanol	Small dead spot, slow death	63 ± 7	54 ± 4
Diisopropanol	Small dead spot, slow death	65 ± 5	52 ± 4
Monoisopropanol	White crystals, no kill	92 ± 5	92 ± 5
Triethyl	Medium dead spot	25 ± 4	42 ± 4
Diethyl	Medium dead spot	27 ± 7	42 ± 4
Ethyl	Medium dead spot	70 ± 7	68 ± 5
Isopropyl	Medium dead spot	35 ± 6	42 ± 4
Diisopropyl†	No kill	50 ± 4	42 ± 4
N-butyl	Crystals, dead spot	75 ± 5	62 ± 5
2-ethylhexyl†	Medium dead spot	20 ± 4	35 ± 4
Di-2-ethylhexyl‡	Small dead spot	30 ± 5	50 ± 5

* Mean and standard error.
† 90 per cent water, 5 per cent ethanol, 5 per cent acetone.
‡ Test A: 88 per cent water, 5 per cent ethanol, 5 per cent acetone, 2 per cent oil emulsion; Test B: 89 per cent water, 5 per cent ethanol, 5.5 per cent acetone, 0.5 per cent oil emulsion.

One of the factors that might influence the results with the different amines is the toxicity of the amines themselves. In order to test the toxicity, aqueous solutions containing 10 per cent of the different amines were applied to bean leaves. Injury after one week was not apparent with the triethyl-, diethyl-, ethyl-, isopropyl-, or n-butylamines. Tissue kill was rapid with the monoethanol- and isopropanolamines. Tissue kill occurred but was slow with the di- and triethanolamines and di- and triisopropanolamines. With respect to possible injury due to treatment, there was evidently an inverse correlation between injury and effectiveness. How important this was in these experiments is questionable, since the actual concentration of the amines was for the most part less than 0.5 per cent. With applications of 3 pounds of 2,4-D per acre in 5 or 10 gallons of water, it is possible that the toxicity of the amines themselves may be an important factor.

Effect of alkyl esters. Because of volatility problems, there has been interest in developing esters of greater molecular weight. There have been differences in the effectiveness of different esters under field conditions, suggesting a need for further study.

As has already been noted, oil is the most effective diluent for esters (on bean plants, with the drop method) and oil solutions are easier to apply than emulsions. An oil (Bayol D) was therefore used as a diluent for several alkyl esters; however, since some of the esters were not sufficiently soluble in this oil, 12 per cent butyl cellosolve was added in all of the tests. Four concentrations of each ester were used. The drop was applied about three-fourths of an inch from the base of the blades to reduce the possibility of the mixture creeping down the petioles. The esters chosen for study were formed from straight-chain alkyl alcohols containing 1 to 18 carbons.

Data on bending are presented in figure 2. With dosages of 3 and 12 μg per plant the methyl ester was the most active, while the octadecyl ester was least active; all of the other esters were intermediate, without any special pattern being evident. Activity with 50 and 200 μg dosages was fairly similar for all except the octadecyl ester, which remained the least active of the esters tested. Crafts (1956) noted a similar relationship between alkyl esters in bending studies conducted over a 1- to 8-hour period. The methyl ester gave the quickest response, followed by the hexyl ester, while the octadecyl ester responded but little within the period studied.

Data in figure 3 show the influence of the different esters in suppressing the growth of the bean plant. The differences in growth response were certainly less marked than were the differences in bending. With dosages of 3 and 12 μg, the methyl ester was more effective than the others, with the octadecyl ester being the least effective, especially with the 12 μg treatment. All esters produced almost identical responses at 50 μg, while at 200 μg the methyl ester was less effective than the others, and the octadecyl ester was the most effective. While most of the differences in response presented in figure 3 are not great, the results suggest that the shorter-chain esters may be more effective when low concentrations are used and the longer-chain esters more effective with the higher concentrations. The reduced effect of the methyl ester at the higher dosages may be related to the somewhat greater contact injury produced by this ester, because of very rapid penetration and absorption.

Placement of the drop and influence of dosage. Crafts (1956) has already presented data on the influence of placement of the drop on the translocation of 2,4-D* out of bean leaves. Applications made to the edge of the blade resulted in less radioactivity in the stems than applications made to the base or central part of the midrib. Weaver and DeRose (1948) noted a similar relationship in regard to growth inhibition in bean plants. Day (1952) showed that placement of the drop influenced the bending response of beans.

The present study involved an amine formulation applied in water containing 0.2 per cent Vatsol OT. The purpose was to obtain a more direct measure of the influence of placement of the drop on the growth retardation of the bean shoot; a further purpose was to investigate the influence of varying dosages of 2,4-D on the above relationships.

The data presented in figure 4 indicate that certain relationships exist between dosage and point of application. With the lowest dosage used (50 μg) the expected relationship between growth suppression and point of application was observed, but the difference was slight. Applications made to the center of the midrib were not much more effective than those made at the tip or edge of the blade. Dosages greater than 50 μg did not appreciably change or increase the effectiveness of applications made to the tip or edge of the blade, but they did increase the effectiveness of applications made to the center of the midrib. Dosages of 100 μg and more made to the base of the blade produced a maximum effect.

A correlation between the size and abundance of vein tissue and effectiveness is evident from these results. The least effective location was the margin of the leaf, where veins are small and the chlorenchyma is most abundant. The next was the apex of the blade, where the midrib terminates, thus resulting in more vein tissue than on the edge of the blade. The next was the central part of the midrib, where the ratio of vein to chlorenchyma is greater. The most effective point of application was the base of the blade, where the midrib is largest and where the two large lateral veins, one coming in on each side, are present. It is therefore evident that the vein plays an important role in the absorption and translocation of 2,4-D in bean leaves. It is possible that the chlorenchyma was not involved in the absorption and translocation of 2,4-D because the high dosages used may have inactivated this tissue. The data suggest that plant sensitivity may be related to size and abundance of veins; evidently, other things being equal, plants with the largest veins should be most sensitive to high concentrations of 2,4-D.

Radioactive tracer studies. To study further the influence of placement of the drop on effectiveness, some 2,4-D* treatments were given. The dosage was 50 μg as described under Methods. Other aspects of this study included the influence of a lanolin ring, the effect of triethanol- and triethylamines, and the influence of Tween 20 and Vatsol OT. The results described below were observed from autographs such as that shown in figure 5.

A lanolin ring confined the solutions containing the triethanolamine formulations without a wetting agent or with Tween 20. The other formulations were not consistently confined by the lanolin ring; evidently the affinity of the alkyl amine or alkyl wetting agent for lanolin was sufficient to overcome the lanolin barrier. This property of the alkyl formulations to spread, especially along the veins, appears to be related to their effectiveness with such dosages as were used in most of the drop tests.

The influence of placement is suggested by the autographs. Movement from applications made at the tip of the blades was intense in the veins of the midrib, the intensity appearing to be as great as from applications made at the base of the blades. However, the total width of the image is greater from applications made at the base of the blades, suggesting that there was movement of 2,4-D* in more of the vascular bundles. Such a distribution would be an expected consequence of the location of the application.

Because the rings of lanolin alone did not necessarily confine the solutions (fig. 5), a ring was made using a mixture of lanolin and starch, and a plastic ring was set in this mixture. With this procedure the applied drops were successfully confined to the ring; any movement beyond the confines of the ring necessarily had to be inside the leaf and not over the surface. In this experiment, 2,4-D* and ATA* were compared, with applications being made at the apex and base of the blades. The procedure is described in detail under Methods.

Results from these tests are shown in figures 6 and 7. There was some movement of 2,4-D* inside the leaf beyond the external limits of the lanolin ring, but this was not extensive. Since the amine salt formulation was completely water soluble, the restricted internal movement of the 2,4-D* must have been due to a change in its physical state. The restricted movement may be due in part to a weak binding of the 2,4-D to the cell walls, and there was probably some precipitation of the 2,4-D as the acid. The amine may be independently absorbed, leaving the acid radical behind. ATA can be considered as a type of amine which moves freely, as shown in figures 7, 9, 10 and as has been shown by Crafts and Yamaguchi (1958). The solubilizing influence of Tween 20 and other solvents did not seem to have any marked influence on the internal spread, so the problem relating to the restricted spread of 2,4-D* beyond the point of application is not clear (figs. 6, 8).

Cross sections of the petioles and stems (fig. 6) indicate that there was movement in more veins from applications made at the base of the blades than from apical applications. In spite of this, 2,4-D* was not present in all of the veins in the stems with either treatment; the opposing leaves would have had to be treated to have accomplished this. The intense image in the petioles and cortex of the stems indicates that 2,4-D* was concentrated here, probably as a 2,4-D-protein complex (Fang, Johnson and Butts, 1955; Butts and Fang, 1957), free 2,4-D, and breakdown products. The results in figures 6 and 8 suggests that applications made at the apex of the blade resulted in 2,4-D* movement mainly downward in the stem, whereas the basal application resulted in movement both up and down.

Results with ATA* were somewhat different from those with 2,4-D* in several respects. For one thing, from applications made at the base of the blade ATA* moved upward and toward the margins. This movement was not in the veins; in fact, the large lateral veins seemed to stop or block the lateral spread of the ATA*. Evidently there was a flow of water in the apoplast (cell walls) toward the leaf's edge and the ATA* was carried along with it. The flow of water outside of the veins through the cells walls is called the "extrafascicular transpiration stream" by Strugger (1949). Apparently an application at the base of the blade is equivalent to treating at least 50 per cent of the entire blade, whereas treatment at the apex results in a treatment of little more than the area of the leaf's surface covered by the external spread of the chemical.

Radioactivity within the petioles and stems was much less with ATA* than with 2,4-D*, indicating much less tie-up or binding of the ATA* by plant constituents. The intensity of the image in the new growth was much greater than for 2,4-D*, indicating that ATA* was much freer to move.

Entire bean plants treated with ATA* and MH* are shown in figures 9 and 10. These figures indicate that the type of movement made by a chemical in the treated leaf and in the plant is to a large extent determined by the chemical and physical characteristics of the chemical itself. In movement MH* resembles ATA* much more closely than it does 2,4-D*. Actually, MH* is much freer to move in a plant than even ATA*, as can be seen by comparing the images in figure 10.

Other tests. In order to check on the translocation of 2,4-D* in the bean plant, 200 μg of 2,4-D* (amine) was applied to the primary leaves. Twenty-four hours later, these were separated into treated leaves, epicotyl, upper hypocotyl, lower hypocotyl, and roots. These were extracted with 1:10 HCl and water, and the 2,4-D* was removed from the water with ethyl ether, following the method of Hay and Thimann (1956). The actual quantities of 2,4-D* in the extracts were determined by the reaction of cotton seedlings and by counting the activity in the extracts. The results were similar to those obtained by Hay and Thimann in that extractable 2,4-D* decreased markedly from the treated leaves to the roots. However, in contrast to the results achieved by these workers, some 2,4-D* was recovered from the lower portions of the hypocotyl and the roots because more sensitive methods of detection were used. At least 1 μg of 2,4-D* was present in the root system of each bean plant. Some 2,4-D* could be recovered from the treatment solutions, indicating that leakage was taking place.

The Spray Method

Effect of wetting agents and other additives. In preliminary experiments about 75 wetting agents and other additives were added to aqueous solutions of 2,4-D which were sprayed on bean and barley plants. The sprays had to be sufficiently dilute to prevent the plants' being killed and yet concentrated enough to partially inhibit growth. Although such an approach is open to criticism, there was no obvious alternative. In the preliminary test the 2,4-D (amine) solutions contained 125 ppm 2,4-D and 250 ppm additive. Both Tween 20 and Vatsol OT were close to the top in effectiveness.

In the barley tests growth in height was taken as a measure of effectiveness. The 2,4-D (amine) was 500 ppm and the additive was 1,000 ppm. Growth was most effectively retarded when some of the more oil-soluble additives (Multifilm MWC-1, etc.) were used, while the more water-soluble materials (glycerol, propyleneglycol) were least effective.

Varying concentrations of 2,4-D and Tween 20. This experiment was conducted to detemine whether the optimum concentration of Tween 20 was influenced by the concentration of 2,4-D. The 2,4-D was formulated as the triethanolamine salt and ranged in concentration from 125 to 1,000 ppm; this was equivalent to an application of 4.2 to 34 gm per acre or 7.4 to 59 μg of 2,4-D per plant. The latter dosage falls within the range of many of the tests conducted by the drop method; however, the important difference is that in this experiment the chemical was applied all over the plant rather than being concentrated in one spot. This makes for a basic difference in the reaction of plants to different wetting agents.

As may be seen in figure 11, the influence of Tween 20 was considerable, but the optimum concentration varied with the concentration of the 2,4-D. The lower concentrations of 2,4-D were not benefited by concentrations of Tween 20 greater than 2,000 ppm, while the highest concentration of 2,4-D used (1,000 ppm) was benefited by the highest concentration of Tween 20 that was used (32,000 ppm). At the latter concentration of 2,4-D, death of bean plants was also related to the concentration of Tween 20: with 2,000 ppm Tween 20 none of the bean plants died; with 8,000 ppm, 25 per cent died; 32,000 ppm, 75 per cent died. As will be noted later, Tween 20 had essentially no toxicity of its own, so that its entire effect can be considered apart from this aspect. The influence of this material is apparently unrelated to surface tension. The solubility of 2,4-D in Tween 20 may partially account for the relationships shown in figure 11.

Influence of ethyl alcohol, Tween 20, and formulation. In the preparation of amine salt formulations from 2,4-D acid, it is convenient to add a small amount of ethyl alcohol to facilitate the reaction. It is not known whether or not there might be an interrelationship between the effectiveness of a given formulation and the presence and percentage of ethyl alcohol. Again, it was considered that Tween 20 might also influence some of these relationships. In order to test some of these factors, the experiment shown in figure 12 was conducted. The 2,4-D acid formulation was prepared in water with ethyl alcohol because of the possibility of some precipitation of the 2,4-D; thus the entire picture of the influence of ethyl alcohol on effectiveness of the 2,4-D acid is not presented; however, an experiment by Pallas[6] showed that the addition of a small quantity of ethanol without a wetting agent brought about an appreciable increase in activity of the 2,4-D acid.

The application rate per acre was 10.2 gm, which was the equivalent of 17.7 μg per plant or about 1 μg per square inch of plant surface. The only evident or marked effect due to treatment was the influence of Tween 20. Ethyl alcohol seemed to have no influence on either the triethanol- or triethylamine formulations and no influence on the acid formulation when Tween 20 was used. Without Tween 20, ethyl alcohol increased the effect of the 2,4-D acid, although not to any great extent. There is evidently some benefit derived from the ethyl alcohol with the acid formulations but none with the amine types.

Influence of sucrose, boric acid, and Tween 20. There seems to be a relationship between the export of sugars and 2,4-D out of leaves (Weintraub and Brown, 1950; Jaworski, Fang, and Freed, 1955), and sucrose seems to be the main sugar in the sieve tubes (Esau, Currier, and Cheadle, 1957). The addition of sucrose to leaves, after a period of exposure to darkness, has stimulated translocation, as evidenced by the export of 2,4-D (Rohr-

[6] J. E. Pallas, Jr., personal communication, 1957.

baugh and Rice, 1949; Hay, 1956); sucrose did not stimulate translocation of 2,4-D in the light (Hay, 1956). It has been suggested that boron is involved in translocation (Gauch and Dugger, 1954). The purpose of the present test was to determine whether the addition of sucrose and/or boric acid might contribute to the effectiveness of 2,4-D (triethylamine) and, if so, whether this relationship might be influenced by Tween 20.

The results of this test are shown in figure 13. Evidently the treatments were influenced chiefly by Tween 20 and sucrose was not a factor at any concentration; boric acid, likewise, had no influence on the effectiveness of 2,4-D. The concentrations of boric acid used were 0, 0.05, 0.2, and 0.8 per cent, and treatments were applied both with and without Tween 20 (0.2 per cent). In some field tests on woody plants, the addition of boric acid and sugar to the acid and amine formulations of 2,4-D was without benefit. It seems evident at this time that the effect of adding sucrose and/or boric acid to solutions of 2,4-D is of physiological interest only and likely to have no place in the practical application of 2,4-D. Possibly, in the case of an extreme boron deficiency, the inclusion of boric acid in the spray might, under some conditions, produce an improvement. These conclusions in no way relate to the possibility of obtaining a growth response by applying boron before spraying with 2,4-D.

Influence of propyleneglycol and glycerol. Both propyleneglycol and glycerol have been used to increase the effectiveness of some materials applied in sprays. These substances are thought to keep the applied chemicals in a liquid state and thus favor absorption. It was further thought that there might be some interrelationship in the effect of propyleneglycol, glycerol, and Tween 20; possibly the concentration of Tween 20 necessary to produce a maximum effect would be affected by these two materials.

Results from a test with propyleneglycol are shown in figure 14. The results with glycerol were identical. As with sucrose, the only discernible effect of the treatment was due to the influence of varying concentrations of Tween 20. No combination of either propyleneglycol or glycerol had any influence on the uptake of the triethylamine salt of 2,4-D. The dosage used was 17 gm per acre, which was equivalent to about 27.6 μg per plant or 1.7 μg per square inch of plant surface. Since all the materials used were hygroscopic, the basic influence of Tween 20 is apparently due to some other property.

Effect of different wetting agents. Several experiments were conducted using different concentrations of wetting agents with the triethylamine formulation of 2,4-D. Results from one of these tests may be seen in figure 15. The solutions were applied at 18 gallons per acre and contained 10.2 gm of 2,4-D, which was equivalent to about 1 μg per square inch of plant surface. The actual dosages of wetting agents ranged from 8.4 to 538 gm per acre, or about 0.9 to 58 μg per square inch of plant surface. Such dosages did not seem detrimental to the bean plants (fig. 16).

The effect of 2,4-D varied with the wetting agent used and its concentration. Tween 20 was the most effective material tested, although at the lowest concentration tested it was only slightly more effective than Vatsol OT and

Amine 220. Tween 20 seemed to reach its maximum effectiveness at 500 ppm, while Amine 220 and Vatsol OT did not increase in effectiveness beyond 125 ppm, the lowest concentration used. It is interesting that G-1006 and G-1036 gave almost identical effects at various concentrations; these two chemicals are of the same composition, except that G-1006 has an appreciably greater molecular weight. Both of these compounds more closely approached Tween 20 in activity than did the other compounds tested. The concentration of Multifilm L required to produce an equal effect was much higher than that of the other preparations used.

There was a general relationship between bending and final response of the bean plants, even though the chemicals were sprayed over the entire plant (readings were made two days after application). The relationship between bending and final response was better than occurred in a test made using the drop method.

Effect of different amines. All of the various amines investigated were applied as sprays. Because of the great sensitivity of the bean plant, it was necessary to use dilute concentrations, such as were generally used with the spray method.

The same amines used with the drop method (table 7) were sprayed onto bean plants, at a concentration of 2,500 ppm and a volume of 18 gallons per acre; this was equivalent to 170 gm of 2,4-D per acre. The effect of this treatment was actually too severe to measure differences between the different amines because considerably more than half the plants died within the experimental period. There was an indication that the alkyl amines were slightly superior to the alkanol amines, especially with the oil-emulsion diluent. There was an outstanding difference between the results obtained with water and with an oil-emulsion diluent. With water as a diluent about 40 per cent of the plants died, while 88 per cent of the plants died with the emulsion.

Relationship between formulation and concentration of Tween 20. It was considered that the best formulation might vary with the concentration of the wetting agent. Tween 20 was used so that the results would be more comparable with those of other studies. The formulations tested were the 2,4-D acid, triethanolamine, triethylamine, and 2-ethylhexylamine. The data are shown in figures 17 and 18. The 2-ethylhexylamine salt of 2,4-D was slightly more effective than the other two amine salts when used without a wetting agent; however, the response it produced when the lowest concentration of Tween 20 was used was less than that of the other amine salts. With 0.2 per cent Tween 20 and more, all amine formulations produced identical effects on growth retardation of the bean plant.

DISCUSSION AND CONCLUSIONS

Results obtained in these studies corresponded in part with various unpublished results obtained under field conditions, which will be drawn on in the following discussion. An attempt will be made to trace the absorption and translocation of 2,4-D out of leaves and its movement through the cuticle and stomata into the cell walls and intercellular spaces. Factors influencing absorption of 2,4-D by cells and translocation processes will then be considered. Results obtained with ATA* and MH* will be drawn on as an aid in interpreting the results obtained with 2,4-D*.

General aspects involved in absorption and translocation. When a solution is first deposited upon a leaf's surface, its spreading characteristics depend upon viscosity and interfacial tension. Spreading ceases to be a factor with complete coverage. Spreading is increased by decreasing the polarity of the formulations, by using, for example, alkyl wetting agents, alkyl amines, oil-soluble esters, oils, etc.

Immediately after application, the solution begins to undergo physical changes. Water quickly evaporates, and as a result crystals of the herbicide may be precipitated. This may be prevented by using certain cosolvents or solutions that are sufficiently dilute that the chemical will dissolve in the cuticle. When air humidity is high, a state of solution may be maintained by humectants, which allow the dissolved herbicide to remain continuously available for absorption. When esters are applied in oils, a continuous state of solution is assured.

The degree of viscosity and the polarity of a formulation influence its rate of penetration and mode of entry. Stomata, cuticular cracks, and the cuticle itself are all involved. Many plants do not possess stomata on the upper surfaces of the leaves, and penetration must of necessity be largely cuticular (stems are not considered in this discussion).

The exact mode of entry through the cuticle will be considered only in a general way. Some cuticular penetration studies have already been presented by Leonard and Crafts (1956) and Crafts and Yamaguchi (1958). The partition of 2,4-D between the cuticle and the constituents in the formulation is a factor influencing the amount of 2,4-D that may pass through the cuticle. Likewise, there may be partition relationships between the cuticle and certain adjuvants, solvents, and 2,4-D itself. The cuticle may protect the leaf against toxic materials, as shown by Leonard and Crafts (1956). In studies of brush species they also found that tetraethoxysilane, a highly toxic chemical, added to 2,4-D* improved the uptake and movement of 2,4-D* from applications made to the upper surface of a leaf (toyon and live oak) and reduced it from applications to the lower surface. The upper surface of leaves of these species contain no stomata, in contrast to the normal abundance of these openings on the lower surface.

Once through the cuticle, the 2,4-D and adjuvants come in contact with cell walls. When solutions pass through stomata, they move first into intercellular spaces and their composition is essentially unchanged. With cuticular absorption, however, toxic additives seem to be "screened out" to some extent.

In the normal process of translocation, sugars are moved out of the chlorenchyma into the border parenchyma and then into the sieve tubes. Available evidence indicates that other substances are similarly moved; however, this process is dependent upon functional chlorenchyma. 2,4-D is a toxicant, its toxicity varying with concentration. If the concentration is low enough, cells can continue to function for a time; with high concentrations, however, cells may quickly become nonfunctional. The epidermal cells will be inactivated first, whereas the dosage arriving in the palisade cells will for a time be below the point of inactivation, thus allowing time for some transport of 2,4-D out of these tissues into the sieve tubes. The spongy mesophyll will next receive and translocate 2,4-D before it becomes inactivated.

Now, when 2,4-D solutions have been applied as a complete coverage over the leaf, translocation must of necessity stop after all of the chlorenchyma cells have been inactivated; however, when the solutions have been applied as discrete drops, the only tissues that will be inactivated are those beneath the drops (plus internal spreading). The remainder of the chlorenchyma should continue to function, thus making possible the continued translocation of 2,4-D already in the sieve tubes because sugar will move out of the functioning chlorenchyma.

Stomatal penetration by nonpolar solvents may occur quite rapidly. (This may easily be observed by placing a drop of aromatic oil on the under surface of a leaf.) If the solutions are toxic, the tissues quickly become inactivated, stopping translocation within a short time; however, if the solutions are of a low order of toxicity, translocation will proceed over a longer period of time. Stomatal penetration is, therefore, extremely desirable if the solution is not highly toxic.

Absorption by veins. The normal function of the vein tissues (aside from xylem and sieve tubes) in translocation is not understood. Present data merely demonstrate the importance of the veins in the absorption and translocation of 2,4-D; it is further indicated that the size of the veins is important, especially where high concentrations of 2,4-D are used. "Border" parenchyma are believed to be involved in piling chemicals into the sieve tubes (Leonard, 1939; Barrier and Loomis, 1957), but through how many layers of cells does this "accumulating" mechanism work?

The number of cells in the veins between the upper surface of a leaf and the sieve tubes may range from a few to more than fifty. Movement can be both through the cell walls and through the cells; some movement through the intercellular spaces may occur, but these are not abundant in the veins. The outer cells of the veins may become inactivated or killed but will continue to act as reservoirs containing diffusible 2,4-D. As the cells deeper and deeper in the veins become nonfunctional, the sieve tubes will continue to function until the border parenchyma and the sieve tubes themselves become nonfunctional as a result of high concentrations of 2,4-D or toxic adjuvants. It is evident that the length of time the sieve tubes can continue to function will depend on (a) the concentration of 2,4-D used and (b) the depth of the protective layer of cells surrounding the sieve tubes. It should not be surprising, therefore, that applications to large veins are more effective than applications to small veins and that toxic additives markedly reduce effectiveness.

Binding factors involved in translocation. What happens to 2,4-D with reference to binding both in the leaf and during translocation? Considering the concentrations of 2,4-D* used in the autoradiographic studies, much of the 2,4-D* should be complexed with a protein, according to Fang, Johnson, and Butts (1955) and Butts and Fang (1957). According to the autoradiographs, 2,4-D* amines spread only slightly internally beyond the external boundaries of the lanolin-starch rings. Some 2,4-D passes into the xylem as it spreads through cell walls and through cells. Evidently some of the 2,4-D is weakly bound after entering the leaf or is precipitated as the 2,4-D acid. The amine radical is probably independently absorbed and translocated, perhaps as ATA is . (ATA is a type of amine and reacts with 2,4-D to form a salt similar to other amines.) Free 2,4-D as such rapidly disappears from the leaf (Hay and Thimann, 1956) and becomes bound with proteins (Fang, Johnson, and Butts, 1955; Butts and Fang, 1957) as well as being decomposed.

The movement of ATA* and MH* within the leaf blade contrasts interestingly with that of 2,4-D* and helps in an understanding of the latter. The movement or spreading of 2,4-D* in a leaf is limited, whereas ATA* and MH* move freely from the point of application to the edge of the blade. This suggests that ATA and MH move in a stream of water flowing in the apoplast (cell walls) from the base of the blade to the margins, which carries soluble substances with it. In some respects, this movement corresponds to the extrafascicular water movement described by Strugger (1949). With chemicals that have capacity to spread, single-drop applications approach complete coverage of the leaf because of this mechanism. Obviously ATA and MH differ considerably from 2,4-D in their characteristics of penetration and spread.

Cross sections of the petioles and stems show much radioactivity (figs. 6, 7) in the cortex, especially with 2,4-D*. It is suggested that some 2,4-D moves out of the sieve tubes during transport, and that this movement may be the result of 2,4-D itself producing a "growth reaction" on the cells surrounding the sieve tubes. That 2,4-D moves out of cotton roots into nutrient solutions has been shown by Crafts (1956), and in the present study the same thing has been shown to occur with beans. The "growth reaction" in these cells would increase their capacity to compete with the meristems (apical and lateral) for more 2,4-D and food materials. Upon entering the cells surrounding the sieve tubes, most of the 2,4-D evidently becomes bound to protein, leaving little free 2,4-D that might move back into the sieve tubes for further translocation. ATA* is stored in the cortex far less than is 2,4-D. It is not a growth stimulant and so may not be diverted from the sieve tubes as readily as 2,4-D; furthermore, it may not be as subject to chemical binding by plant constituents.

Correlation of results with different formulations. How do some of the results correlate with the discussion above? It will be recalled that when low concentrations of 2,4-D were used, there was little if any advantage to alkyl formulations (esters, oils, alkyl amines, alkyl wetting agents). Under these conditions, Tween 20 was the most effective material studied, and it very greatly improved the uptake and movement of 2,4-D. How this chemical acted to produce this effect is not known, but somehow it increased the capacity of the cells to absorb and translocate 2,4-D. Possibly a part of its effect was to "protect" the cells from some of the inhibitory effects of 2,4-D or to reduce "binding." Neither sucrose, propyleneglycol, nor glycerol began to approach this effect of Tween 20. It is suggested that Tween 20 exerts its main effect on the chlorenchyma and/or the small veins. Mitchell and Linder (1950) noted that Tween 20 was the most effective surface agent used in their tests on bean plants with 2,4-DI^{131} (2,4-dichloro-5-iodophenoxyacetic acid).

With high concentrations of 2,4-D applied as drops, the alkyl or less polar formulations were more effective than the more polar types. When high concentrations are used, the only tissues of importance that are involved in absorption are the large veins; therefore, substances like Tween 20, which probably exert their effect on the chlorenchyma, cannot function or be of value. The alkyl formulations spread along the veins and in the veins, thus increasing the uptake of 2,4-D. Translocation can proceed because the chlorenchyma not covered by the drop can continue to carry on photosynthesis and thus export sugar.

Substances in the treatment solutions which increase the toxicity beyond that due to 2,4-D itself are likely to be detrimental. The most favorable result was brought about by a completely nontoxic carrier, one of the silicones. Certain high-UR oils, however, were almost as effective and were more practical because of cost. As has already been mentioned, the effect of adjuvants with 2,4-D solutions may be quite different from their effects with ATA and MH. Currier and Dybing (1957) have observed that the slight toxicity of Vatsol OT may actually favor the absorption and translocation of MH, which has very little toxicity in comparison with 2,4-D, which has both acute and chronic toxic properties. Certainly any additional toxicity beyond that present in 2,4-D itself would be undesirable.

Correlation with field results. Studies on the control of woody plants with 2,4-D and 2,4,5-T were initiated in 1950. It was soon found that formulation, concentration, method of application, and diluents had a pronounced influence on the effectiveness of these chemicals. For example, isopropyl ester was considerably inferior to the butoxyethanol and PGBE esters by every method studied; however, the polyethyleneglycol 200 and 600 monoesters of 2,4-D were as good as these esters when good coverage was achieved with ground equipment but were almost completely nonlethal when applied by aircraft (Leonard, 1956*a*). Offord[7] has found that *Ribes roezli* from the central Sierra Nevada is appreciably more sensitive to low concentrations of the sodium salt of 2,4-D than the esters. With this species the greater toxicity the esters have for the leaf chlorenchyma is possibly an important factor. Coyote brush (*Baccharis pilularis*) is slightly more sensitive to amine formulations of 2,4-D than to esters when applied by aircraft; the addition of some nontoxic emulsifiable oil to the spray mixture improves the kill, especially when plant sensitivity is not optimum (Leonard and Harvey, 1956). Hull (1956) has observed that amine formulations of 2,4,5-T translocated better than esters in mesquite seedlings grown under greenhouse conditions; under field conditions, the trees are rather consistently more effectively killed with esters (Fisher, Meadors, and Behrens, 1956). Under

[7] H. R. Offord, personal communication, 1957.

field conditions cutinization evidently limited penetration, whereas under greenhouse conditions injury was a limiting factor.

Valley oak (*Quercus lobata*) was more readily killed by aircraft application of 2,4,5-T (PGBE ester) than was blue oak (*Quercus douglasii*). Both are species of white oaks. The differences in sensitivity might have been predicted since valley oak leaves have a larger midrib, furnishing a great blanket of protecting cells over the sieve tubes. Actual plant sensitivity to 2,4,5-T was about the same with the two species, as determined by the cut-surface method of treatment (Leonard, 1956*b*).

Trials using Tween 20 with 2,4-D amine to control woody plants have not shown any special promise. It is necessary to use 5,000 ppm of 2,4-D to control many of the woody plants studied, and this concentration, according to most tests on the bean plant, is too high to benefit by the addition of Tween 20.

Aside from formulation, the type of diluent can be an important factor determining kill. Live oak sprouts sprayed (complete coverage) with esters of 2,4-D plus 2,4,5-T (brush-killers) in diesel oil quickly become brown, but this condition is followed by vigorous sprouting. Similar treatments applied in water (containing 0.5 to 1 per cent oil) may result in relatively good kills by comparison. Penetration and quick absorption, effected by the oil carrier, quickly kill the chlorenchyma in the leaf, thus stopping translocation before much movement can occur.

Other physiological factors affecting translocation. From the preceding discussion, it is evident that many factors are involved in determining how effective a given 2,4-D spray may be. Even though a 2,4-D spray may be ideal insofar as foliar absorption is concerned, translocation problems may make it ineffective. Plants that are growing slowly or not at all "bind" most of the 2,4-D before much movement can take place. Sprays should be applied during the period of active growth. Leonard and Crafts (1956) have shown that the movement of 2,4-D* in woody plants is greatest or most intense when growth is active. Moisture deficiencies and decreased growth are correlated with an increased resistance of chamise to 2,4-D (Leonard, 1956*a*). In poison oak (*Rhus diversiloba*) Leonard (1958) observed that both 2,4-D* and ATA* were actively translocated when the plants were growing; after growth had ceased, 2,4-D* failed to translocate appreciably, while the movement of ATA* was essentially the same in intensity as it was during the period of active growth. The herbicidal effectiveness of the two compounds on poison oak was correlated with the observations on translocation. *Zebrina* growing in nutrient solutions translocated 2,4-D* quite well, whereas the translocation of 2,4-D* in nutrient-starved plants was slight (Crafts and Yamaguchi, 1958); in contrast, ATA* translocated well under both conditions of nutrition in *Zebrina*.

It is sometimes possible to improve conditions so as to make a normally "resistant" plant "sensitive"—for example by burning chamise (*Adenostoma fasciculatum*), as has been shown by Leonard (1956*a*) and Leonard and Carlson (1957). The succulent young shoots are sensitive to 2,4-D, and it is possible that this sensitivity might be further increased with irrigation and fertilization.

Translocation of 2,4-D is also influenced by the "relative" competition of different parts of a plant for food materials. For example, in the dormant season (late fall, winter, and early spring) evergreen woody plants may be quite sensitive to 2,4-D (Leonard, 1956*a;* Emrick and Leonard, 1954). During this period biochemical activity in the shoots is evidently low, and this should reduce the storage and binding of 2,4-D as well as the rate of decomposition. Active root growth during this period (Harris, 1926) furnishes a "sink" for food materials and 2,4-D.

The results of this study suggest certain formulations and additives that may improve 2,4-D sprays. They further suggest that the factors involved in the absorption of 2,4-D may be quite different from those connected with ATA and MH. A correlation of factors involved in the absorption of different foliage-applied herbicides is needed.

SUMMARY

Relationships between the composition of 2,4-D solutions and their effectiveness as herbicides were investigated. The measure of effectiveness was the reduction in fresh weight of the growth above the primary leaves of bean plants. Two methods of application were used—the drop method (0.01 ml of solution) and the spray method. Variables studied were: diluents, humectants, surfactants, types of amines and esters, concentration, and dosage. Other studies included the effect of point of application on effectiveness and on the distribution of radioactive 2,4-D in the treated leaf and in the plant. For comparative purposes, some similar studies were conducted with radioactive aminotriazole (ATA) and maleic hydrazide (MH).

Studies with the drop method of application produced the following results:

1. A marked difference was observed in the effectiveness of different oils, hydrocarbons, and silicones as diluents of the n-hexyl ester of 2,4-D. With every mixture used, there was a definite relationship between injury and weight reduction. One silicone was slightly superior to some of the highly refined oils.

2. Vatsol OT was the most effective surfactant, while Tween 20 was poor (with 25 μg or more of 2,4-D).

3. Dosage and formulation were found to have a definite relationship to effectiveness. At low dosages (less than 12 μg) the triethylamine salt of 2,4-D was more effective with Tween 20 than with Vatsol OT; this formulation was also more effective than the n-octyl ester or the propyleneglycolbutylether ester applied in a nontoxic oil. With dosages of 12 μg or more, however, the esters were the most effective.

4. Alkyl amines tended to be more effective than alkanol amines. For example, triethyl-, diethyl-, and isopropylamines were more effective than the corresponding alkanol amines. The most effective amine appeared to be the 2-ethylhexylamine.

5. In tests with a series of alkyl esters, the methyl appeared to be the most effective at 12 μg or less and perhaps the least effective at 200 μg.

6. A relationship was evident between placement of the drop and effectiveness. At dosages of 50 μg or more, the effectiveness of 2,4-D was not improved when the drop was placed on the margin or apex of the blade, but effectiveness was increased when it was placed on the central part of the midrib or at its base. The latter was the most effective position.

7. With radioactive 2,4-D the less polar formulations tended to spread more on the leaf blade than did the more polar formulations. Lanolin rings prevented the spread of the latter but not of the former. Except for translocation, there was very little internal spread of 2,4-D within the leaf.

8. Bean plants treated with labeled 2,4-D placed in a lanolin-starch ring were sectioned to determine the distribution of radioactivity within the cross sections. Radioactivity was distributed around the sections to a greater extent from applications made at the base of the blade than from applications made at the apex. This suggests that 2,4-D moves in certain vascular bundles, but that some exchange between bundles takes place. The general distribution of radioactivity around the centers of movement indicates considerable loss or escape of 2,4-D from the sieve tubes during transport.

9. Radioactive ATA under the conditions described above moved quite differently, both within the treated leaf and through the plant. The type of movement within the treated leaf indicates that the ATA is caught in the "extrafascicular transpiration stream" in the cell walls and is moved toward the margin of the leaf. Evidently a single drop of ATA applied at the base of the leaf effectively treated half of the leaf. Since the ATA was surrounded by a ring, the radioactivity beyond the ring must be evidence of movement inside the leaf. During transport, ATA was not taken out of the sieve tubes and held in the petioles and stems as much as was 2,4-D. More chemical was available to go to the growing portions of the plant, indicating that ATA is freer to move in the bean plant than is 2,4-D. In movement, MH was much more similar to ATA than to 2,4-D.

10. Cotton seedlings used as indicator plants showed that 2,4-D applied to bean leaves was distributed throughout the bean plant, including the roots, within 24 hours; however, free or readily extractable 2,4-D decreased from the point of application to the roots.

Studies with the spray method of application produced these results:

1. With 1,000 ppm of 2,4-D the concentrations of Tween 20 and 2,4-D showed a relationship to weight reduction. With 1,000 ppm of 2,4-D, effectiveness increased progressively as concentrations of Tween 20 increased from 125 to 32,000 ppm.

2. Substances such as ethyl alcohol, sucrose, propyleneglycol, and glycerol had no influence on the effectiveness of 2,4-D amine, either alone or in combination with different concentrations of Tween 20. Similar tests with boric acid showed it to have no influence on 2,4-D effectiveness.

3. When a low concentration (150 ppm) of 2,4-D was used, Tween 20 was outstandingly the most effective surfactant tested, regardless of concentration. The influence of Vatsol OT in this study was only slight. The sur-

factants by themselves did not bring about any weight reduction of the bean plants.

4. The influence of the type of amine was insignificant at concentrations of 125 and 250 ppm 2,4-D. The 2-ethylhexylamine was slightly more effective than the triethyl- and triethanolamines and the acid formulations of 2,4-D when no Tween 20 was used; however all differences between formulations disappeared with the use of Tween 20. Concentrations of 2,4-D had to be kept low to keep from killing the plants. Greater concentrations killed most or all of the plants, thus preventing comparisons.

ACKNOWLEDGMENTS

Mr. Harold Drever, Mr. Carl Hamilton, Mr. A. L. Johnston, and Dr. Shogo Yamaguchi all helped with various aspects of these studies, and their help is gratefully acknowledged.

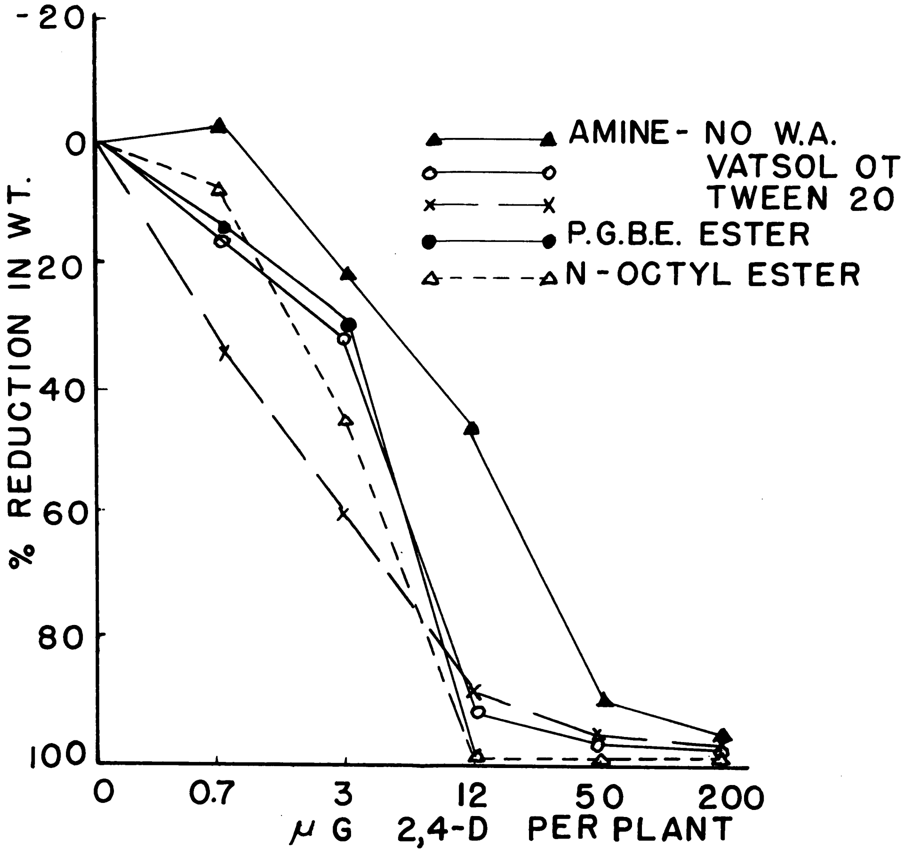

Fig. 1. The relationship of dosage and formulation of 2,4-D to reduction in fresh weight (above the primary leaves) of the bean plant. The triethylamine salt of 2,4-D was applied in water containing either no wetting agent or 0.8 per cent wetting agent. The esters (unformulated) were applied in oil (Bayol D) containing 5 per cent butyl cellosolve.

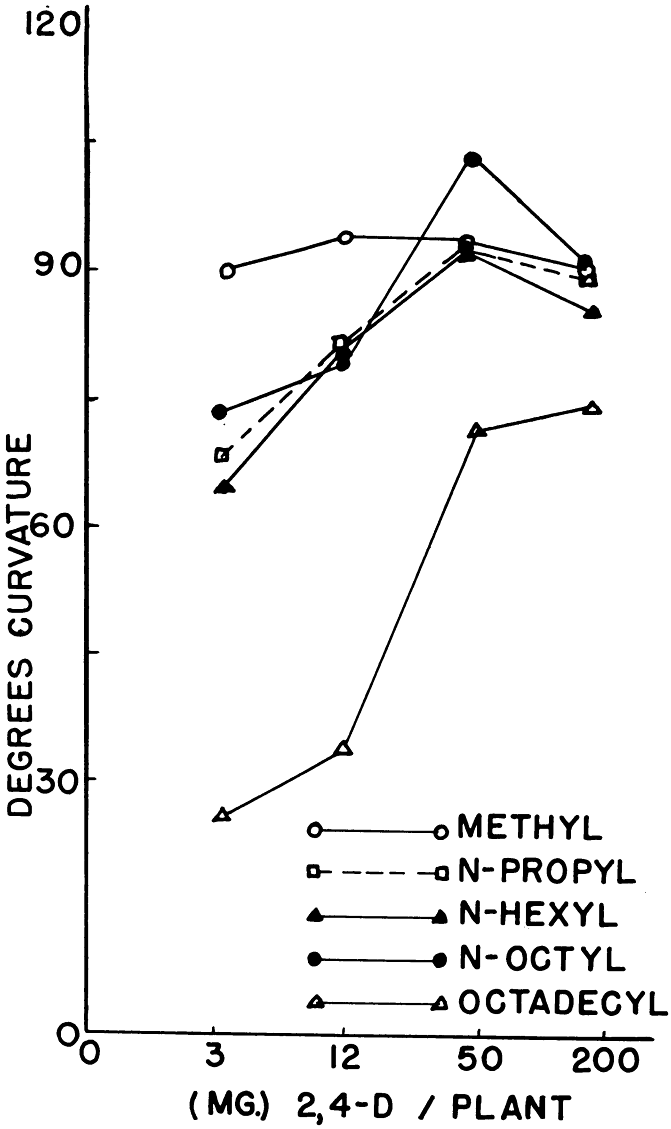

Fig. 2. The relationship of dosage and type of ester to bean curvature two days after treatment.

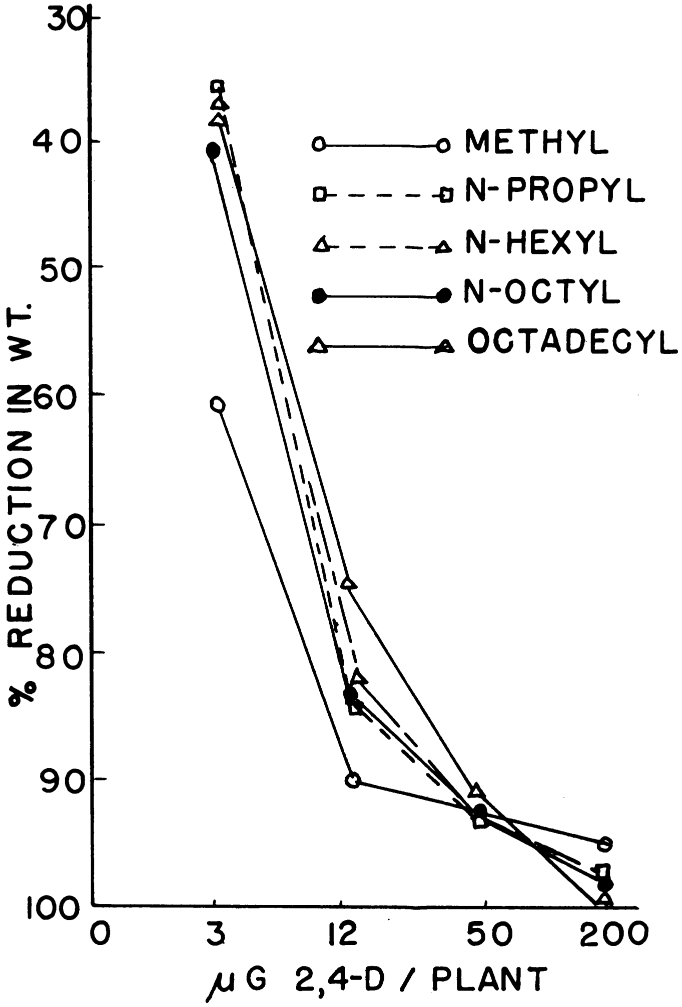

Fig. 3. The relationship of dosage and type of ester to reduction in fresh weight (above the primary leaves) of bean plants. The 2,4-D esters (unformulated) were dissolved in a mixture containing 88 per cent Bayol D and 12 per cent butyl cellosolve.

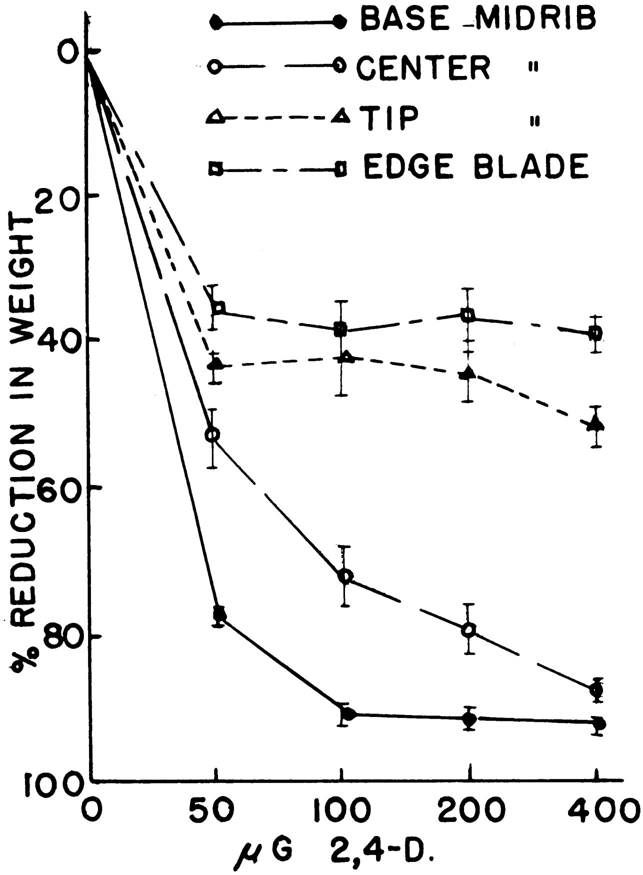

Fig. 4. Influence of dosage and point of application on reduction in fresh weight (above the primary leaves) of the bean plant. The 2,4-D amine was applied in water to which had been added 0.2 per cent Vatsol OT. The standard errors are indicated on the graph.

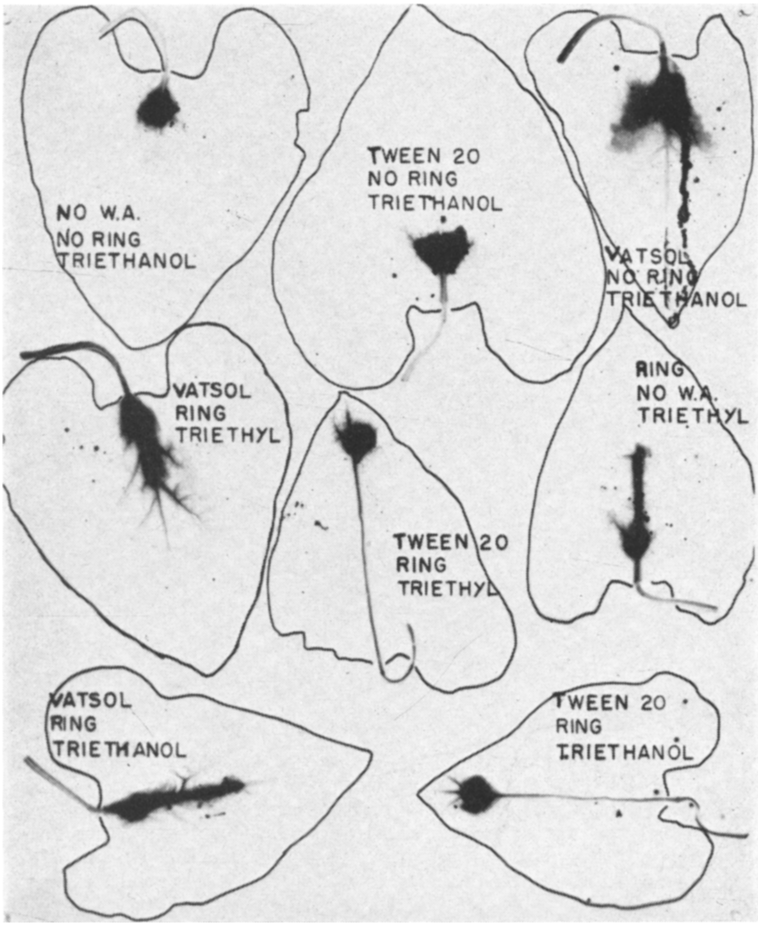

Fig. 5. The influence of wetting agents, type of amine, lanolin ring, and placement of the drop on the distribution of 2,4-D* within treated bean leaves three days after treatment. These autographs indicate the general nature of the results that were obtained. Freeze-dried.

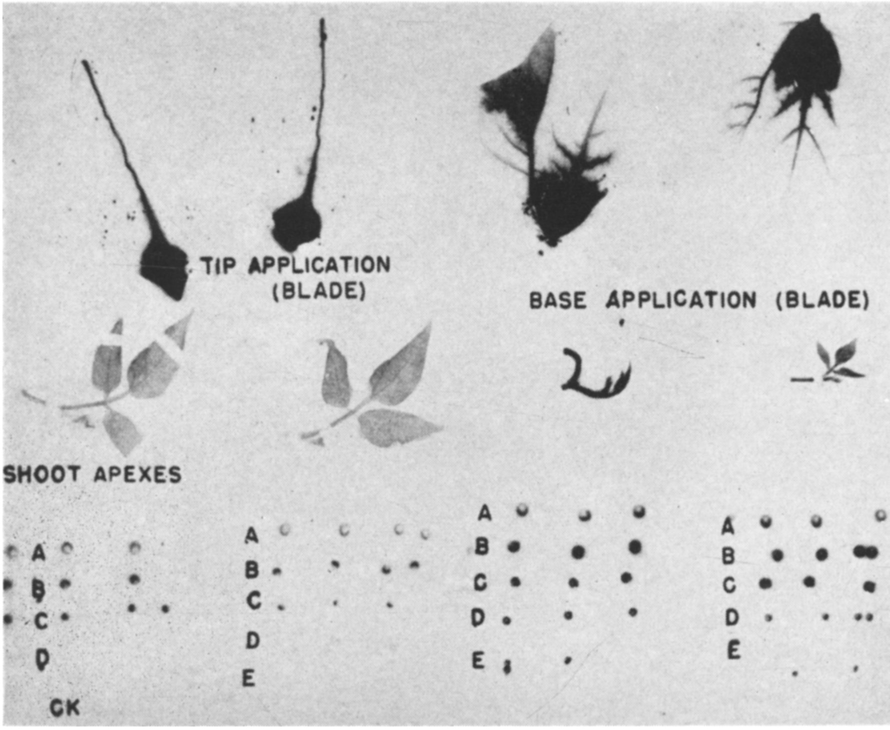

Fig. 6. The influence of placement of 2,4-D* on the bean leaf (primary leaf) on the distribution of radioactivity in different parts of the plant. A drop of 2,4-D* was placed in a lanolin ring (see Methods) and plant samples were taken three days later. The autographs shown above are of the treated leaf blade, the shoot apex, and cross sections of *A* the lower part of the hypocotyl, *B* the epicotyl below the primary leaves, *C* the petiole of the treated leaf, *D* the epicotyl above the treated leaf, and *E* the epicotyl above the first trifoliate leaf. Freeze-dried.

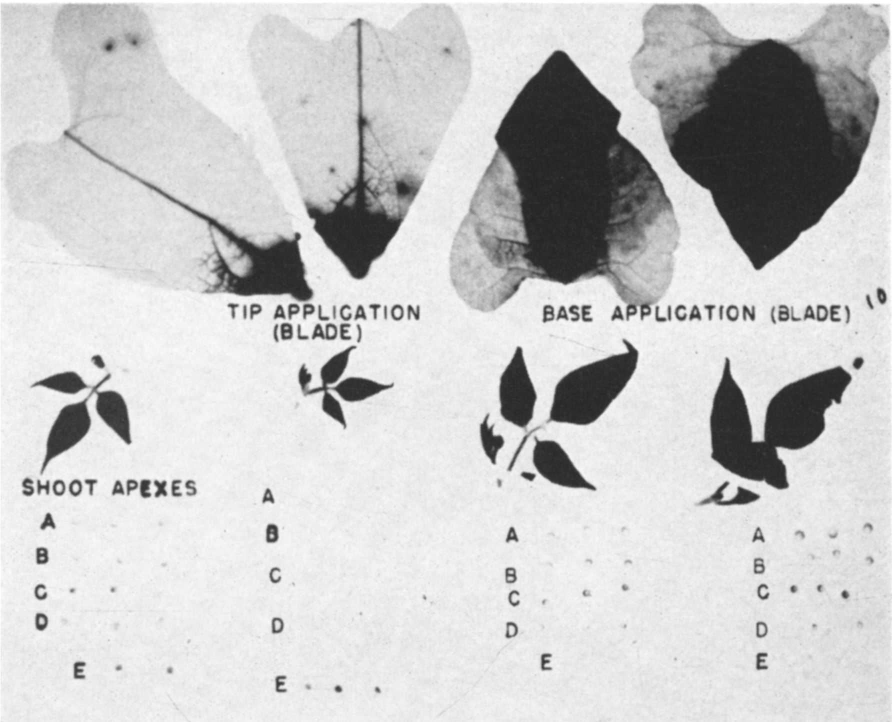

Fig. 7. The influence of placement of ATA* on the bean leaf (primary leaf) on the distribution of radioactivity in different parts of the plant. The ATA* was placed in a ring (see Methods) and sampled three days later. The plant parts shown here correspond to those in figure 6.

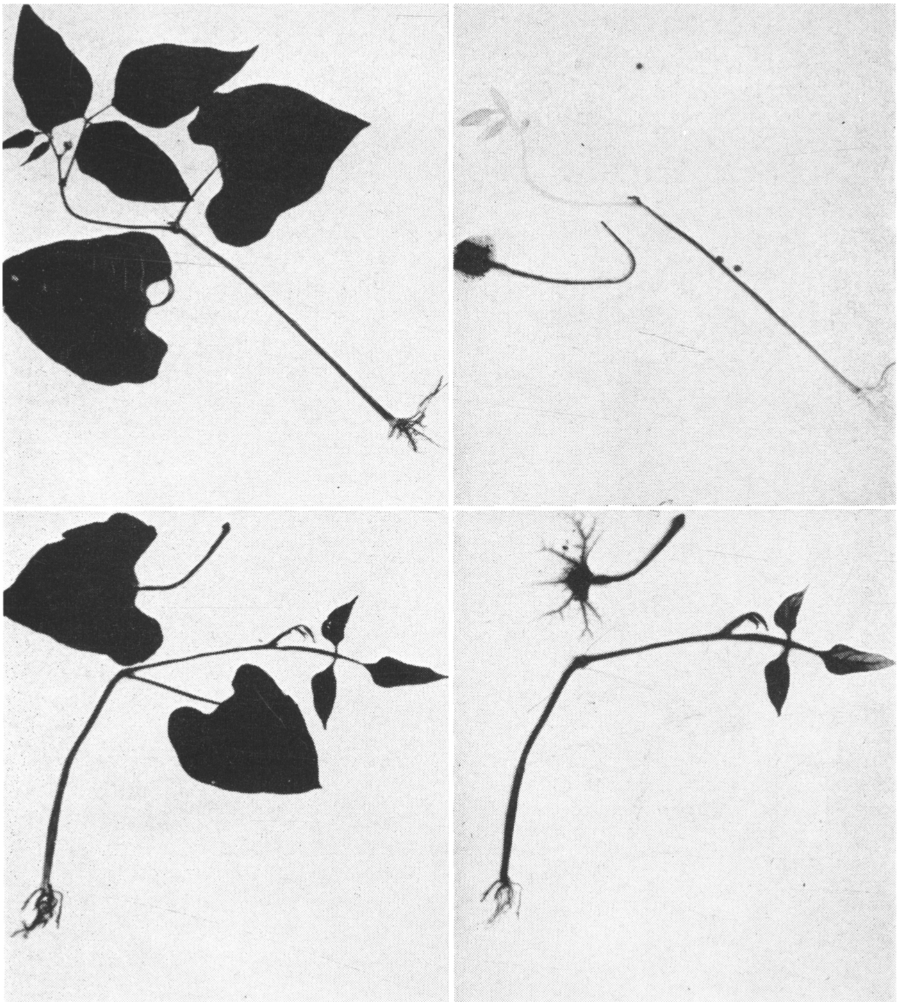

Fig. 8. Autographs of whole plants given the treatment described in figure 6. 2,4-D* was applied to the tip of the blade (upper row) and to the base of the blade (lower row). The dried plants are on the left, the autographs on the right.

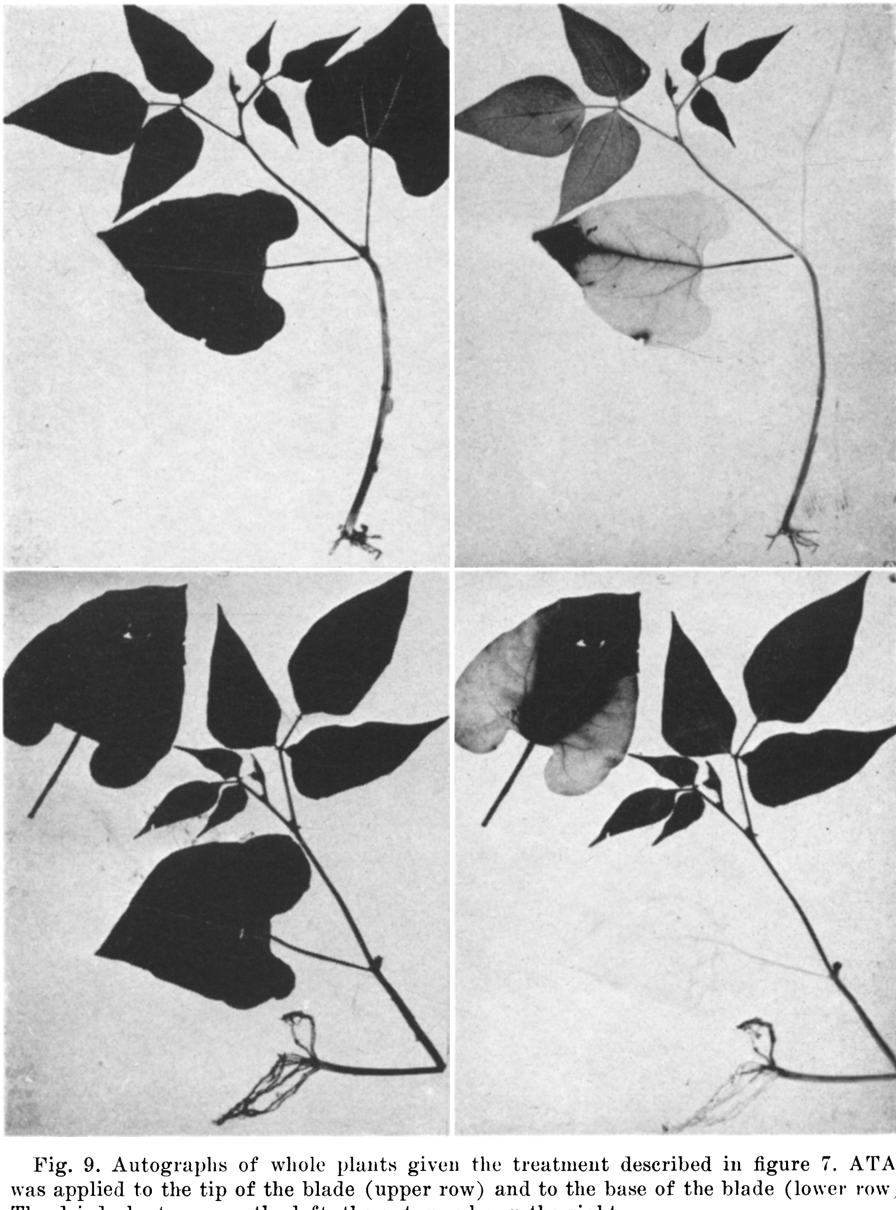

Fig. 9. Autographs of whole plants given the treatment described in figure 7. ATA* was applied to the tip of the blade (upper row) and to the base of the blade (lower row). The dried plants are on the left, the autographs on the right.

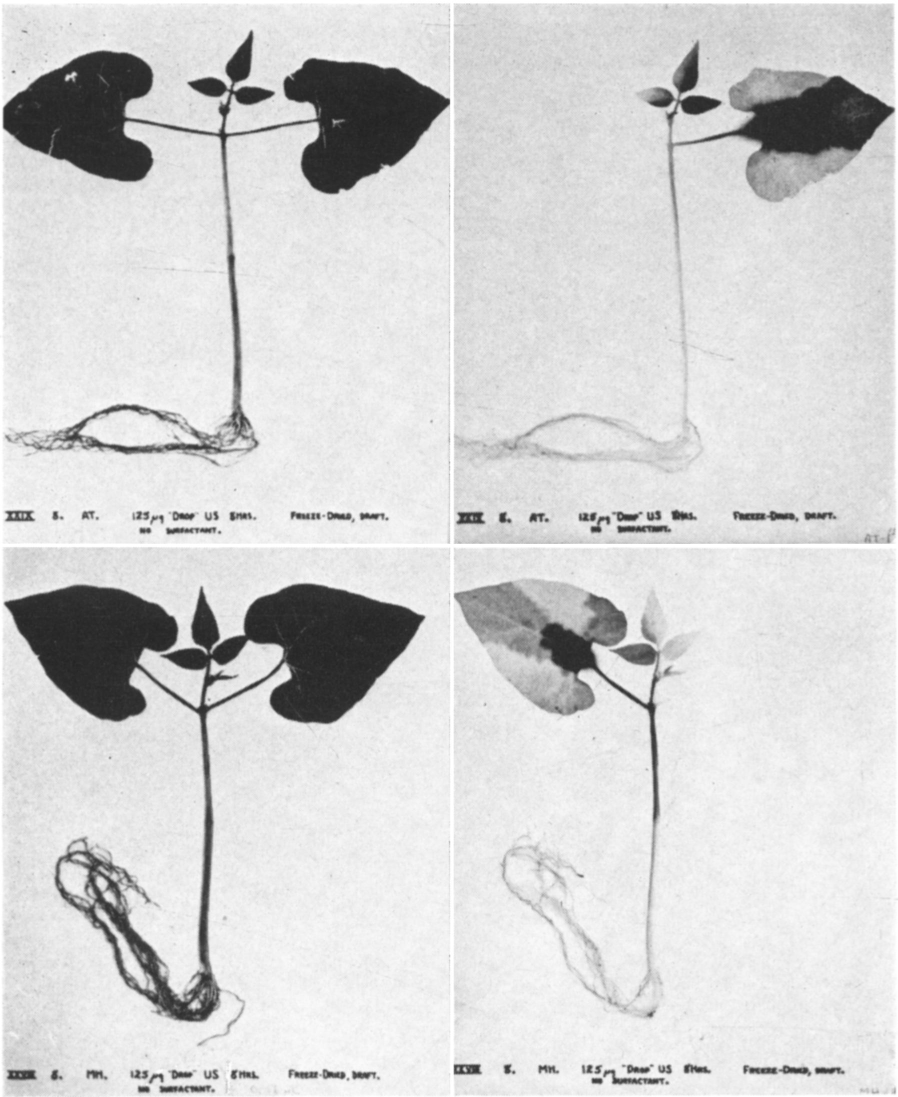

Fig. 10. The influence of ATA* (upper row) and MH* (lower row) on the distribution of radioactivity 8 hours after treatment; 125 μg of each chemical was placed within a lanolin ring at the base of the blade. The radioactivity of the ATA* was 1.4 μc and of the MH* 0.9 μc. No surfactant was used. The plants were freeze-dried. The dried plants are on the left, the autographs on the right.

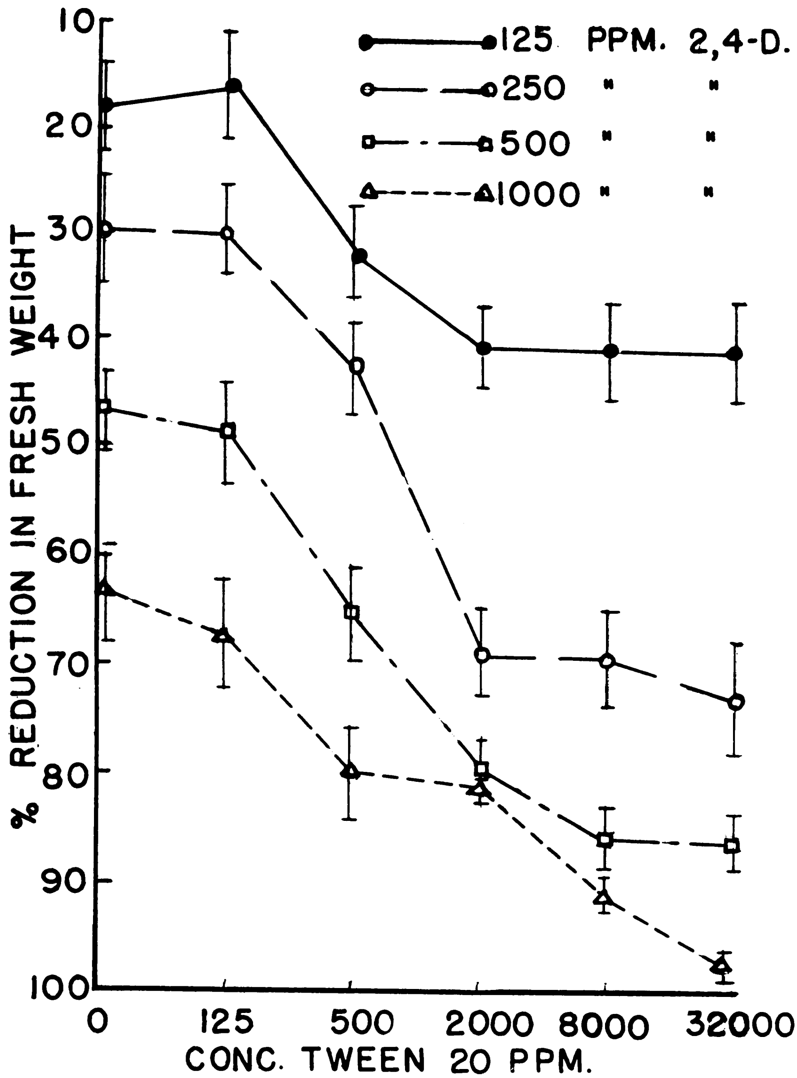

Fig. 11. The influence of varying concentrations of Tween 20 in combination with different concentrations of the triethanolamine salt of 2,4-D on the bean plant (reduction in fresh weight above the primary leaves). The standard errors are indicated on the graph. Spray application.

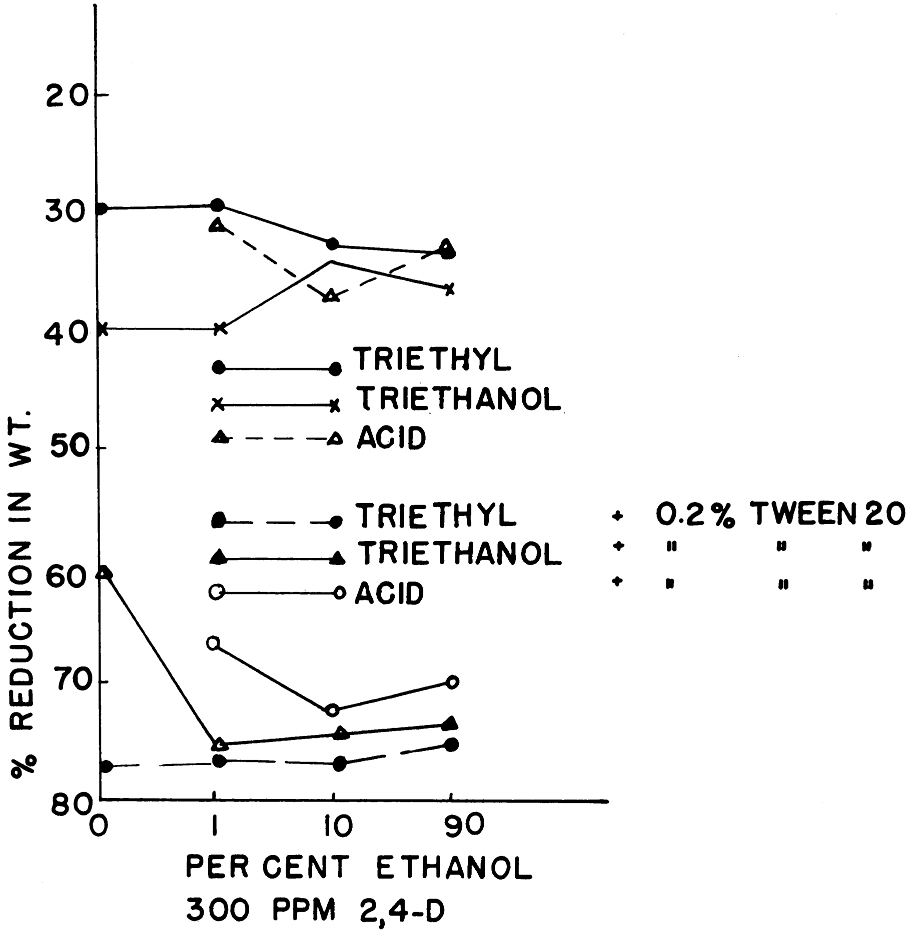

Fig. 12. The influence of various concentrations of ethyl alcohol on the reduction in fresh weight (above the primary leaves) of bean plants sprayed with three formulations of 2,4-D with and without Tween 20.

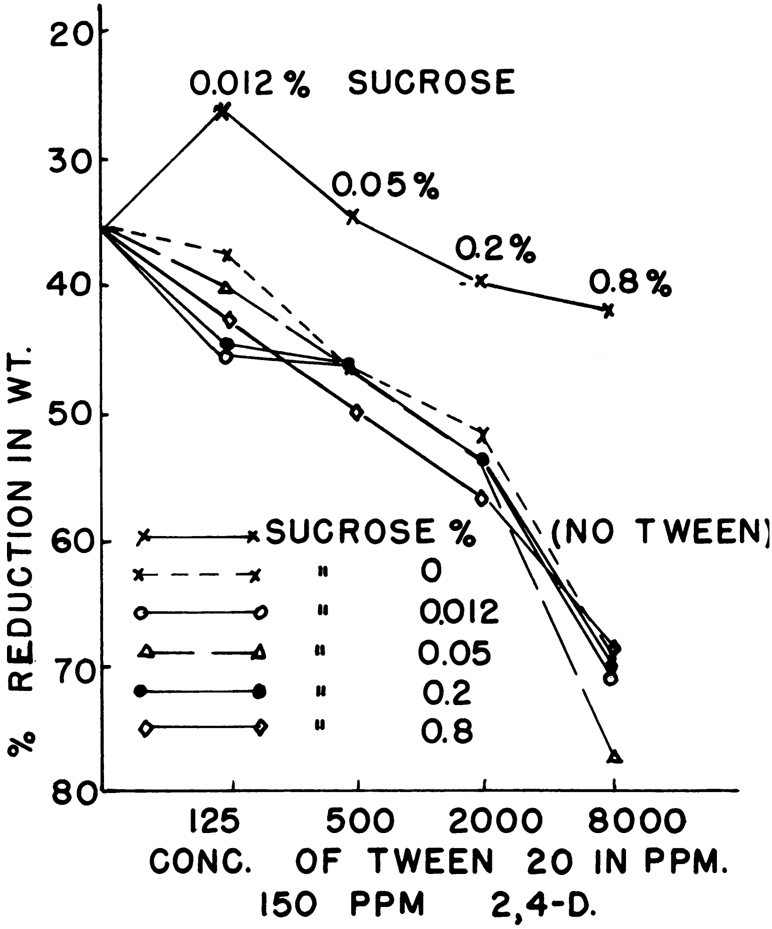

Fig. 13. The influence of different concentrations of sucrose and Tween 20 on the reduction in fresh weight (above the primary leaves) of bean plants treated with the triethylamine salt of 2,4-D. Sprays were applied at the rate of 18 gallons per acre.

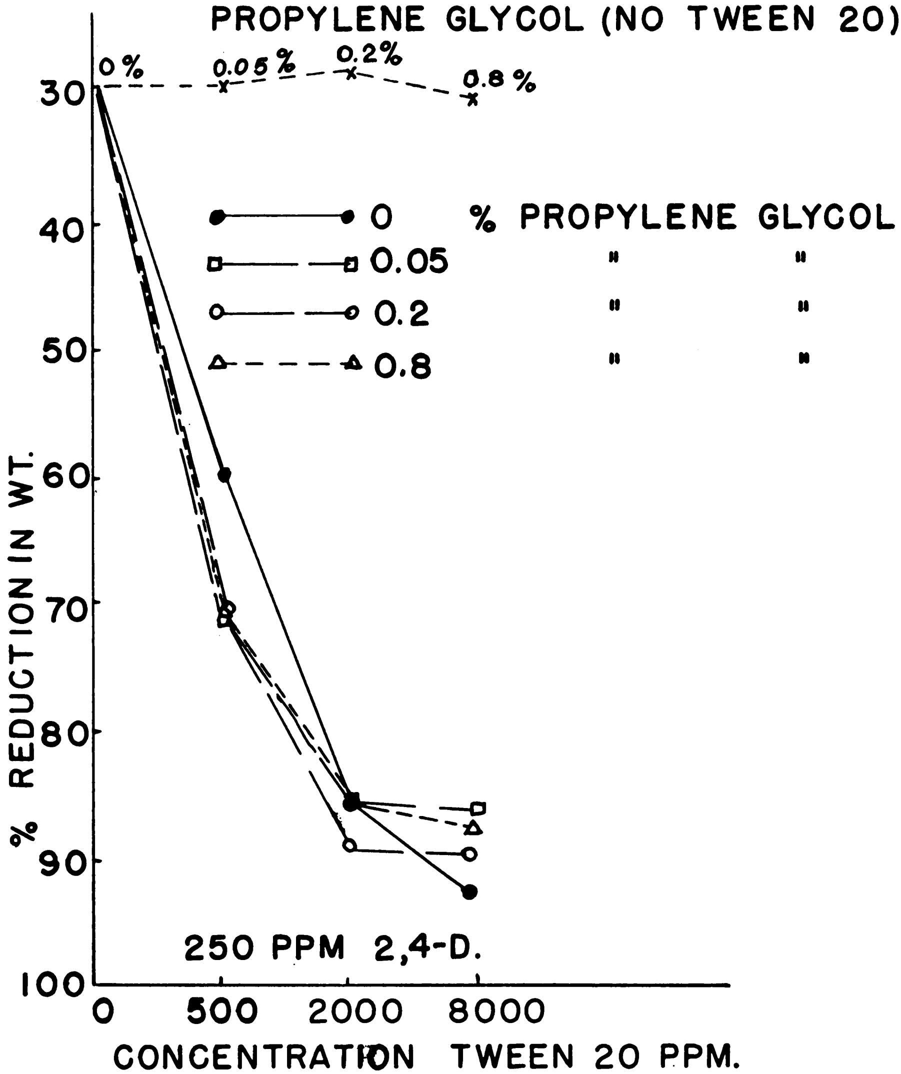

Fig. 14. The influence of different concentrations of propylene glycol on the reduction in fresh weight (above the primary leaves) of bean plants sprayed with the triethylamine salt of 2,4-D.

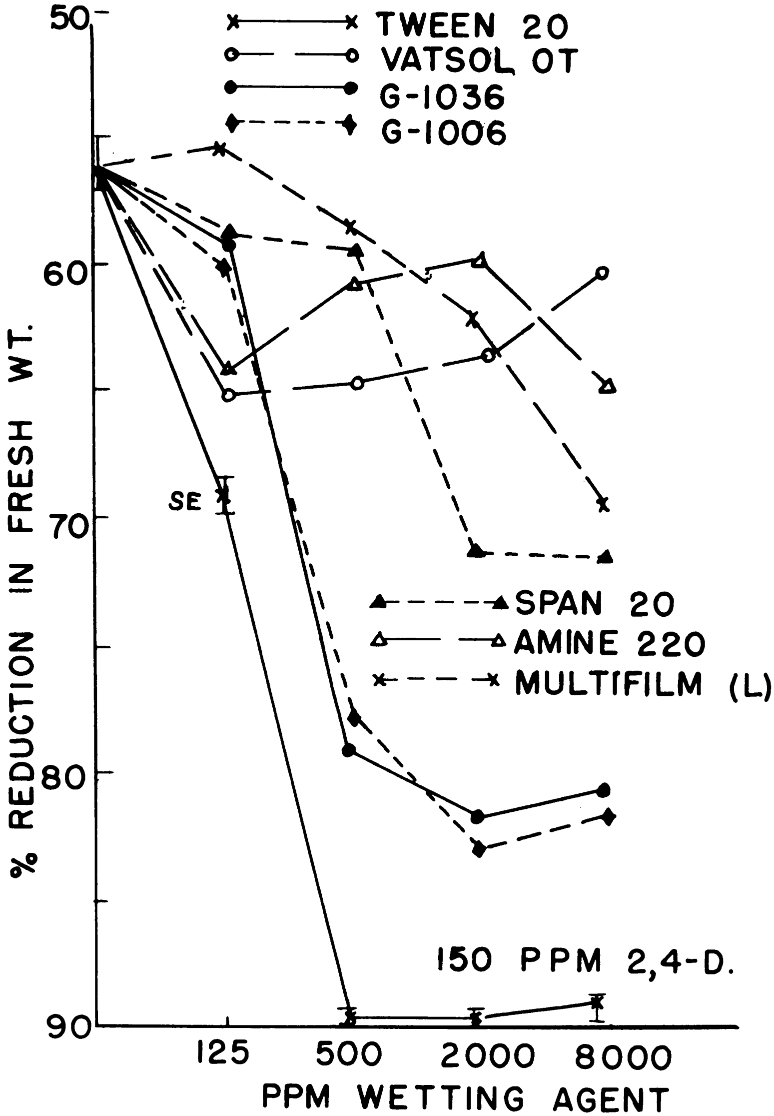

Fig. 15. The influence of several concentrations of different wetting agents on the reduction in fresh weight (above the primary leaves) of bean plants treated with the triethylamine salt of 2,4-D. Sprays were applied at 18 gallons per acre. The standard errors are shown for the Tween 20 treatments.

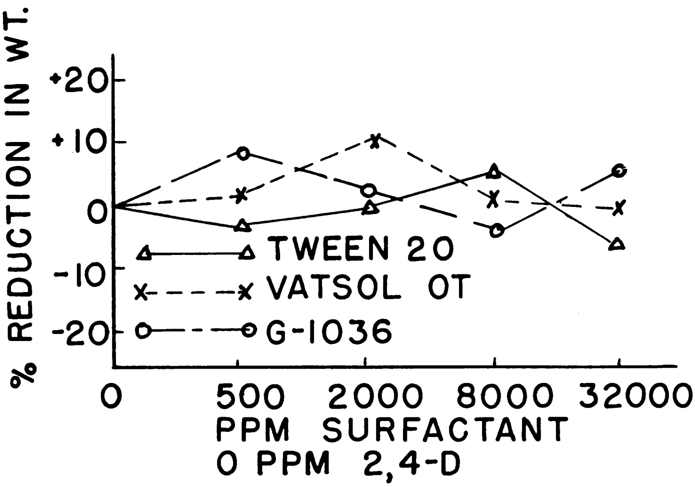

Fig. 16. The influence of several wetting agents on the reduction in fresh weight (above the primary leaves) of bean plants. The plants were sprayed.

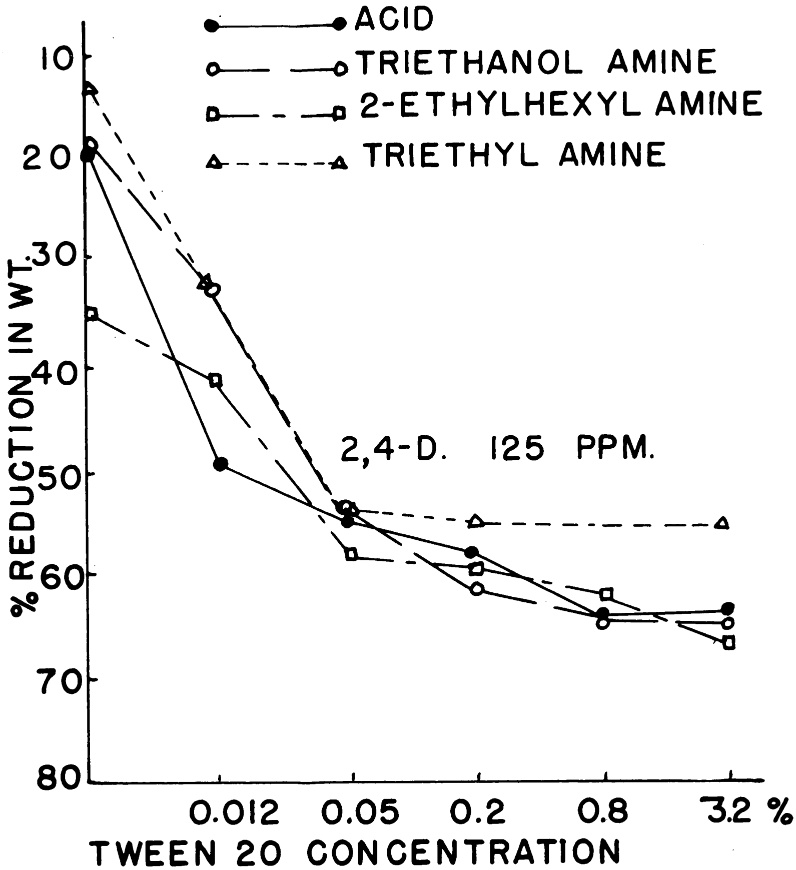

Fig. 17. The influence of different concentrations of Tween 20 on the effect of several formulations of 2,4-D in reducing fresh weight (above the primary leaves) of bean plants. The sprays were applied at 18 gallons per acre.

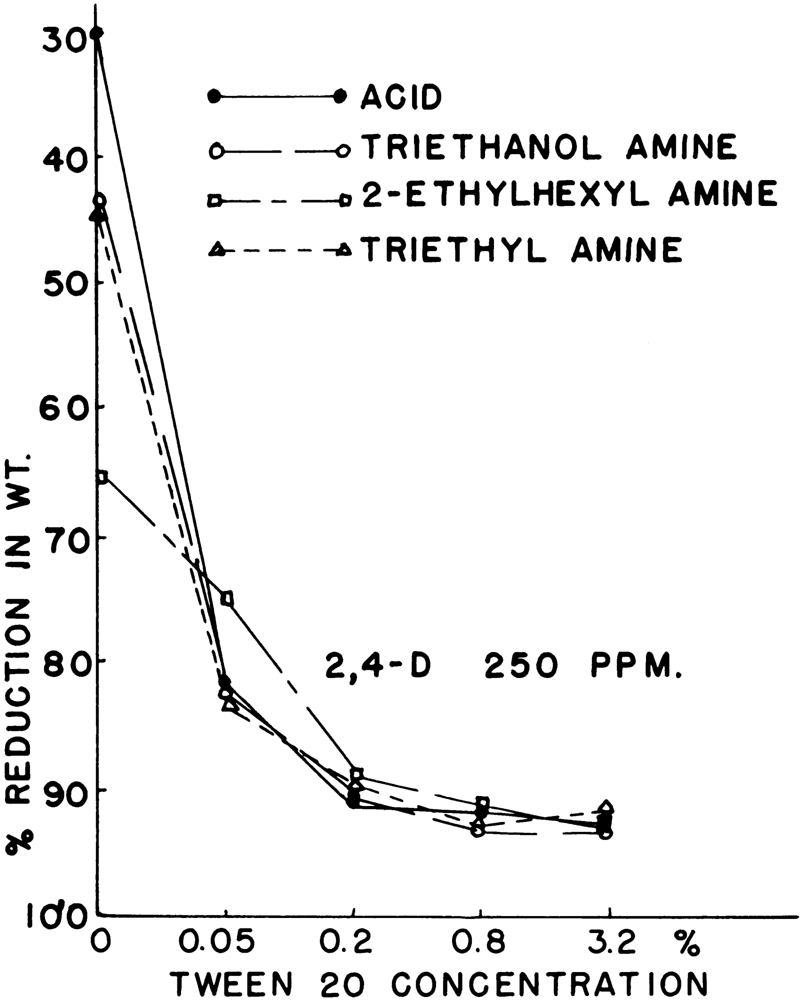

Fig. 18. The influence of different concentrations of Tween 20 on the effect of several formulations of 2,4-D in reducing the fresh weight (above the primary leaves) of bean plants. The sprays were applied at 18 gallons per acre.

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