

FACTORS EFFECTING THE ACTION
OF
2,4-DICHLOROPHENOXYACETIC ACID

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A THESIS

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TABLE OF CONTENTS

	Page No.
I. Introduction	1
II. Review of Literature	
1. Carrier and solvent	3
2. Mixtures of non-hormone and hormone with 2,4-D	4
3. Activated Carbon	7
4. Cation exchanger	8
5. Plant extract	9
6. pH level	10
7. Age of plant	11
8. Species of plants	12
9. Temperature	14
10. Light	16
11. Rainfall	18
12. Carbon dioxide	19
13. Soil moisture, organic matter, temperature and lime on retention of toxicity	19
14. Leaching of soil	21
15. Soil micro-organisms	22
III. Experimental	
1. Materials which greatly increase the action of 2,4-D	
A. Plastic material Geon 31X	24
B. Comparison and discussion of Geon 31X and other coating materials	33
C. pH level in solution of Geon 31X	37
D. 2 Amino-2-Methyl-1-3-propanediol and its related material	42
E. Discussion of 2 Amino-2-Methyl-1-3-propanediol	55
2. Materials which moderately increase the action of 2,4-D	
A. Sucrose with potassium phosphate	57
B. Sucrose with potassium sulfate, Magnesium sulfate, Ammonium sulfate, and other compounds	61
C. Discussion of sucrose combinations	62
3. Materials which decrease the action of 2,4-D	
A. Allyl alcohol	63
B. Potassium ferro-cyanide	67
C. Sodium hypochlorite	68

TABLE OF CONTENTS (cont'd)

4. Materials which have no definite effect on 2,4-D	71
5. Natural factors	
A. Light	74
B. Carbon dioxide	76
C. Humidity	78
D. Infra-red and plant temperature	81
E. Undetermined seasonal factor	83
6. Inhibition of photosynthesis by 2,4-D	84
7. Plant factors	
A. Varietal difference	87
B. Strain difference	90
C. Diploid and tetraploid plants	94
D. Observation of toxicity inheritance	94
E. Doubling and shortening of onion chromosome induced by 2,4-D treatment	96
IV. Summary	104
V. Literature cited	107

I. INTRODUCTION

The development of 2,4-D as a selective herbicide has been rapid and significant. However, it is still not clear how 2,4-D kills plants or why its action varies under different circumstances. 2,4-D in very minute amounts produces symptoms in plants somewhat similar to those caused by disease, and it has been suggested that there may be substances which act as anti-toxins to overcome the effect of 2,4-D and that there may be still other compounds which have synergistic effects increasing or promoting its toxic action.

Since 2,4-D is effective in such small quantities, its mode of action may be modified by numerous unknown factors both internal and external. Experimental data show a great range of variation in response of plants to 2,4-D from species to species and between the result of experimental work in the laboratory or in the greenhouse and its application in the field.

The mechanism of action of 2,4-D represents a scientific picture puzzle. The puzzle needs the assembling of various pieces so that eventually a true picture can be obtained. The present investigation was undertaken in the hope that it would contribute some pieces in the puzzle. Materials which might modify the action of 2,4-D were tested. In

addition some of the natural factors which might have some influence on the action of 2,4-D were also investigated.

Within the plant aberrations and morphological changes of the chromosomes resulting from 2,4-D treatment were observed. It is the hope that the findings reported here would provide some additional clues regarding the behavior of growth regulators on plant growth and also provide information which might aid in increasing the practical use of 2,4-D as an herbicide.

II. REVIEW OF LITERATURE

1. Carrier and Solvent

Mitchell and Hammer (46) first found that the addition of carbowax compound to aqueous solution of 2,4-D increased the effectiveness of the acid in bringing about growth responses and morphological change in kidney bean plants. While a 0.5% solution of carbowax 1500 was proved to be non-toxic. Application of relatively high concentrations of 2,4-D in solution with carbowax killed the bean plants when applied either to the soil or above ground portions.

However, Ennis et al (19) reported that solutions containing above 9% carbowax 1500 were toxic to greenhouse growing tomato plants and that soybean and kidney bean leaves became yellowish when sprayed with solution containing 10 and 15% by weight of carbowax 1500. The 2,4-D produced more highly significant inhibition of growth in kidney bean when sprayed in a solution containing 0.5% of carbowax 1500, 1540, or 4000 than when applied in a wholly aqueous spray.

Zimmerman (70) suggested the value of carbowax as a co-solvent for increasing the concentration of 2,4-D in aqueous solution.

In laboratory studies Hopp and Linder (29) reported that the addition of glycerin to various water soluble

herbicidal sprays, including sodium trichlorophenoxyacetate and sodium pentachlorophenoxyacetate, improved the effectiveness of such sprays.

According to Weaver et al (65) oil is a better carrier of 2,4-D preparations than water under certain conditions as in rainy areas where the oil is less likely than water to be washed off the plant.

Of some 50 organic solvents tested by Ennis et al (18), only tributyl phosphate proved satisfactory as a solvent for preparing oil-miscible solutions of 2,4-D. The 2,4-D readily dissolves in mineral oils. Tributyl phosphate causes local burning of plant tissues at points of direct contact; such an effect may be desirable for herbicidal purposes. This solvent is also capable of dissolving large amounts of 2,4,5-trichlorophenoxyacetic acid, parachlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and other substituted phenoxyacetic acids.

- 2. Mixtures of non-hormone and hormone with 2,4-D

Mangual (41) reported that concentrate 40 + 2,4-D and oil emulsion fortified with Santophen 20 + 2,4-D both suppressed the population of *commelina langicaulis* jacq. and *ipomoea* sp. more than the same nonselective sprays without 2,4-D. The combination sprays also suppressed more weeds than 2,4-D alone. The results indicated that con-

concentrate 40 and oil emulsion fortified with Santophen 20, when used as a combination spray with 2,4-D did not inhibit the lethal effects of 2,4-D and that it may be more effective than either non-selective herbicide when used alone on grass control. In another experiment on Bermuda grass control, the same author found that 2,4-D possibly activated the constituents in concentrate 40, or vice versa with a resulting synergistic reaction.

Crafts (14) recommended that 1 lb. of 2,4-D or its equivalent as an ester can be added to the concentrate consisting of 30 lbs. of medium gravity highly aromatic oil, 2 lbs. of pentachlorophenol, 2 lbs. of oronite wetting agent and 95 gals. of water and that the resulting emulsion spray will kill grasses commelium, coque, bepicos, morivivi (*mimosa pudica*) and many other weeds. In other words it combines the virtues of a general contact herbicide with those of 2,4-D to give a spray that kills most of the weeds of tropical crops.

King (33) reported that certain indole naphthalene and phenyl compounds and those containing various forms of nitrogen, potassium and phosphorus increase the herbicidal activity of 2,4-D in the case of water hyacinth.

A number of workers (62, 4, 41, 14, 20) indicated that the herbicidal activity of 2,4-D was greatly enhanced by the use of highly toxic activators such as arsenicals,

chlorates, sulfamates, and chlorinated phenols; when relatively small amounts of these toxicants were combined with 2,4-D in spray mixtures.

Hitchcock and Zimmerman (28) found that mixtures of α -naphthaleneacetic acid and 2,4-D were more effective in killing the roots of dandelion than either of the individual components.

Southwick (59) suggested that mixtures of 2,4-D and 2,4,5-trichlorophenoxyacetic acid should be more effective, particularly on woody plants, than 2,4-D alone, on the basis that 2,4,5-trichlorophenoxyacetic acid has proven more effective than 2,4-D for killing the shrubs and trees and certain species of *Rubus*.

In some unpublished experiments of Zimmerman and Hitchcock (71) it was found that 2,4-D mixtures containing Benoclor 3C, Sulfamate, or arsenicals are more effective in killing tomato plants than either component alone. These mixtures were also more effective in killing volunteer seedlings of crabgrass, smartweed, purslane, and chickweed. Solutions containing 1 to 3 per cent Benoclor 3C and 0.05 to 0.1 per cent 2,4-D were nearly as effective when applied successively to tomatoes after an interval of twenty-four hours, as when applied as mixtures.

The same authors (71) also found that similar increasing activation of 2,4-D was obtained with

2,4,5-triiodobenzoic acid.

3. Activated Carbon

Lucas and Hamner (39) first found that preparations of 2,4-D are inactivated^a by adsorption on activated charcoal. A solution of the water soluble powder containing 1,000 p.p.m. of the active principle can be safely sprayed on all but very young bean plants after being mixed and shaken with 1 per cent activated charcoal, Norit A.

Weaver (68) demonstrated that plants which are sensitive to the effects of 2,4-D could be protected by dusting or by spraying (aqueous suspension) with an activated carbon preparation (Norit A), or with a cation exchanger (Zeo-Karb H) just prior to, or immediately after, dusting or spraying those plants with 2,4-D preparations. The ion exchange material seemed to be less effective in this respect than the activated carbon; lamp black and bone black were only partially effective.

Arle et al. (9) reported that when roots of sweet potato slips were treated with activated carbon prior to planting in 2,4-D treated soil, the 2,4-D injury to sprouts was minimized while the control plants were seriously injured.

4. Cation Exchanger

Weaver (67,68) reported that cation exchangers in the hydrogen-cycle adsorbed much 2,4-D acid and its ammonium, calcium and cupric salts, and in about equal amounts. Anion exchangers adsorbed much 2,4-D and its salts but not always in equal amounts. Little or no 2,4-D or its salts was adsorbed by cation exchanger in the sodium or calcium form. Much less 2,4-D was adsorbed by Amberlite IR 100 H when the pH of the solution was 3.3 than when it was 2.5 or lower. The amount of 2,4-D adsorbed by ion exchangers from solution varying in concentration from 20 to 200 ppm was in about direct proportion to the concentration of 2,4-D in initial solution.

The same author (69) employed varying ion exchangers to inactivate 2,4-D. He found addition of Zeo-Karb H or Norite A to soil containing 2,4-D resulted in a decrease or in complete elimination of toxicity. In a greenhouse experiment addition of Norite A at a concentration of about 218 ppm decreased the toxic effects of 2,4-D present in the soil. Finely ground Norite A or Zeo-Karb H was dusted or sprayed in aqueous suspension on Red Kidney bean, soybean, white mustard or marigold. The plants were then sprayed with aqueous 0.1% solutions of ammonium 2,4-D, sodium 2,4-D, triethanolamine salt of 2,4-D isopropyl ester of

2,4-D or BE 2,4-D or were dusted with a dust containing BE 2,4-D. In most cases the toxic effects of the growth regulators were greatly decreased or completely eliminated by Norit A or Zeo-Karb H.

5. Plant Extract

The addition of onion extracts to the sodium salts of 2,4-D solution enhanced the activity of the herbicide according to studies made by Lucas and Hammer (38). The activating effect of the onion extract is optimal if it is prepared by diluting 1 part of onion with 20 to 30 parts by weight of distilled water. Lower and higher dilutions are less effective. If a dilution of approximate 1:50 is exceeded, the activating effect is lost. These studies also indicated that water extracts of garlic were less effective than onion extracts in increasing the activity of 2,4-D, that tomato extracts have no effect and that red beet extracts actually inactivate it. They found no significant change of pH whether in the addition of onion extracts to 2,4-D salt solution or in the introduction of the 2,4-D salt into onion extracts, change of pH is not the cause of activation of 2,4-D by onion extracts.

Spear and Thimann (58) tried to determine the effect of the onion juice on 2,4-D and found the effect is not due to an auxin in the onion juice, but it resides in the

ether-insoluble fraction of the juice. Analysis of the juice demonstrated a considerable content of reducing sugar, potassium and phosphate. When these constituents in an approximate concentration as in the juice are substituted for onion juice, they have nearly the same effect. It is concluded that the action of onion is due to its content of sugar, phosphate and potassium ions.

6. pH Level

It had been reported by Dolk and Thimann (17) that auxin solutions caused greater curvatures in the Avena test when applied at a lower pH.

Lucas and Hamner (38) succeeded in increasing the action of 2,4-D salt by the addition of certain concentrations of citric and l-malic acid. Other organic acids having a similar pH did not produce the same effect.

Hamner, Lucas and Sell (34) reported later that the pH range of 2.0 to 3.0 of unbuffered acid solutions gave optimum results if sufficient phosphoric acid was used. The titratable acid within its noninjurious range, rather than the pH value of a solution, accounts for the increased effect of the herbicide.

Lucas, Felber, Hamner and Sell (40) found that at pH₂ and pH₃ buffered and unbuffered solutions of 2,4-D exerted similarly strong inhibiting effects on bean plants.

At pH₄, pH₅, pH₆, and pH₇, however, the inhibiting effect of 2,4-D was far greater in buffered than in unbuffered solutions. At pH levels of 8 and above, the relationship between buffered and unbuffered solutions became erratic and requires further investigations.

7. Age of Plant

Weaver, Swanson, Ennis and Boyd (66) studied the effect of 2,4-D in relation to stages of development of certain dicotyledonous plants. They found that cabbages at younger stages were killed by the 2,4-D at rates of 0.3 or 3.0 pounds per acre but the headed plants were little inhibited in growth. Soybeans at younger stages were for a time severely retarded in growth and distorted by this compound at a rate of 0.5 pound per acre but at older stages they showed less visible response immediately following treatment although subsequent yields of beans were significantly reduced. At rates of 0.1 and 1.0 pound per acre of ammonium 2,4-D young plants (8 to 12 inches high) could be killed, plants at the early flowering stage set no fruit and plants at the early fruiting stage showed a reduction in yield. At rates of 0.25 or 1.0 pound per acre of 2,4-D young sweet potato plants were killed and root development of older plants was greatly reduced. The susceptibility of the sweet potato to the growth regulator decreased with increasing age

and size of plant.

8. Species of Plants

A. Grass family. Marth and Mitchell (42, 44) and Avery (10) first reported that it is possible by spraying an established lawn of bluegrass or mixed grasses with 2,4-D to destroy the dandelion, narrow leaf plantain and numerous other weeds while leaving the grass intact. The bluegrass may turn a darker green but it seems to be unharmed.

Avery (10) found that certain bent grasses (*Agrostis* sp.) are much less resistant to 2,4-D than the bluegrass (*Poa* sp.).

In England Slade (55) found the same results in controlling yellow charlock (*Brassica Kaber*) in oat fields.

Both in Louisiana and Puerto Rico treatment of weeds without damage to sugar cane has been obtained by Arceneaux (8), Brown (11) and Van Overbeck (63).

To control weeds in rice fields in Louisiana and Texas 2,4-D is being applied in dust form from airplanes. However, Kraus and Mitchell (35) reported that in laboratory experiments, rice plant 10 to 12 inches tall have been damaged by emulsions of hormones applied to the water in which they were growing.

There are numerous experimental data on 2,4-D control

of weeds for wheat, flax, corn and other grains. Recommendations are made regionally and will not be reviewed here.

B. Herbaceous Plants. Marth and Mitchell (42) listed the following weeds as relatively sensitive to 2,4-D: dandelion, narrow leaf plantain, Dutch white clover, chickweed, pigweed, woodsorrel, knotweed, broadleaf dock, bindweed, and shiny pennywort.

Hildebrandt (27) had controlled water-hyacinth successfully by 2,4-D application.

Pratically all broad-leaved garden plants are injured by 2,4-D.

However, certain broadleaved weeds are slightly resistant to 2,4-D; for example, broadleaf plantain (*Plantago Major*) and yarrow (*Achillea millafolium*). Some of the most troublesome weeds of the South are at least moderately susceptible to 2,4-D control; examples of these are alligator weed (*Alternanthera philoxeroides*) (8,11), Wild garlic (*Allium vineale*) (57) and water-hyacinth (*Eichornia crassipes*) (27).

C. Woody Plants. Hamner and Tukey (23) and Anonymous (3) found that woody plants whether vines, shrubs or trees are less susceptible to 2,4-D than the herbacious plants. They usually require larger amounts of the hormone for killing dose.

Anonymous (3) reported that ester of 2,4-D kills

woody plants more readily than do the free acids or the salts. Sprays are more effective in killing suckers and stump sprouts than the main body of trees.

Of the conifers it was reported by Johnson (30) that pines are susceptible, at least, in the seedling stage. On the contract Hamner and Tukey (23) found juniper is resistant.

According to Southwick (59) the use of 2,4,5-trichlorophenoxyacetic acid alone or combined with 2,4-D give a best killing of brush.

Many important limiting factors in brush control were described at the Northeastern Weed Control Conference held in New York City, February 1948.

9. Temperature

In studying the selective herbicidal action of mid-summer and fall application of 2,4-D Hamner and Tukey (22) found responses of common weeds were least during hot, dry conditions of July. The responses were much more intense and affected a greater portion of the plant when growing conditions were more favorable in late August.

Marth and Davis (43) reported 2,4-D treatment at the greenhouse temperature of 75 to 90° F resulted in rapid killing of *Barbarea verna* and *Plantago lanceolata* at 18 to 21 days. At 50 to 60° F, however, the time required to kill

plants with the acid treatment was extended for 11 to 15 days longer while at 32 to 40° F the treated plants were still living at the end of 50 days following spray application.

Also it was reported by Hamner and Tukey (23) that application of 2,4-D as a foliage spray resulted in better killing of several shrubs, vines and trees at time of leaf emergence during a warm period of 80 to 85° F in early April than during a cool period below 50° F in late April.

Under Wyoming conditions Klingman (34) found that the best time to kill dandelion was from July 16 to August 15 treatment.

Hitchcock and Zimmerman (28) found that 2,4-D treatments applied in July were as effective or more so for killing dandelion and also for minimizing recovery, as were sprays applied in May, June, or September. This discovery according to the investigators, is not in agreement with the prevalent concept that dandelions are more effectively eradicated in spring and fall than in midsummer.

Kelly (32) recently reported that the minimum concentration of 2,4-D that brought about killing of Red Kidney bean plants varied from less than 0.5% at 5° C, 0.5 at 15° C and 0.01% at 25° C. In crabgrass about 30 to 40 per cent of the killing at 25° C was caused by the high

temperature itself or by some secondary factor since the unsprayed control plants exhibited yellowing at that temperature.

10. Light

In studying the movement of the 2,4-D stimulus Mitchell and Brown (47) reported that the leaves of snap bean seedlings responded 2,4-D more slowly when the plants were in darkness than did the leaves on illuminated plants.

Weaver and DeRose (64) found that Red Kidney bean plants growing in the light showed stem curvature more rapidly than those growing in shade after 2,4-D treatment. Average fresh weights of trifoliate leaf blades also showed significant difference between treated plants in sunlight and shade.

However, Penfound and Minyard (52) found that the effect of 2,4-D on bean plants was the same whether they were placed in the dark, in diffused light or in direct sunlight. Difference in the 2,4-D carriers used was suggested as the source of the discrepancy in these investigations where kerosene was the carrier used by Penfound and Minyard and water was used by the other workers mentioned above.

Penfound and Minyard (52) also reported that 2,4-D in kerosene caused greater epinasty and much greater necrosis

of water hyacinth in shaded conditions while complete destruction did not occur in any plants in full sunlight, although several leaves of small plants exhibited considerable necrosis. In the shade the parent plant was killed quickly, and all the offshoots showed considerable necrosis by the end of the experimental period. In full sunlight, however, only a few leaves of the parent plant were killed, the offshoots not only being injured but increasing in number by 300%.

Thimann (61) studied the influence of sun and shade on the action of 2,4-D on some woody tropical plants. He reported that the plants in partial shade and those sprayed on the underside were slightly less affected by 2,4-D after 12 days than those sprayed on the top in full sun.

Payne and Fults (50) activated 2,4-D the sodium salt and butyl ester of 2,4-D, and 2-methyl-4-chlorophenoxyacetic acid with ultraviolet light in the laboratory. In view of their findings and because ultraviolet light quality and intensity vary with changes in atmospheric conditions, altitude, and season of year, these investigators suggested that comparative tests of the herbicidal effects of these activated and nonactivated chemicals be conducted to unearth a possible explanation of the variable results secured from uniform trials with 2,4-D and other similar compounds at different places and times.

Rice (53) reported there was no significant difference between the amount of 2,4-D salt absorbed at 100 foot-candles of light and at 900 foot-candles at the same air temperature; the amount absorbed in the dark was significantly greater. But the relative fresh weights of the trifoliate leaf blades of plants treated at 100 foot-candles of light were considerably greater than those of plants treated at 900 foot-candles. He suggested that the rate of translocation of NH_4 (2,4-D) from the leaf in the Kidney bean is positively correlated with light intensity.

11. Rainfall

Weaver, Minarik and Boyd (65) studied the influence of rainfall on the effectiveness of 2,4-D sprayed for herbicidal purposes. They found when, 2,4-D was applied in oil solution, an immediate heavy rain caused no diminution in the response to the herbicide. When aqueous solutions of the compound were used, rainfall often decreased plant response. In greenhouse experiments the plant responses were not decreased by artificial rainfall if 6 hours or more elapsed between application of the chemical and artificial rainfall, but in a field trial rainfall occurring within 24 hours of application reduced effectiveness of the herbicide. They suggested that in regions of frequent rainfall the use of an oil carrier for

a herbicide is advantageous.

Ennis, Thompson, and Smith (18) found in the preparation of oil sprays, tributyl phosphate can be used as a co-solvent of 2,4-D.

12. Carbon Dioxide

Mitchell and Brown (47) reported that the stem of a bean plant whose leaf was treated with 2,4-D in aqueous carbowax mixture and was supplied with air containing a normal amount of CO₂ developed a marked curvature within 6 hours following treatment. In contrast the stem of the plant whose leaf was supplied with CO₂-free air failed to develop a curvature during the same period. Stem curvature did not develop in plants whose treated leaves were surrounded by CO₂-free air, even during a period of 17 hours following treatment.

13. Soil Moisture, Organic Matter, Temperature and Lime on Retention of Toxicity

Mitchell and Marth (48) found that the toxic effect of 2,4-D was reduced more rapidly in moist than in dry soil. In warm, moist soil the toxicity of 2,4-D lasted for only 2 weeks, while in dry soil it persisted for as long as 18 months. They concluded that "it is apparent that the effectiveness of the acid in killing weed seeds and seedlings in

soil will depend in part upon the organic and moisture content of the soil, upon the temperature to which it is subjected, and upon the soil flora and fauna present." The findings of DeFrance et al (15), Jorgensen and Hamner (31) and Brown and Mitchell (12) also seemed to indicate that increase in moisture content tends to hasten the dissipation of the 2,4-D effect in the soil.

High temperatures were more favorable to the disappearance of 2,4-D (Na salt) than freezing or subfreezing temperatures, the compound being equally effective as a weed seed killer under all these temperatures (31). Brown and Mitchell (12) showed that with soils treated with 2,4-D and stored, the higher the storage temperature, the greater was the rate of inactivation of the compound.

The relation of moisture content, organic matter content, and lime to the persistence of 2,4-D toxicity in the soil has been studied by Kries (36). Her work indicates that the residual effect of 2,4-D persists for a long period in dry soil and the toxicity in the limed soil lasts for a longer period than in the unlimed soil. By adding large amounts of leaf mold to the limed soil, she was able to cause a striking reduction in the degree and persistence of the 2,4-D. However, according to Hanks (26) the leachates from limed and unlimed soils showed no significant difference in toxicity. In contrast to the findings of

Kries, Jorgensen and Hamner (31) found that the difference in soil pH (4.5, 6.0, 7.5) had no effect on the rate of loss of 2,4-D toxicity.

14. Leaching of Soil

In studying the persistence of 2,4-D in soils DeRose (16) found that when a soil was contaminated with 2,4-D and then leached, the leachate contained the compound. Under greenhouse conditions this herbicide did not remain active in unleached soil for longer than 8 weeks. In the field it did not persist in the soil for more than 80 days after treatment.

Hanks (26) treated several soils representing a wide range in pH and physical properties with lime, 2,4-D and calcium salt of 2,4-D. After treatment these soils were leached, and their leachates were tested for relative toxicities. Leachates from the peat soil were relatively non-toxic at the end of 2 weeks and the leachates of all soils tested except those of the naturally alkaline one, were relatively free of the herbicide at the end of 6 weeks. There was no significant difference in toxicity between the leachates from the limed and the unlimed soil; nor was there any significant difference in toxicity between leachates from the soil treated with 2,4-D and from the soil treated with the calcium salt of 2,4-D.

15. Soil Micro-organisms

Brown and Mitchell (12) indicated that a light application of manure to soil low in organic matter hastened the inactivation of 2,4-D. Since micro-organisms thrive on organic matter and 2,4-D is an organic compound, it is assured that the higher the organic matter content, the greater will be the number of micro-organisms to decompose the 2,4-D. Where the soil was autoclaved which destroyed the soil micro-organisms, the 2,4-D toxicity remained at a high level during a period of at least 2 weeks. In contrast, a large part of the 2,4-D in unautoclaved soil stored under the same condition of soil moisture and temperature was inactivated during the same period of time.

Martin (45) reported that soil microbes are apparently able to decompose 2,4-D when grown in artificial media containing the substance.

It has also been demonstrated by Stevenson and Mitchell (60) that some fungi and bacteria grow readily on agar media containing as much as 1,000 ppm 2,4-D. (1)

Smith et al (56) indicated that concentration of 1 to 100 ppm of 2,4-D in silt loam and 55 ppm in sandy soil "had no significant effect on the total plate counts, actinomyces, fungi, and protozoa."

However, nodulation in legumes had either been re-

duced or prevented by 2,4-D (13, 21, 51, 18).

III. EXPERIMENTAL

1. Materials Which Greatly Increase the Action of 2,4-D

A. Plastic Material Geon 31X

Geon 31X is a polymer, furnished as a colloidal dispersion of modified vinyl resin in water containing 55% total solids. It is negatively charged, spherical particles have a diameter of about 0.2 micron. By drying the new Geon latex at room temperature, strong films and coatings having good adhesion to paper, fabric, leather, wood, etc., may be prepared. Films normally contain no plasticizer and have low moisture vapor transmission. Besides forming films, this new latex can be used as a laminating agent which requires neither heat nor pressure. Furthermore, it demonstrates good coagulant dip characteristics.

Physical properties of the latex are listed as follows (table 1), (2).

Table 1

Physical Properties of Geon 31X Latex

Physical properties	Geon 31X
Color	White
Mechanical Stability	Good
pH	7-8.5
Total Solids	50-55%
Specific Gravity	1.25
Surface Tension (dynes/cm)	34-40
Viscosity (centipoises)	10-20
Particle Size (microns)	0.2

Experiment I

(1) Method and Material

Seeds of Red Kidney beans selected for uniformity, were planted November 1947 in 4" pots in the greenhouse. Each pot contained two plants that were treated when the first trifoliate leaf was expanding. Twenty plants were used for each treatment.

Application of 2,4-D was made by dipping one of the primary leaves of each treated plant into solutions containing the sodium salt of 2,4-D in varying concentrations of 1, 5, 50, 250, and 500 ppm. After the solutions had dried, the treated leaves of half of the plants were sprayed with a dispersion of 5% Geon 31X latex.

(2) Result

Within 48 hours after treatment marked differences were noted between the plastic and nonplastic treated lots. Curvature of the first internode and epinasty of the leaves were much more pronounced in the plants which had received a plastic coating in addition to 2,4-D treatment. Nine days after treatment, all the plants that had been treated with 500 ppm of 2,4-D were dead. In the group tested with 250 ppm, death occurred in all the plants which had received, in addition to the 2,4-D, the 5% plastic coating. Those plants that received only 2,4-D were beginning to resume new growth. Although the stems were swollen and the leaves

and petioles somewhat twisted, the plants were definitely recovering from the treatment (Fig.1). In the group treated with 50 ppm of 2,4-D, only those plants that had received, in addition, a 5% spray of Geon 31X latex were severely affected; some of the plants were dead, and those not killed by the treatment showed no new growth and no sign of recovery (Fig.2). Where 5 ppm of 2,4-D was used, the addition of the plastic did not increase the response of the plants.

Plate I

The effect of Geon 3lx in increasing the action of 2,4-D salt.

Fig. 1 Bean plants on the left were treated with 250 ppm of 2,4-D salt by dipping one of the primary leaves in the solution of 2,4-D. Plants on the right received the same concentration of 2,4-D, but, in addition, the treated leaves were sprayed with 5% of Geon 3lx.

Fig. 2 By the same method of application as mentioned above bean plants on the left were treated with 50 ppm of 2,4-D. Plants on the right received the same concentration of 2,4-D, but, in addition, with 5% of Geon 3lx.

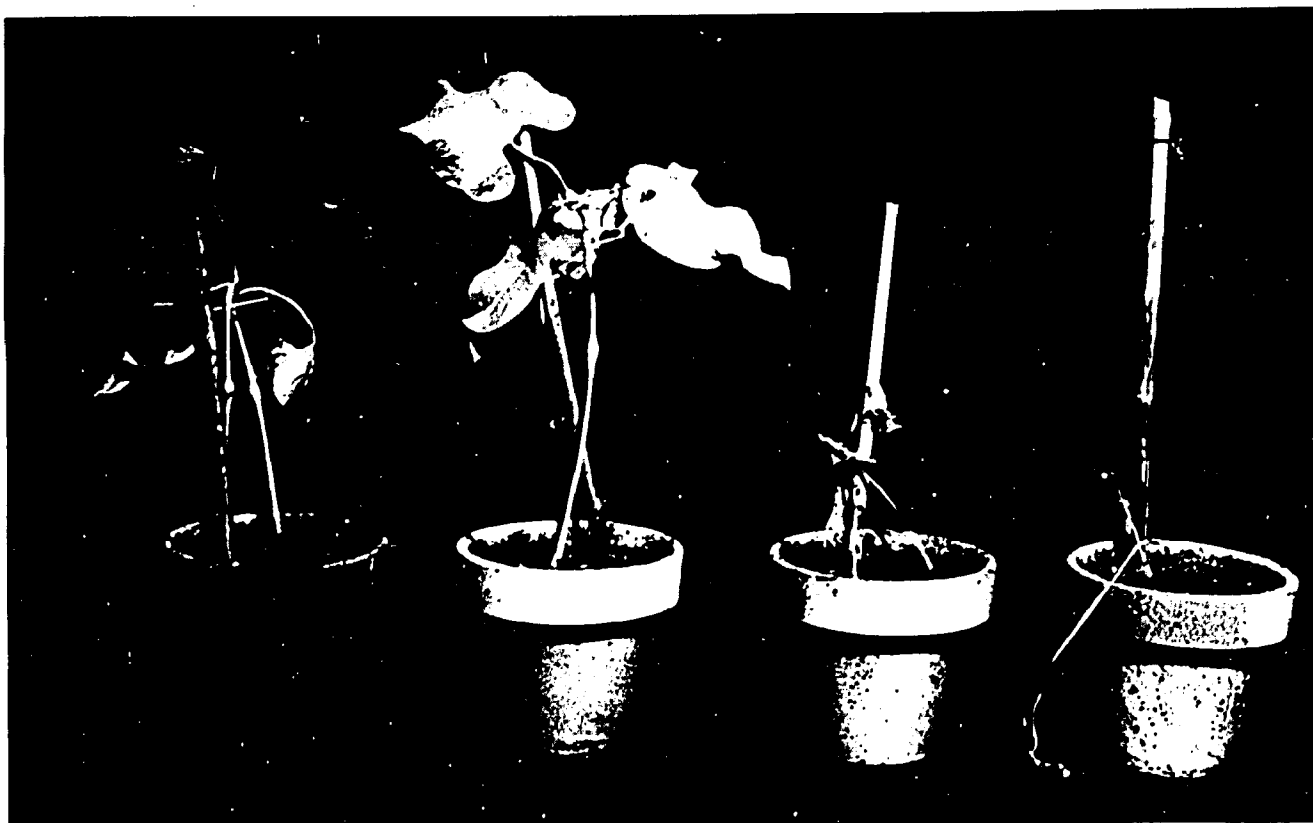


Fig. 1



Fig. 2

Experiment II

(1) Method and Material

In this experiment the plastic material was added directly to the solution of $\text{Na}_2,4\text{-D}$. Ten-day-old Red Kidney beans planted December 1947 were used as the experimental material, and applications were made by placing a single drop of the solution on the base of one of the primary leaf-blades. Pipettes which released 0.05 ml of solution were used for applying the drops. The amount of $\text{Na}_2,4\text{-D}$ used in all the solutions remained constant at 1,000 ppm; the amount of plastic material added to the 2,4-D however, ranged from 0% to 1, 5, and 10%. The plants were harvested 2 weeks after treatment, and the average fresh weight of growth above the primary leaves was recorded for each treatment.

(2) Result

Differences between treatments were noted a few hours after application. It was evident that the response of the bean plants was directly proportional to the concentration of the plastic in the solution. The plants treated with 2,4-D plus a 10% concentration of the plastic showed the greatest degree of inhibition. Those treated with 2,4-D and a 1% concentration of the plastic, although not so severely inhibited as those with the 10% plastic, were nevertheless much more affected than those receiving only

2,4-D (Table 2). No inhibiting effect was noticed when the entire plant was sprayed only with the Geon 31X latex in concentrations of 1, 5, and 10%.

Table 2

Fresh Weight of bean shoots determined 14 days after treatment with 2,4-D salt and Geon 31X latex

Treatment (2,4-D at 1,000 ppm)	Average Weight of Shoot/ plant (gm) //	Average Weight of shoots on per cent basis 2,4-D=100
Untreated	2.45	144
2,4-D salt	1.70	100
2,4-D salt plus 1% Geon 31X latex	.39	23
2,4-D salt plus 5% Geon 31X latex	.25	14
2,4-D salt plus 10% Geon 31X latex	.23	13

// The weight represents all growth above the primary leaves.
// Twenty plants were used for each treatment.

Experiment III

(1) Method and Material

Two methods of treatment were used:

a. Young oat seedlings approximately 4 inches tall were treated January 1948 with sprays containing 1,000 and 3,000 ppm of Na2,4-D. In addition to 2,4-D some of the sprays contained Geon 31X latex at 6, 12, and 25%. Since water spray of 2,4-D did not stick well on the leaf surface of oats when it was used alone, a little soap powder was

added as sticker.

b. The same oat seedlings were treated with a few drops of the above materials around the base of plants near the soil.

(2) Result

Two days after treatment plants treated with 2,4-D alone showed no effect. Those which received 2,4-D at 3,000 ppm together with 25% Geon 31X latex became pale in color and leaf blades turned down, shrinking and finally yellowish. Those which received 2,4-D at 1,000 ppm in addition with 25% Geon 31X showed the same response but not as severely. Plants which were treated by covering the base of plant body with 2,4-D at 3,000 ppm and 25% Geon 31X bent in the treated region near the soil surface. No visible response was seen with the rest of the treatments.

Ten days later almost all the plants treated with 2,4-D at 3,000 ppm together with Geon 31X at 25% were dead. Scattered survivals from treatment were very weak, could not stand up straight and sprawled on the pots. New growth of them was arrested. Plants treated with 2,4-D at 1,000 ppm together with Geon 31X at 25% were also injured, but otherwise not as severely and only few of them died. Those treated on the base portion of plant body with 2,4-D at 3,000 ppm and 25% of Geon 31X were greatly stunted (Fig. 3).

After twenty days affected plants resumed their new growth. But they showed different abnormalities: leaves developed with onion-like appearances. In some of them the tips did not separate from the sheath and thus formed a loop of leaf. Some of the leaves separated from the sheath but twisted into spiral shapes (Fig. 4). These abnormalities were not frequently found on 2,4-D treated plants, but were commonly found on plants treated with 2,4-D and Geon 31X.

At the time the untreated plants produced seeds, those treated with 2,4-D together with Geon 31X below 6% were delayed. Plants which received 2,4-D at 1,000 and 3,000 ppm in addition with 25% Geon 31X were still in the vegetative stage and grew rapidly and vigorously. Finally they reached 4 to 6 inches taller than the untreated plants or plants which were uninjured. It took almost one month longer for these plants to produce mature seeds. Maturity was thus delayed for almost one month.(25).

Plate II

The effect of Geon 3lx in increasing the action
of 2,4-D salt

Fig. 3

Oats seedlings on the right were sprayed with 1,000 ppm of 2,4-D. Seedlings on the left were sprayed with the same concentration of 2,4-D plus 25% of Geon 3lx, and seedlings in the middle were treated with the same concentration of 2,4-D and Geon 3lx by placing the solution on the base of each plant stem.

Fig. 4

Oats seedlings were sprayed with 1,000 ppm of 2,4-D and 6% of Geon 3lx. Abnormal growth showed on the leaves of some plants.

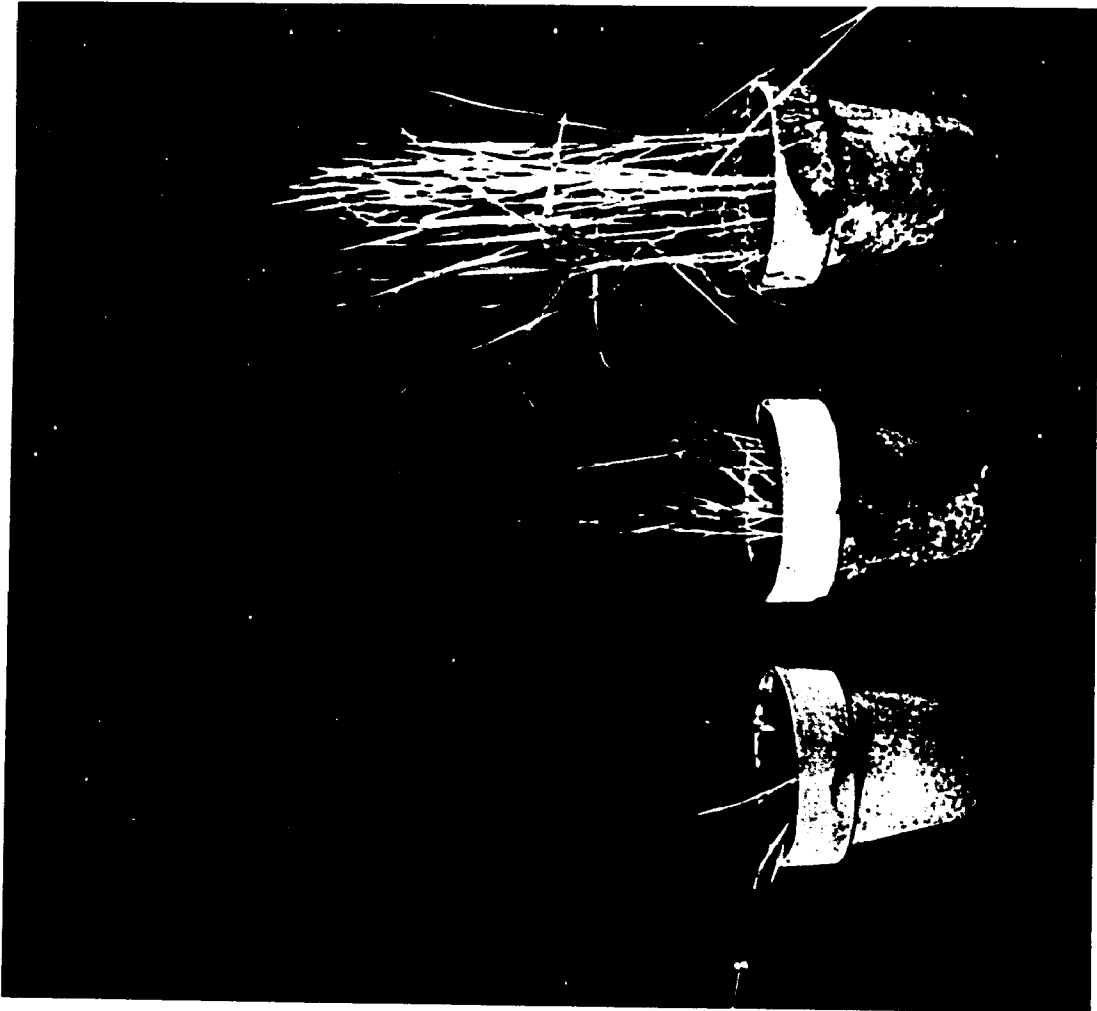


Fig. 3

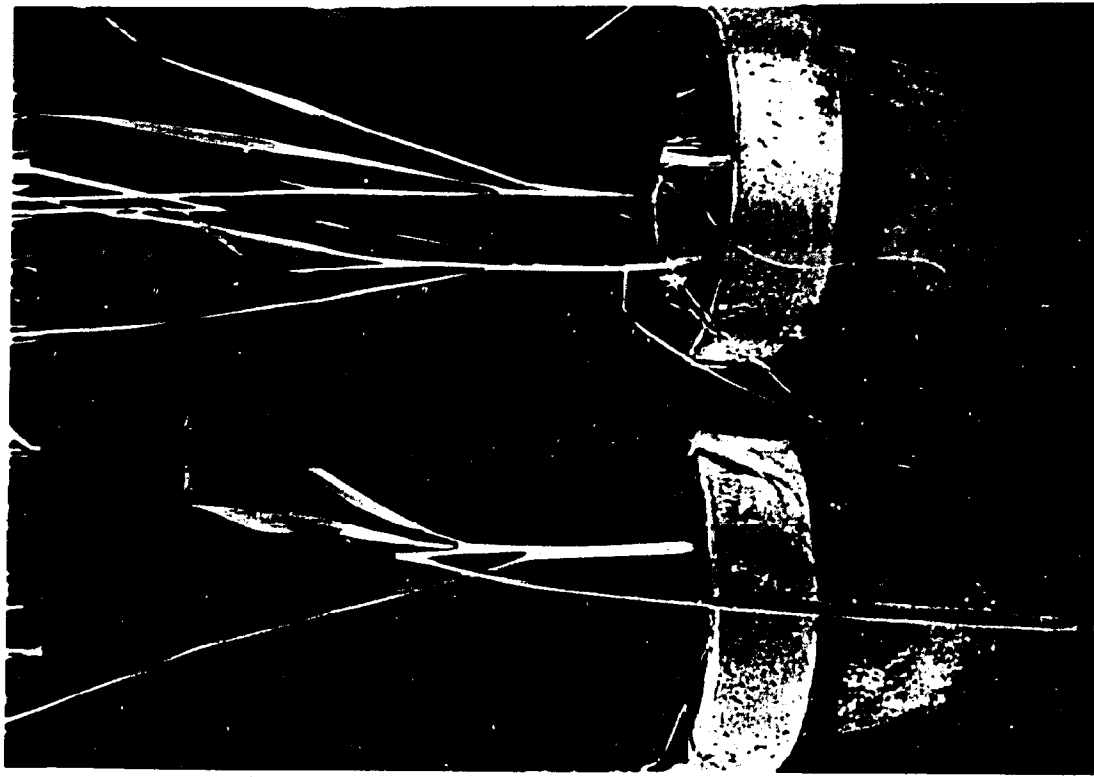


Fig. 4

B. Comparison and Discussion of Geon 31X and Other Coating Materials

Experiment I

(1) Method and Materials

Ten day-old Red Kidney beans planted June 1948 were used as the experimental materials, and applications of Na2,4-D were made by placing a single drop of the solutions on the base of one of the primary leaf blades. The amount of 2,4-D used in all the solutions remained constant at 1,000 ppm. In addition to these solutions they were given a combination of 1% of Geon 31X, sodium silicate, agar, gum arabic and Canada balsam.

(2) Result

Plants treated with Geon 31X showed the increasing effect on 2,4-D as in previous experiments. However, neither of the other materials had any visible effect although they formed various kinds of coating on the spots where they were located.

(3) Discussion

The manner in which the plastic material acts to increase the effectiveness of 2,4-D was assumed that the carbon dioxide and oxygen relationships may be changed as a result of the coating and hence affect the physiology of the cells, making them more susceptible to 2,4-D injury. However, this experiment showed simply that coating effect

could not be enough to explain the behavior of Geon 31X.

Experiment II*

(1) Method and Material

Application of Na2,4-D was made by placing two drops of the solutions on the base of both primary leaf blades of Red Kidney bean plants. The amount of 2,4-D used in these solutions remained constant at 500 ppm. Some of the solutions contained "Elvanol" polyvinyl alcohol at 2.5 and 5%. Generally this material forms a temporary coating which can be easily removed and which is often used for articles in process or in storage as protection against abrasion, grease, solvents and certain gases. Films deposited from water solutions are nontoxic, transparent, tough, and highly resistant to oils, greases, fats, hydrocarbons, and most organic solvents. They are highly impervious to many gases such as hydrogen, hydrogen sulfide, carbon disulfide, oxygen and nitrogen. There are, however, a few exceptions including ammonia gas and water vapor (7).

(2) Result and Discussion

Since this material also has plastic coating characteristics, the same kind of effectiveness as in Geon 31X might be obtained in increasing the action of 2,4-D, but it showed no effect in this experiment.

It might be due to that plastic film of "Elvanol" is

*Conducted in August, 1948.

permeable to water vapor and ammonia gas, whereas Geon 31X is not, and hence their effect on 2,4-D are different (Ref. (2) and (7)).

Experiment III*

(1) Method and Material

a. Application of Na2,4-D was made by placing two drops of the solutions on the base of both primary leaf blades of Red Kidney bean plants. The amount of 2,4-D used in these solutions remained constant at 500 ppm. Some of the solutions contained "Elvanol" polyvinyl acetate at 5 and 10%. This emulsion of polyvinyl acetate resin in water possesses a transparent synthetic resin characterized by excellent thermoplastic adhesive and film-forming properties. It is not necessary to apply it as a hot melt or as a solution in an organic solvent. In physical property this emulsion is viscous, milk-white liquids containing 55 to 57% solids with pH level from 4 to 6. Such materials as metal, wood, cloth, paper and ceramic-ware can be given highly adherent coatings by treating with "Elvacet" emulsion and air drying. These films are very stable to light and oxidation, and are not affected by aliphatic hydrocarbons, vegetable oils, or animal fats (6).

b. Bean plants were treated with sprays containing 100 ppm of 2,4-D. In addition to it some of the sprays contained "Elvacet" polyvinyl acetate in water emulsion at

*Conducted in August, 1948.

5 and 10%. A hand sprayer was used and plants were thoroughly wetted by spraying.

(2) Result

It was erratic that different results were obtained by different methods of application. While plants treated with 2,4-D at 500 ppm and also "Elvacet" at 10% by placing two drops on the base of primary leaf blades showed some increasing effect of 2,4-D, those by spraying 2,4-D at 100 ppm plus the same amount of "Elvacet" showed no effect. On the other hand, addition of "Elvacet" at 5% to 500 ppm of 2,4-D had no effect in application of two drops on the leaf base, but some increasing effect was shown with the spraying method. Because of this uncertain result and since its effect was not great, further experiments were not conducted.

(3) Discussion

Since this compound is very similar to Geon-31X in characteristics, it might also increase the action of 2,4-D but this was not the case. As a matter of fact, it is not necessarily true that the similar plastic material possesses the same behavior on 2,4-D.

Experiment IV*

(1) Method and Material

One group of bean plants were treated by dipping one

*Conducted in March, 1950.

of the primary leaves into the solution of Na2,4-D at 1,000 ppm and the other group, into the solution of 2,4-D in addition to Geon 31X at 5%. After the solutions were air-dried, all the plants were, one by one, moved in the infra-red and measured for their temperature by the use of Potentiometer and Thermocouple connection.

(2) Result and Conclusion

Under Infra-red lamp which was set around 15 inches above the leaf surface, untreated leaf at right angle to the light source were measured with their temperature from 93 to 94° F. Leaf treated with 2,4-D at 1,000 ppm gave the same measurements. However, one of the primary leaves treated with 2,4-D in addition to Geon 31X gradually increased the temperature to 102 to 104° F as the time elapsed, and the other untreated primary leaf of the same plant remained from 93 to 94° F.

From this result it seems that the building up of temperature and the sealing up of 2,4-D in contact with leaf surface by the use of Geon 31X are in part responsible for facilitating the penetration of 2,4-D into the plant cell especially in cool weather.

C. pH Level in Solution of Geon 31X

Experiment I*

(1) Method and Material

*Conducted in August, 1949.

Applications were made by placing a single drop of the solution on the base of one of the primary leaf blades of bean plants. The amount of $\text{Na}_2,4\text{-D}$ used in all the solutions remained constant at 1,000 ppm; 5% of Geon 31X with its pH ranging from 2.5 to 7.5 were added to the 2,4-D. The pH of Geon 31X originally ranges between 7 and 8.5 and may drop slightly on prolonged storage. To lower the pH level into a 2.5 reading acetic acid was used in a prepared solution.

(2) Result

Four days after treatment all the plants that had been treated with 1,000 ppm of 2,4-D showed curvature of the first internode and epinasty of the leaves. Their stems became swollen and yellowish. Those treated with 2,4-D at 1,000 ppm in addition of Geon 31X with pH 7.5 or in its original pH level showed increasing herbicidal effect on bean plants. Plants treated with 2,4-D and Geon 31X with pH lowered to 2.5 by acetic acid, however, showed decreasing effect of 2,4-D. Their treated leaves burned and died. Their terminal buds also burned and died, although they were not in contact with the solution. It is obvious that acetic acid is not suitable to lower the pH level in this kind of treatment, and low pH level may burn the plant's tissue and prevent the penetration and translocation of 2,4-D instead of increasing its effectiveness.

Experiment II*

(1) Method and Material

Amounts and applications of Na2,4-D and Geon 31X were the same as Experiment I. However, their pH levels were lowered to 2.5 by the addition of citric acid and raised to 9-10 by the addition of potassium hydroxide.

(2) Result

Except plants treated with 2,4-D alone, all the other plants treated with 2,4-D in addition of Geon 31X showed the same increasing herbicidal effect. It made no difference whether the pH levels in these solutions were 2.5, 7 or 10. Twenty days after treatment all these plants had no new growth at all, while those treated with 2,4-D alone were flowering (Fig. 5).

* Conducted in October, 1949.

Plate III

Different pH levels showed no effect on the herbicidal action of 2,4-D salt plus Geon 31x

Fig. 5 Bean plants from right to left were treated with (1) 2,4-D, (2) 2,4-D plus Geon 31x at pH 10, (3) 2,4-D plus Geon 31x at pH 7, and (4) 2,4-D plus Geon 31x at pH 2.5

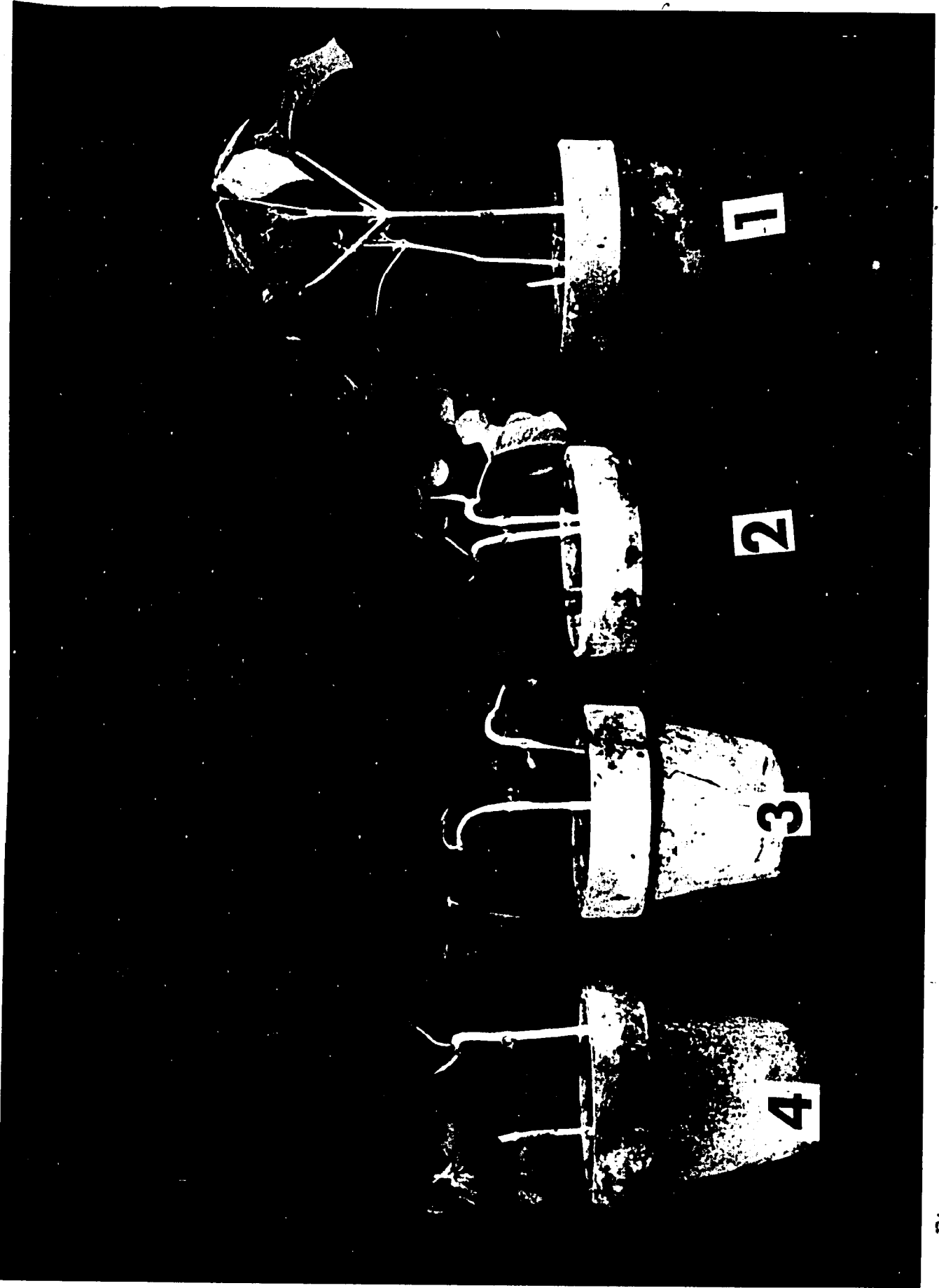


Fig. 5

Plants were harvested and weighed as shown in

Table 3.

Table 3.

Fresh weight of bean shoots determined 20 days after treatment with 2,4-D salt and Geon 31X latex with its pH level ranging from 2.5, 7, and 10.

Treatments (2,4-D at 1,000 ppm and Geon 31X at 5%)	Average Weight of Shoot/plant (gm)	Average Weight of shoots on per cent basis 2,4-D=100
Untreated	2.92	194
2,4-D	1.50	100
2,4-D + Geon 31X pH 9-10	0.09	6
2,4-D + Geon 31X pH 7.0	.09	6
2,4-D + Geon 31X pH 2.5	.05	3

* The weight represents growth above the primary leaves whether it is a shoot or a swollen terminal bud.

** Twenty plants were used for each treatment.

(3) Discussion

Since Geon 31X is at pH 7 to 8.5, that by dropping the pH level to 2.5 it might furthermore increase the action of 2,4-D. Yet, present result showed that the pH level had little to do with this combined solution.

Geon 31X is negatively charged. According to Lloyd (37) the mechanism of entry of one element to another through a semi-permeable membrane is based upon the following consideration, that if a non diffusible ion is present in a system bounded by a membrane, it will exert an electrostatic repulsion on the diffusible ion of the same sign leading to the repulsion of these to the opposite side of

the membrane. Meyer and Anderson (49) stated that cytoplasm is usually on the alkaline side of its iso-electric range and therefore, it should be expected that the constituent micelles or non diffusible ions are negative charged and hence would be repulsed by the cytoplasm. Under this condition if by lowering the pH of the solution of 2,4-D the cytoplasm would change from the alkaline side of the iso-electric range to the acid and hence the micelles would attract the 2,4-D Molecule and 2,4-D would enter, but present experiment is not agreed with these theories. It is not known why negatively charged Geon 31X could not decrease the entry of negatively charged Na-2,4-D into negatively charged cytoplasm. It is not understood why increasing the pH of this solution did not increase the repulsion of cytoplasm which is already alkaline.

D. 2 Amino 2-Methyl 1-3 propanediol and its related material

Both 2 Amino 2-Methyl 1-3 propanediol and 2 Amino 2 Methyl 1-propanol are members of Aminohydroxy compounds. They form substituted amides with esters, anhydrides, and acyl halides. They also readily react with alkyl halides, aldehydes, ketones, and carbon disulfide. Usually these compounds are being used for the synthesis of wetting and surface-active agents, pharmaceuticals, photographic developers, resins, and dyestuffs. In aqueous solutions they absorb acidic gases such as CO_2 and H_2S when cold, and

liberate them when heated. Both of them are powerful emulsifying agents for preparing water dispersions of fats, oils, waxes, and resins.

In Table 4 are listed the important physical properties of these new compounds (5):

Table 4

Physical properties of Aminohydroxy Compounds

	2-Amino-2Methyl-1,3-Propanediol	2-Amino-2-Methyl-1-Propanol
Formula	$\begin{array}{c} \text{NH}_2 \\ \\ \text{CH}_2\text{OH}-\text{C}-\text{CH}_2\text{OH} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{NH}_2 \\ \\ \text{CH}_3-\text{C}-\text{CH}_2\text{OH} \\ \\ \text{CH}_3 \end{array}$
Molecular Weight	105.14	89.14
Melting point, °C	109 to 111	30 to 31
Boiling point, °C	151 to 152 10 mm	165 760 mm
Specific Gravity at $\frac{20^\circ}{20^\circ}\text{C}$		0.934
pH of 0.1M Aqueous solution at 20° C	10.8	11.3
Solubility in water -grams per 100 ml at 20°C	250	Completely miscible

Experiment I*

(1) Method and Material

Ten-day-old Red Kidney bean plants were used as the experimental material. Applications were made by placing a

*Conducted in January, 1949.

single drop of the solution on the base of one of the primary leaf blades. The amount of Na2,4-D used in all the solutions remained constant at 1,000 ppm. In addition to 2,4-D some of the solutions contained 5g./l. of 2 Amino-2Methyl-1-3 propanediol and of 2 Amino-2Methyl-1 propanol.

(2) Result

One day after treatment, marked differences were noted between the lot treated with 2,4-D and those treated with 2,4-D plus 2-Amino-2Methyl 1-propanol and 2-Amino-2Methyl-1-3 propanediol. Curvature of the first internode and epinasty of the leaves were very pronounced in the latter two treatments, while the former or plants treated with 2,4-D alone showed no visible effect, and their growth was not checked. One week after treatment plants treated with 2,4-D in addition of 2-Amino-2Methyl-1-propanol recovered slowly, and weakly. New growth took place. However, those treated with 2,4-D in addition of 2-Amino-2Methyl-1-3 propanediol had worse appearance; their stems became swollen and yellowish, and their terminal buds were completely arrested in development. They stayed in this condition until they were harvested; it was 19 days after treatment (Table 5).

Table 5

Fresh weight of bean shoots determined 19 days after treatment with 2,4-D salt and Aminohydroxy compounds

Treatment (2,4-D at 1,000 ppm and Aminohydroxy Comp. at 5g/l.)	Average weight of Shoot/plant (Gm)	Average Weight of shoots on per cent basis 2,4-D=100
Untreated	3.25	136
2,4-D salt	2.38	100
2,4-D salt + 2 Amino-2Methyl 1 propanol	.95	40
2,4-D salt + 2 Amino-2Methyl 1-3-propanediol	.23	10

The weight represents growth above the primary leaves.

Sixteen plants were used for each treatment.

Experiment II*

(1) Method and Material

This was a duplication of "Experiment I". The same methods and materials were applied to another lot of bean plants.

(2) Result

The same results were obtained in this duplicated experiment. In addition to the 2,4-D symptom described above it was noted that 2-Amino-2-Methyl-1-3-propanediol delayed the flowering much more than the other treatments, causing a yellowing of stem below the first node and a marked yellow spot developed on the leaf where the solution was located. Plants were harvested 19 days after treatment

*Conducted in January, 1949.

(Table 6).

Table 6

Fresh weight of bean shoots determined 19 days after treatment with 2,4-D salt and Aminohydroxy compounds*

Treatment (2,4-D at 1,000 ppm and Aminohydroxy comp. at 5g/l.)	Average weight of shoot/plant (gm.)**	Average weight of shoots on percent basis 2,4-D=100
Untreated	1.95	113
2,4-D salt	1.73	100
2,4-D salt + 2 Amino-2Methyl 1-propanol	.90	52
2,4-D salt + 2 Amino-2Methyl 1-3-propanediol	.24	13

*The weight represents growth above the primary leaves.

**Sixteen plants were used for each treatment.

Experiment III*

(1) Method and Material

In this experiment the application of Na₂2,4-D was made by placing a single drop of the solution on the base of one of the primary leaf blades of bean plants. The amount of 2,4-D used varied from 50 ppm to 100, 500 and 1,000 ppm. In addition to each of these concentrations 2 Amino-2 Methyl-1-3 propanediol ranged from 1 to 5, 10 and 20 g/l. were added, and thus formed 16 different combinations of solutions. Except these 16 combinations, some plants were simply treated with 1,000 ppm of 2,4-D and other with 10 g/l. of

*Conducted in February, 1949.

2 Amino-2Methyl-1-3 propanediol for comparison.

(2) Result

One day after treatment all plants treated with 2,4-D at 500 and 1,000 ppm in addition of 2-Amino-2Methyl-1-3-propanediol at any concentrations showed the increasing effect of 2,4-D with the epinasty of leaves and curvature of stems. While treatment which contained only 2,4-D at 1,000 ppm and those which contained only 2 Amino-2Methyl-1-3-propanediol at 10 g/l. showed no effect. With 2,4-D at 500 ppm in addition of 2 Amino-2Methyl-1-3-propanediol, the effect showed faster than those with 2,4-D at 1,000 ppm in addition of 2 Amino-2Methyl-1-3-propanediol. However, after one week the latter showed greater effect.

In the groups treated with 500 ppm of 2,4-D plus 2 Amino-2Methyl-1-3-propanediol it was found that 1 and 5g/l. of the additions produced faster effect than 10 and 20g/l. but after few days 10g/l. showed greatest effect.

In the groups treated with 1,000 ppm of 2,4-D plus 2 Amino-2Methyl-1-3-propanediol, 5g./l. of the addition showed the effect faster but 10 and 20 g./l. of the addition produced greater effects. One g./l. gave no visible effect. It was interesting that while 2,4-D at 1,000 ppm in addition of 2 Amino-2-Methyl-1-3-propanediol at 5, 10 and 20 g./l. showed higher increasing effect on 2,4-D than any other treatments in this whole experiment, when it was in addition of

one g./l., the effect reduced to the lowest class (Table 7, 7 and Fig. 6). In fact it showed less effectiveness than 2,4-D at 50 ppm plus one g./l. of 2-Amino-2-Methyl-1-3-propanediol. This result was at first suggested that there might be a ratio between these two compounds. But the later experiments did not support it, and the effective rate of 2-Amino-2-Methyl-1-3-propanediol fell into the range of 5 to 10 g./l. in increasing the action of 2,4-D.

Plants treated with 2,4-D at 100 ppm in addition of 2-Amino-2-Methyl-3-propanediol at 1 and 5g./l. showed visible effect. But it was surprising that 10 and 20g./l. did not show any effect. The same also happened in plants treated with 2,4-D at 50 ppm in addition of 10 and 20 g./l. while 1 and 5 grams additions caused curvature of the bean plants.

It was also interesting to find that when plants were affected by these combined solutions, many of them had the petiole of treated leaves elongated from $\frac{2}{5}$ to $\frac{1}{2}$ longer than the petiole of untreated leaf on the same plant. This might be induced by the combined effect of 2,4-D and 2 Amino-2-Methyl-1-3-propanediol.

Plants treated with 2 Amino-2-Methyl-1-3-propanediol alone at 10 g./l. had no other effect except it marked a yellow colored spot on the leaf blade where the solution was placed. The same was seen when this concentration was com-

bined with 2,4-D. However, 1g./l. of it did not cause this mark on the leaves.

Table 7

Growth responses of bean plants treated with Na-2,4-D
and 2 Amino-2-Methyl-1-3-propanediol

Treatment		Epinas- ty of leaf	Elonga- tion of petiole	Curva- ture of stem	Inhibition of new growth
2,4-D	2 Amino-2-Methyl-1-3-propanediol				
1,000 ppm	1 g./l.	+		+	+
	5 g./l.	+++	+++	+++	+++
	10 g./l.	+++	+++	+++	+++
	20 g./l.	+++	+++	+++	+++
500 ppm	1 g./l.	++		++	+
	5 g./l.	+++	+	++	++
	10 g./l.	+++	+++	+++	+++
	20 g./l.	++	++	++	++
100 ppm	1 g./l.	++	+	++	++
	5 g./l.	++	+	++	++
	10 g./l.				+
	20 g./l.				
50 ppm	1 g./l.	++	+	++	
	5 g./l.	++		++	+
	10 g./l.				
	20 g./l.				
1,000 ppm	None				
None	10 g./l.				
Untreated					

+ = Slightly affected
++ = Moderately affected
+++ = Greatly affected

Table 8

Fresh weight of bean shoots determined 20 days after treatment with 2,4-D salt and 2 Amino-2-Methyl-1-3-propanediol

Treatment		Average weight of shoot/plant (Gm) ^{1/2}	Average weight of shoots on per cent basis 2,4-D=100
2,4-D	2 Amino-2-Methyl-1-3-propanediol		
1,000 ppm	1 g./l.	.53	54
	5 g./l.	.35	36
	10 g./l.	.30	31
	20 g./l.	.28	29
500 ppm	1 g./l.	.39	40
	5 g./l.	.34	35
	10 g./l.	.30	31
	20 g./l.	.35	36
100 ppm	1 g./l.	.47	48
	5 g./l.	.41	42
	10 g./l.	.75	76
	20 g./l.	.75	76
50 ppm	1 g./l.	.48	50
	5 g./l.	.48	50
	10 g./l.	.93	95
	20 g./l.	.98	101
1,000 ppm	None	.97	100
None	10 g./l.	1.00	103
Untreated		1.30	134

^{1/2} The weight represents growth above the primary leaves.

^{1/2} Twenty plants were used for each treatment.

Experiment IV*

*Conducted in April, 1949.

(1) Method and Material

The method of applying Na2,4-D on bean plants was the same as in "Experiment III." However, the amounts of 2,4-D were cut down to 25, 50, 100 and 250 ppm. Also the percentages of 2-Amino-2-Methyl-1-3-propanediol which were added to each of the different concentrations of 2,4-D were reduced to 1 and 2 g./l. The 2,4-D solutions which had no additions of 2-Amino-2-Methyl-1-3-propanediol were used as checks.

(2) Result

Four days after treatment, it was noted that the plants showed increasing effects as the concentration of 2,4-D was increased in different treatments. However, the additions of 2 Amino-2-Methyl-1-3-propanediol, whether in 1 or in 2g./l., did not show greater effect than 2,4-D used alone. In the group treated with 25 ppm of 2,4-D, the addition of 2g./l. of 2-Amino-2-Methyl-1-3-propanediol rather gave decreasing effect. It was not known what was the reason, and those previous experiments were reconducted. But as the weather got warmer and warmer the results became erratic, and previous records could not be duplicated again in that summer time. As a matter of fact when the days were hot, the addition of 2-Amino-2-Methyl-1-3-propanediol prevented the translocation of 2,4-D beyond the petiole by burning the tissue of treated leaves.

Experiment V*

(1) Method and Material

Ten-day-old Red Kidney bean plants were used as the experimental material, and applications were made by placing a single drop of the solution on the base of one of the primary leaf blades. The amount of Na2,4-D used in all the solutions remained constant at 1,000 ppm; the amount of 2-Amino-2-Methyl-1-3-propanediol added to the 2,4-D however, ranged from 1, 5, 10, and 20 g./l.

(2) Result

Four days after treatment, plants that received only 2,4-D at 1,000 ppm and 2,4-D plus one g./l. of 2 Amino-2-Methyl-1-3-propanediol did not show visible effect. Those received 2,4-D plus 5 and 10 g./l. of 2 Amino-2-Methyl-1-3-propanediol showed curvature of the stem and epinasty of the leaves. However, the addition of 20 g./l. did not show much curvature. Twenty days after treatment, no new growth had been found in those plants treated with 2,4-D in addition of 5 and 10 g./l. of 2-Amino-2-Mythyl-1-3-propanediol, while those with the additions of 1 and 20 g./l. resumed growth weakly (Fig. 6). The plants were harvested and the average fresh weight of growth or swollen buds above the primary leaves was recorded (Table 9).

*Conducted in November, 1949.

Plate IV

The effect of different concentrations of 2-Amino-2-methyl-1-3 propanediol in increasing the action of 2,4-D salt.

Fig. 6 Bean plants from right to left were treated with 2,4-D (1), 2,4-D plus 2-Amino-2-methyl-1-3-propanediol at 1 g./l (2), 5 g./l (3), 10 g./l (4), and 20 g.l (5).

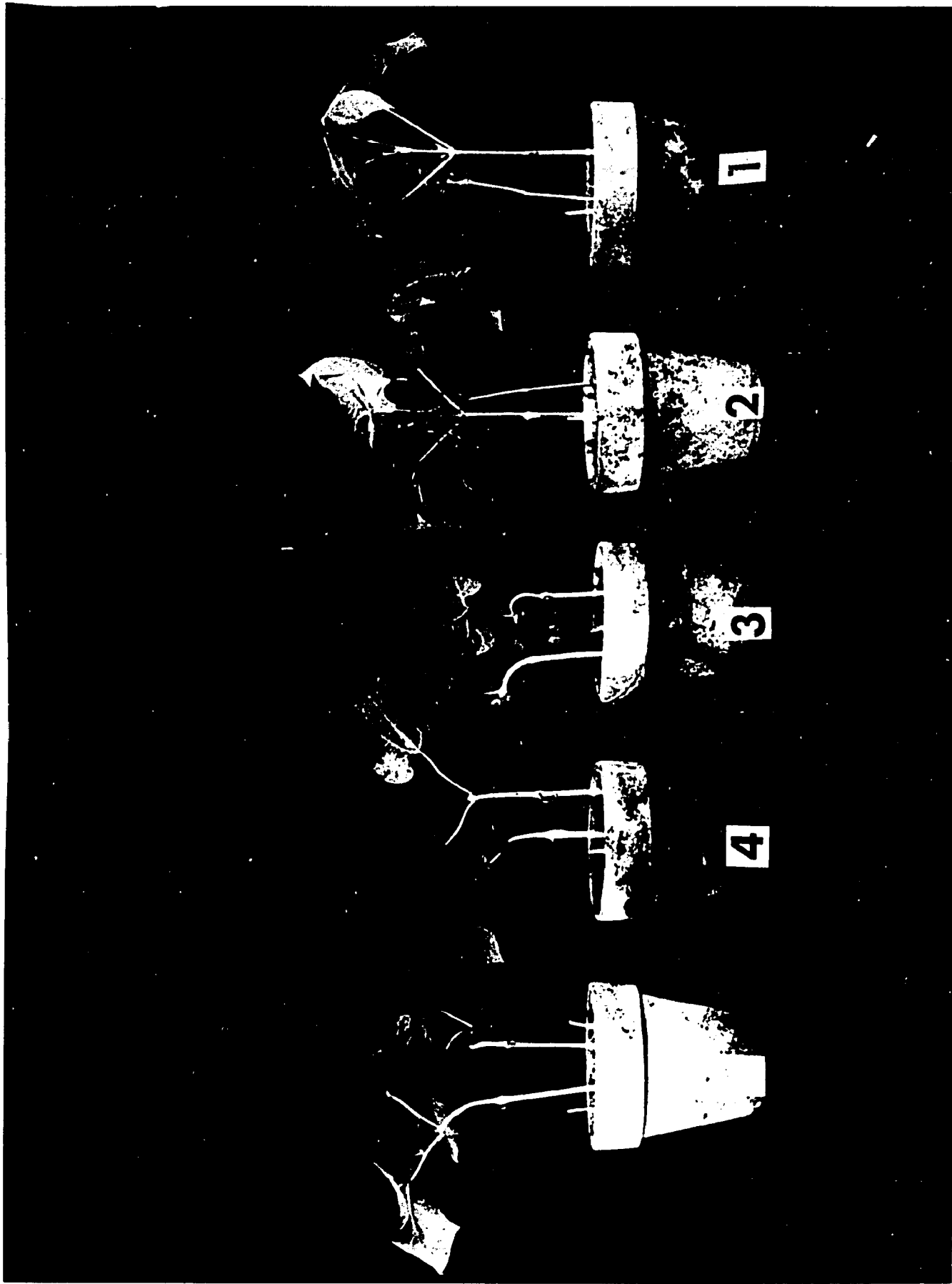


Fig. 6

Table 9 .

Fresh weight of bean plants determined 20 days after treatment with treatment with 2,4-D salt and 2-Amino-2-Methyl-1-3-propanediol^{*}

Treatment (2,4-D at 1,000 ppm)	Average weight of shoot/plant (gm) ⁺⁺	Average weight of shoots on per cent basis 2,4-D=100
Untreated	6.72	445
2,4-D salt	1.51	100
2,4-D salt + 1g./l. AMPI ⁺⁺⁺	1.07	70
2,4-D salt + 5g./l. "	.53	35
2,4-D salt +10g./l. "	.54	35
2,4-D salt +20g./l. "	1.05	70

^{*}The weight represents growth above the primary leaves.

⁺⁺Sixteen plants were used for each treatment.

⁺⁺⁺Abbreviation of 2-Amino-2-Methyl-1-3-propanediol.

Experiment VI^{*}

(1) Method and Material

Application of 2,4-D was made by dipping one of the primary leaves of each bean plant into the solutions, containing 1,000 ppm of sodium salts of 2,4-D, and 2,4-D plus 5 grams per liter of 2-Amino-2-Methyl-1-3-propanediol.

(2) Result

Twenty two days after treatment, plants receiving 2,4-D at 1,000 ppm had completely recovered, and new growth developed rapidly. However, those treated with the same amount of 2,4-D in addition to 5 grams per liter of 2-Amino-

^{*}Conducted in January, 1950.

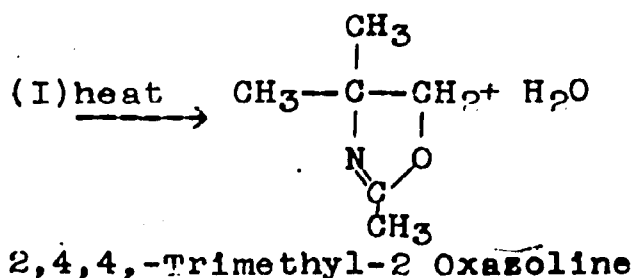
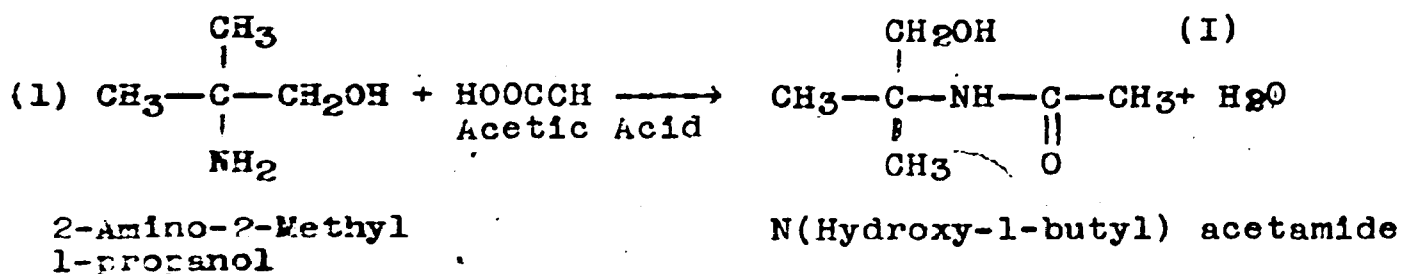
2-Methyl-1-3-propanediol had half of the treated plants dead; the survivor showed no sign of recovery, and sooner or later would have died.

E. Discussion of 2-Amino-2-Methyl-1-3-propanediol

From these experiments carried on over 2 years, it is definitely concluded that 2-Amino-2-Methyl-1-3-propanediol has the property to increase the action of 2,4-D. The effective rate of it falls into the range of 5 to 10 grams per liter. But unfortunately it works only in the winter time under greenhouse conditions.

Since it reacts readily with a great many other chemicals and even with acidic gases, it is not known how it increases the action of 2,4-D. But according to its physical properties, the emulsifying, wetting and surface-acting effects may have some quality which facilitates the penetration of 2,4-D during the winter time.

However, it is difficult to explain why it does not work well in summer time. The only thing which gives some speculation is from the chemical reaction of 2-Amino-2-Methyl-1-propanol with acetic acid and heat. The equations are shown as follows: (5)



To test whether heat and acetic acid have some effect to 2-Amino-2-Methyl-1-3-propanediol as they did to 2-Amino-2-Methyl-1-propanol, acetic acid was added with sufficient amount of 2-Amino-2-Methyl-1-3-propanediol. Half amount of this solution was reserved for comparison, and other half was heated. Then these two solutions as well as 2-Amino-2-Methyl-1-3-propanediol were, at 500 per liter, combined with Na2,4-D at 1,000 ppm. Treatments were made on Red Kidney bean plants with their first trifoliate leaf expanding. A single drop of each of these solutions was placed on the base of one of the primary leaf blades. The 2,4-D at 1,000 ppm was used alone as control plants. One day after treatment all the plants which received 2,4-D at 1,000 ppm with the addition of 2-Amino-2-Methyl-1-3-propanediol alone showed severe curvature while other combinations showed no effect. By this result it was

speculated whether or not the same reaction could happen in hot season and produce some products which would not increase the action of 2,4-D.

2. Materials Which Moderately Increase the Action of 2,4-D

A. Sucrose with Potassium phosphate

Experiment I*

(1) Method and Material

In this experiment Red Kidney bean plants which had two or three trifoliate leaves expanded were used. Application was made by spraying the solutions on the whole plant of each group until they were thoroughly wetted both upper and lower sides of the leaves. A solution containing Na2,4-D at 500 ppm and a solution containing the same amount of 2,4-D with the addition of sucrose at 3 grams per liter and potassium phosphate at 0.5 gram per liter were used.

(2) Result

One day after treatment marked differences were noted between the 2,4-D and 2,4-D plus sucrose and potassium phosphate lots. Curvature of the petiole and epinasty of the leaves were much more pronounced in the plants which had been sprayed with 2,4-D plus sucrose and potassium phosphate.

One week after treatment plants that had been treated

* Conducted in January, 1950.

with 2,4-D at 500 ppm were growing unchecked, although they showed more or less 2,4-D symptoms and some degree of curvature on the new shoots. Those plants that received 2,4-D in addition of sucrose and potassium phosphate had no new growth. Their stems were swollen, leaves chlorotic and with greater degree of curvature on the smaller shoots.

Around three weeks later plants treated with only 2,4-D were flowering. However, those treated with 2,4-D in addition of sucrose and potassium phosphate still had no new growth. Picture was taken to show their response at this period. (Fig. 7)

Experiment II*

(1) Method and Material

Around ten-day-old Red Kidney bean plants were used as the experimental material, and applications were made by dipping one of the primary leaves of each plant into a solution containing the same amount of 2,4-D with the addition of sucrose at 3 grams per liter and potassium phosphate at 1 gram per liter.

(2) Result

One day after treatment, all the plants showed curvature of the first internode. However, 3 weeks later plants that received only 2,4-D, although the stems were swollen and the leaves and petiole somewhat twisted, were definitely

*Conducted in January, 1950.

recovered. In the group treated with the same amount of 24-D in addition of sucrose and potassium phosphate showed no new growth at all. The plants were harvested 23 days after treatment, and the average fresh weight above the primary leaves was recorded in Table 10.

Table 10/

Fresh weight of bean shoots determined 23 days after treatment with 2,4-D and with 2,4-D plus sucrose and potassium phosphate//

Treatment (2,4-D at 1,000 ppm)	Average weight of shoot/plant (gm)///	Average weight of shoots on per cent basis 2,4-D=100
Untreated	2.25	320
2,4-D salt	.70	100
2,4-D + 3g./l. sucrose and 1g./l. potassium phosphate	.17	24

/ Data from Table 11.

// The weight represents growth above the primary leaves.

/// Sixteen plants were used for each treatment.

Plate V

The effect of sucrose and potassium phosphate in increasing the herbicidal action of 2,4-D salt.

Fig. 7 Bean plants on the left were untreated, in the middle treated with 2,4-D and on the right treated with 2,4-D plus sucrose and potassium phosphate.



Fig. 7

B. Sucrose with Potassium Sulfate, Magnesium Sulfate,
Ammonium Sulfate and Other Compounds

Experiment I*

(1) Method and Material

Young Red Kidney bean plants were used as the experimental material. Application of Na₂2,4-D was made by dipping one of the primary leaves of each treated plant into the prepared solutions. The amount of 2,4-D used in all the solutions remained constant at 1,000 ppm and the amount of sucrose, at 3 grams per liter. The addition of the other compounds was at 1 gram per liter.

(2) Result

No marked differences between these treatments were noted within one week. After 10 days, plants which received only 2,4-D were beginning to resume new growth. The same behavior was observed in those treated with the addition of 1-2-1 complete fertilizer, manganate sulfate, ferrous sulfate, calcium phosphate and borax; the addition of borax even showed more new growth. With the addition of sucrose alone much less new growth was found. The addition of potassium sulfate, potassium phosphate, magnesium sulfate and ammonium sulfate showed no new growth at all. The plants were harvested and recorded in Table 11.

*Conducted in January, 1950.

Table 11

Fresh weight of bean shoots determined 20 days after treatment with 2,4-D, sucrose and other compounds.

Treatment (2,4-D at 1,000 ppm sucrose at 3g./l.)	Average weight of shoot/plant (gm) //	Average weight of shoots on per cent basis 2,4-D =100
Untreated	2.25	320
2,4-D	.70	100
2,4-D plus Sucrose	.22	31
2,4-D plus Sucrose plus (NH ₄) ₂ SO ₄	.13	20
2,4-D plus Sucrose plus K ₂ (PO ₄) ₂	.17	24
2,4-D plus Sucrose plus K ₂ SO ₄	.13	20
2,4-D plus Sucrose plus MgSO ₄	.18	26
2,4-D plus Sucrose plus Ca ₃ (PO ₄) ₂	.22	31
2,4-D plus Sucrose plus 1-2-1 fertilizer	.50	71
2,4-D plus Sucrose plus FeSO ₄	.71	101
2,4-D plus Sucrose plus MnSO ₄	.75	107
2,4-D plus Sucrose plus Borax	1.25	178

// The weight represents growth above the primary leaves.

// Sixteen plants were used for each treatment.

C. Discussion of Sucrose Combinations

Lucas and Hamner (38) found that onion extract increased the herbicidal action of 2,4-D. Spear and Thimann

(58) analyzed the onion extract and found that the effective fractions which increased the action of 2,4-D were reducing sugar and potassium phosphate. They mixed the sucrose and potassium phosphate in the same proportion was present in onion extract and obtained the same result of increasing effect of 2,4-D. Present experiments agree with all the results of above investigators.

However, when sucrose combinations were compared side by side with Geon 31X and 2-Amino-2-Methyl-1,3-propanediol under green house condition, the former showed less effect in increasing the action of 2,4-D.

Spear and Thimann (58) also found that potassium chloride when combined with sucrose would give the same result on 2,4-D as that of potassium phosphate. Present experiments, otherwise, found that potassium sulfate, ammonium sulfate and magnesium sulfate worked the same as that of potassium phosphate when these materials were mixed with sucrose. Incidentally, elements of these compounds are required by plants. But this does not imply that all elements required by the plant will increase the action of 2,4-D in sucrose combination, since some other elements used in this experiment did not show any effect at all.

3. Materials Which Decrease the Action of 2,4-D

A. Allyl alcohol

Experiment I*

(1) Method and Material

Application of Na2,4-D was made by dipping one of the primary leaves of Red Kidney bean plants into solutions containing the sodium salt of 2,4-D at 10 ppm and the same amount of 2,4-D plus 5 CC per liter of allyl alcohol.

(2) Result

Plants treated with only 2,4-D showed curvature of stem although they kept on growing rather weakly. Those treated with 2,4-D in addition of allyl alcohol showed no visible effect. These plants were harvested two weeks after treatment, and the average fresh weight above the primary leaves was recorded in Table 12.

Table 12

Fresh weight of bean shoots determined 14 days after treatment with 2,4-D and allyl alcohol

Treatment (2,4-D at 10 ppm)	Average weight of shoot/plant (gm) //	Average weight of shoots on per cent basis 2,4-D=100
Untreated	5.38	180
2,4-D	2.98	100
2,4-D + Allyl alcohol (5CC/l)	4.20	141

//The weight represents all new growth above the primary leaves
//Twenty plants were used for each treatment.

*Experiment I conducted in July, 1947.

Experiment II*

(1) Method and Material

The same method and material were used as in previous experiment, with the exception that the concentration of 2,4-D was raised to 100 ppm and the additions of allyl alcohol ranged from 5 CC to 10 CC per liter.

(2) Result

One day after treatment plants which received only 2,4-D showed curvature of the stem and epinasty of the leaves. All the other treated plants showed no 2,4-D symptoms. A few days later those treated with 2,4-D plus 10 CC per liter of allyl alcohol produced more or less chlorotic spots on the surface of the treated leaves. The average fresh weight of growth is shown in Table.13.

Table 13

Fresh weight of bean shoots determined 20 days after treatment with 2,4-D and allyl alcohol

Treatment (2,4-D at 100 ppm)	Average weight of shoot/plant (gm) //	Average weight of shoots on per cent basis 2,4-D=100
Untreated	4.93	172
2,4-D	2.84	100
2,4-D + 5CC/l of Allyl alcohol	3.50	122
2,4-D + 10CC of Allyl alcohol	3.90	140

//The weight represents all growth above the primary leaves.
//Eighteen plants were used for each treatment.

//Thenducted in August, 1947.

//E1

Experiment III*

(1) Method and Material

Applications were made by dipping one of the primary leaves of bean plants into the solutions prepared. The amount of Na2,4-D varied from 1,000 to 2,000 ppm and the amount of allyl alcohol added to the 2,4-D ranged from 0 to 1, 2 and 5 CC per liter.

(2) Result

One day after treatment plants treated with only 2,4-D at 1,000 ppm and the same amount of 2,4-D in addition of 1CC per liter of allyl alcohol showed curvature badly. Those with the addition of 2 CC and 5 CC per liter to 1,000 ppm of 2,4-D showed much less curvature. In the group which received 2,000 ppm of 2,4-D all were dying.

One week after treatment plants treated with 2,4-D alone had no new growth, while those treated with 2,4-D in addition of 2 CC and 5 CC per liter of allyl alcohol were beginning to resume new growth and were definitely recovering from the treatment.

In this experiment allyl alcohol alone at 10 CC per liter was also used to treat the bean plants. It caused slight drooping of the treated leaves for about a day. But the leaves recovered and plants grew normally. Some chlorotic spots occasionally showed on treated leaves.

(3) Discussion

*Conducted in April, 1947.

Allyl alcohol was one of many compounds which reduced the action of 2,4-D in plants. It is possible that the reduced effect was caused by either injury to tissues by the alcohol and hence reduced translocation of 2,4-D, or perhaps by changing the electrical charge in 2,4-D and thus preventing its entry into the plant.

B. Potassium Ferrocyanide

Experiment I*

(1) Method and Material

Applications were made by dipping one of the primary leaf blades of young bean plants into the solutions. The amount of Na2,4-D used in all the solutions remained constant at 100 ppm; the amount of potassium ferrocyanide added to the 2,4-D, however, ranged from 0 to 0.1, 0.5, 1 and 5 grams per liter. Potassium ferrocyanide at 1 gram per liter was also used to treat the plants.

(2) Result

One-day-after treatment the plants treated with 2,4-D plus potassium ferrocyanide at 1 and 5 grams per liter showed no curvature of the stem and epinasty of the leaves, while all other treated plants showed 2,4-D symptoms. Those which received only potassium ferrocyanide without 2,4-D had more or less chlorotic spots on the margin of leaf blade.

*Conducted in July, 1947.

Experiment II*

(1) Method and Material

Application of 2,4-D was made by dipping one of the primary leaves of young bean plants into solutions containing the sodium salt of 2,4-D in a concentration of 1,000 ppm. In addition to the 2,4-D solution, the other treatment contained also potassium ferrocyanide at 5 grams per liter.

(2) Result

The plants which were treated with 2,4-D alone died a few days after treatment. Those receiving 2,4-D in addition to potassium ferrocyanide, at least in part, overcame the toxic action of 2,4-D.

C. Sodium Hypochlorite

Experiment I**

(1) Method and Material

Red Kidney bean plants with their first trifoliate leaf not yet expanded were used as the experimental material. Application was made by dipping one of their primary leaf blades into the solution. The amount of Na₂,4-D used in all the solutions remained constant at 2,000 ppm; the amount of sodium hypochlorite added to the 2,4-D, however, ranged from 0 to 2 CC per liter. The color of the solution of sodium

*Conducted in July, 1947.

**Conducted in January, 1950.

salt of 2,4-D is pink-red, but when sodium hypochlorite is added, the solution was clear.

(2) Result

One day after treatment plants treated with only 2,4-D showed curvature badly. Those treated with 2,4-D plus sodium hypochlorite showed slight curvature of the stem and recovered a few days later. Twenty days after treatment, plants receiving 2,4-D in addition of sodium hypochlorite were flowering. Those which received only 2,4-D had no new growth at all. This experiment showed that sodium hypochlorite, an oxidizing agent, partially overcame the action of 2,4-D. The plants were harvested 20 days after treatment, and the average fresh weight above the primary leaves was recorded in Table 14.

Table 14

Fresh weight of bean shoots determined 20 days after treatment with 2,4-D and sodium hypochlorite

Treatment (2,4-D at 2,000 ppm)	Average weight of shoot/plant(gm)	Average weight of shoots on per cent basis 2,4-D=100
Untreated	2.25	900
2,4-D	0.25	100
2,4-D + 2 g/l NaClO	1.11	444

The weight represents new growth above the primary leaves.
 //Eighteen plants were used for each treatment.

Experiment II*

(1) Method and Material

Treatments were made by dipping one of the primary leaves of bean plants into solutions containing $\text{Na}_2,4\text{-D}$ at 1,000 ppm. In addition to 2,4-D, some of the solutions contained sodium hypochlorite at 0.2% per liter and chloride of lime at 0.5 gram per liter. Both of these chemicals changed the color of $\text{Na}_2,4\text{-D}$ solution from red pink to clear.

(2) Result

One day after treatment, plants treated with 2,4-D alone showed severe curvature. Both of those treated with 2,4-D in addition to sodium hypochlorite and chloride of lime showed slight curvature. Twenty days after treatment, plants that received 2,4-D alone had no new growth. Those treated with 2,4-D with chloride of lime partially recovered and in those with the addition of sodium hypochlorite to 2,4-D had almost completely recovered (Table 15).

*Conducted in March, 1950.

Table 15

Fresh weight of bean shoots determined 20 days after treatment with 2,4-D and 2,4-D with sodium hypochlorite and chloride of lime

Treatment (2,4-D at 1,000 ppm)	Average weight of shoot/plant(gm)	Average weight of shoots on per cent basis (2,4-D=100)
Untreated	2.25	1730
2,4-D salt	0.13	100
2,4-D salt + 0.2% NaClO	0.63	484
2,4-D salt + 0.5% CaOCl ₂	0.30	230

The weight represents growth above the primary leaves.

Sixteen plants were used for each treatment.

4. Materials Which Have No Definite Effect on 2,4-D

For all these experiments young Red Kidney bean plants with the first trifoliate leaf not yet expanded were used. Materials were added to the solution containing the sodium salt of 2,4-D. The indefinite results are summarized in Table 16.

Table 16

Summary of indefinite results caused by the addition
of unrelated materials to the sodium salt of 2,4-D

Name of Material	Concentration used	Remarks
Cyanamide	56gm./l.	Burned the leaf & reduced 2,4-D trans- location.
Lime sulfate	10gm./l.	No increased action over 2,4-D alone
Urea	500 ppm	"
Chlorohydrate	5CC/l.	"
P.E.P.S.	5 gm./l.	"
Carboxy methocel	5CC/l.	"
Calcium chloride	3,000 ppm	"
Sodium silicate	1 per cent	"
Gum Arabic	1 per cent	"
Canada Balsam	1 per cent	"
Phosphoric acid	5 CC/l.	"
Elvacet	5 per cent	Slightly in- creased the action of 2,4-D
Elvanol	5 per cent	No increased action over 2,4-D
Yeast		Very slight increasing of 2,4-D

Continued 16

Yeast	(inject)	Slightly in- creased the action of 2,4-D
Lanthanum nitrite	0.5 gm./l.	No increase over 2,4-D alone
Thorium nitrite	0.5 gm./l.	"
Cupra Ammonium hydroxide	5CC/l.	"
Ethylene glycol	5 gm/l.	"
Chloral hydrate	5gm/l.	"
Triphenyl tetrazolim chloride	1gm/l.	"
Acetic acid	5CC/l.	Burned the leaf & reduced the action of 2,4-D
Amyl acetate	0.2CC/l.	No increased action over 2,4-D alone
Nucleic acid	1CC/l.	"
Thymol	5gm/l.	"
Resorein	5gm/l.	"
Palmatic acid	5gm/l.	"
Levo-2,3-Butylene Glycol	5gm/l.	"
RX-89-D Melemine Resin	5gm/l.	"
Tri-Ethol amino	1gm/l.	"
Acid Amino	1gm/l.	"

Continued 16

Amino Benzoic	1gm/l.	No increased action over 2,4-D alone
Calcium pertohinate E,56	5gm/l.	"
Calcium phosphate	1gm/l.	"
Ferrous sulfate	1gm/l.	"
Borax	5gm/l.	Slightly decreased action of 2,4-D
Potassium permanganate	2gm/l.	No increased action over 2,4-D alone
H ₂ O ₂	5gm/l.	Changes red-pink color of sodium salt of 2,4-D to colorless, but no effect on 2,4-D
N(Hydroxy-1-butyl acetamide	5CC/l.	No increased action over 2,4-D alone
2,4,4, Trimethyl-2-Oxazoline	5CC/l.	"

5. Natural Factors

A. Light

Experiment I*

*Conducted in October, 1949.

(1) Method and Material

Fully grown Red Kidney bean plants with 3 expanded trifoliate leaves were used as the experimental material. Application was made by spraying the plants with $\text{Na}_2,4\text{-D}$ at 2,000 ppm solution. Plants were then divided into 3 groups and kept under 3 different light conditions; one group was in the greenhouse, another group in darkness and a third group in the continuous illumination of a 2,000 watt light bulb.

(2) Result

One day after treatment, all the treated plants showed curvature of young shoots. No differences in response to 2,4-D was observed whether plants were illuminated or in darkness. After one week treated plants set in different light conditions showed the same response to 2,4-D. There was no new growth in any of them and no sign of recovery from the treatment. The only difference which had been noted was that the plants in darkness completely lost their chlorophyll at this period, those in artificial continuous illumination lost most of their chlorophyll, and those in greenhouse kept their old leaves green. However, this is more concerned with light influence on chlorophyll of plant rather than being connected with 2,4-D effect.

Experiment II*

(1) Method and Material

Young Red Kidney bean plants with their trifoliate leaf not yet expanded were used as experimental material, and application of $\text{Na}_2,4\text{-D}$ at 2,000 ppm was made by placing a single drop of the solution on the base of one of the primary leaf blades. Then these plants were separately put into darkness, continuous illumination and diffused light near the window.

(2) Result

One day after treatment, all the treated plants showed slight curvature of the stems, and no significant difference had been found between the different light conditions and the action of 2,4-D.

This experiment was repeated for several times, but each time showed that the presence or absence of light had very little effect on either increasing or decreasing of the action of 2,4-D.

B. Carbon Dioxide

Experiment II**

(1) Method and Material

Young Red Kidney bean plants were used as the experimental material and application of $\text{Na}_2,4\text{-D}$ at 500 ppm was

*Conducted in December, 1949.

**Conducted in August, 1949.

made by placing two drops of the solution on the base of each primary leaf blade. Treated plants were, then, divided into two groups and set into two different desiccators. One of the desiccators contained 10% of carbon dioxide gas produced by a piece of dry ice. The other desiccator had a beaker of potassium hydroxide solution which was used to remove the carbon dioxide from the air in this desiccator.

(2) Result

There was no visible effect of high and low carbon dioxide content of air upon the action of 2,4-D. In both desiccators, plants were susceptible to decay because of the high humidity. Plants in the desiccator of high carbon dioxide content seemed to suffer less decay. However, the curvature of the stem by the action of 2,4-D was the same whether the plants were supplied with carbon dioxide or not.

Experiment II*

(1) Method and Material

The same application of Na₂2,4-D was made on bean plants as described in previous experiments and the same high and low carbon dioxide desiccators were set up for treated plants. However, in both desiccators, calcium chloride was spread on the bottom of these containers to dry the air.

(2) Result

*Conducted in August, 1949.

Plants in desiccator of high carbon dioxide showed less curvature of the stem and less translocation of 2,4-D. They showed petioles swollen in the middle portion, while those in low carbon dioxide desiccator had curved and swollen stems, indicating that 2,4-D had been translocated much farther as CO₂ was present in small amount.

Experiment III*

(1) Method and Material

This is the duplication of experiment II. All the set-up and materials were the same as described in that experiment.

(2) Result

Plants in high carbon dioxide desiccator were dead one week of treatment. Those in low carbon dioxide desiccator were alive. This result was just contrary to that of previous experiment. No further experiment was, then, conducted to test the variable results.

C. Humidity

Experiment I**

(1) Method and Material

Two glass chambers about one meter long, one half meter wide and one meter high were set on the sand in the greenhouse. One of them contained calcium chloride on the

*Conducted in September, 1949.

**Conducted in August, 1949.

bottom to absorb the air moisture. Another one was mulched with paper towels on the bottom and frequently sprayed with water to keep the humidity near the saturation point. Young Red Kidney bean plants were treated with sodium salt of 2,4-D at 1,000 ppm by placing a single drop of the solution on the base of one of the primary leaf blades. Then they were divided into two groups. One group was put into the high humidity chamber and the other, into low humidity chamber. Untreated plants were also put in each chamber as check.

(2) Result

One day after treatment, all treated plants showed curvature of the first internode and epinasty of the leaves. There was no difference between the high or low humidity chambers.

One week after treatment, treated leaves in low humidity chamber dried off; whereas in high humidity chamber they kept turgid although the petioles twisted. The same condition was seen on the cotyledons. In both chambers, 2,4-D treated plants did not have their cotyledons abscised automatically as the untreated plants did, and thus were first subjected to decay on this part when the plants were in high humidity chamber.

Adventitious aerial-roots were formed along the first internode of the 2,4-D treated plants in the high humidity

chamber. However, in the low humidity, there was no such root initiated, and plants had not decayed.

Experiment II*

(1) Method and Material

Application of methyl ester of 2,4-D at 1,000 ppm by weight was made by placing a single drop of the solution on the base of one of the primary leaf blades of young Red Kidney bean plants. Then, they were divided into two groups and separately put into different desiccators. One of the desiccators contained calcium chloride on the bottom to remove the air moisture. Another one had moistened paper towel on the bottom to keep the high humidity condition within this desiccator. Artificial light was applied over these desiccators.

(2) Result

In both desiccators treated plants showed almost the same degree of curvature of their stems. However, plants in the high humidity desiccator had greater swelling of their petioles and stems than those in low humidity desiccator. Since those plants grew more rapidly in that high humidity environment, it is difficult to draw a conclusion whether this difference was due to increasing effect of 2,4-D or due to succulent growth.

*Conducted in December, 1949.

D. Infra-red and Plant Temperature

Experiment I*

(1) Method and Material

Young bean plants were used as the experimental material and applications of $\text{Na}_2,4\text{-D}$ at 1,000 ppm were made both by placing a single drop of the solution on the base of one of the primary leaf blades and by dipping one of the primary leaf blades in the solution. In each day four plants were treated for this experiment; two of them were set in the Infra-red rays for 24 hours and the other two in the same greenhouse as check plants. The distance from the Infra-red lamp to the leaf surface was 12 inches and between them a specific colored filter had been set up to absorb all the visible light. The leaf temperature of the plants under the filter measured constantly from 90 to 93° F depending upon the angle of the leaves, while the check plants changed their temperature from 65° F at night to 80° F on sunny days coincided with the change of air temperature in that greenhouse. For these air and plant temperature measurements Potentiometer and Thermocouple were used. To keep the water supply of the plant in infra-red constant, a water saucer was used under the pot which contained the bean plants.

(2) Result

Plants in infra-red or in other words, plants which

*Conducted in March, 1950.

had higher leaf temperature resulting from infra-red showed no increasing effect on 2,4-D within this range of temperature than the check plants which had normal greenhouse temperature. Sometimes plants in infra-red showed light-green color of their leaves after 24 hours in the high heat ray and shaded condition.

Experiment II*

(1) Method and Material

Material and application of Na₂,4-D were the same as in the previous experiment. However, there was no filter being used to absorb the visible ray, and the light intensity was, therefore, much higher than the check plants in the same greenhouse. The distance from the infra-red lamp to the leaf surface was 15 inches, and the leaf temperature of the plants in infra-red was 92 to 94° F measured by the Potentiometer and Thermocouple. Water saucers were set under the pots of infra-red treated plants to keep the water supply constant. Two lamps were used, and directly under each lamp two Na₂,4-D treated plants were placed.

(2) Result

Plants in infra-red when treated with one drop of 2,4-D solution by placing it on the leaf base even showed less effect than control plants. When they were treated by dipping one primary leaf into the 2,4-D solution, there was no such differences between the plants in infra-red and

*Conducted in March, 1950.

control plants in greenhouse. Sometimes plants in infra-red showed greater swelling of stems. But this effect was too slight to be significant.

E. Undetermined Seasonal Factor

Under greenhouse conditions it was observed through a period of 3 years that 2,4-D showed much higher effect on Red Kidney bean plants in summer time and much less in winter time. For example, one single drop of water solution of sodium salt of 2,4-D at 1,000 ppm when placed on the base of primary leaf blade during August, will cause great curvature of stem; whereas in January the same amount of 2,4-D and the same method of application showed no visible effect at all (Ref. Fig. 5 and Fig. 6). It was observed that Na2,4-D at 500 ppm could cause killing of young bean plants when one of the primary leaves was dipped into the solution during early October (Ref. Fig.1); whereas by the same method of application of 2,4-D at 1,000 ppm (Ref. Table 10) or even as high as 2,000 ppm (Ref. Table 14) during January plants were recovered from treatment after two or three weeks. No attempt was made to find out this seasonal change statistically. But as a matter of fact under Michigan condition 2,4-D alone causes less damage on bean plant from the end of December to the beginning of March in greenhouse experiments.

It is generally believed that photoperiod, light intensity and heat of the sun are the main factors determining this seasonal variation. But according to the previous experiments even if the light period, light intensity and heat ray were increased by the use of infra-red lamp, no greater effect of 2,4-D could be obtained. What factor or factors playing this role in nature are still waiting for determination.

6. Inhibition of Photosynthesis by 2,4-D

The rate of photosynthesis may be measured either by determining the concentration of materials entering into the reaction (carbon dioxide) or by determining the concentration of the end products (Oxygen, sugars, dry weight, starch, etc.). One of the oldest and simplest method is based on the fact that bubbles of oxygen are liberated from the stems and leaves of submerged aquatic plants during photosynthesis. By measuring the amount of bubbles per unit time under one set of conditions and then another, one may obtain information as to the factors effecting the rate of photosynthesis. The method is not highly accurate but may be used if sufficiently great changes in the environmental factors are involved. The following experiment was based on this principle and the action of 2,4-D upon photosynthesis was tested.

Experiment I*

(1) Method and Material

Vigorous twigs of *Elodes* had been cut into one inch cuttings with their terminal and lateral buds trimmed off. These cuttings were immersed in beakers containing the solution of the sodium salt of 2,4-D in varying concentrations of 50, 100, 500, 1,000 and 2,000 ppm. An inverted funnel was put in each beaker to hold the *Elodes* cutting under it. The stem of each funnel was, then, covered by an inverted vial filled with the solution; so by replacing the solution the liberated bubbles of oxygen would be collected in this vial. The cuttings began to liberate bubbles as soon as a 200 watt light bulb was turned on. After 20 hours the vials containing oxygen bubbles were separately submerged in a water tank. The gas passed through the inverted funnel and then into scaled pipettes to be measured. The temperature of the solutions was also measured. It varied from 29 to 30° C depending upon the concentration of the solution, although these beakers were kept in same distance from the light bulb.

(2) Result

By five repeated experiments it was found that 2,4-D did inhibit photosynthesis gradually as the concentration increased and the time elapsed. The data are presented in Table 17.

*Conducted in November, 1949.

Table 17

Average amount of Oxygen liberated by one gram Elodea cuttings after 20 hours in the solution of 2,4-D salt.

Treatment	Average amount of oxygen liberated (ML)
Water	2.14
2,4-D at 50 ppm	1.75
2,4-D at 100 ppm	1.79
2,4-D at 500 ppm	1.64
2,4-D at 1000 ppm	1.47
2,4-D at 2000 ppm	1.01

However, the rate of photosynthesis was not significantly reduced until the concentration of 2,4-D was higher than 500 ppm. The amount of oxygen liberated by the cuttings submerged in 50 or 100 ppm of 2,4-D varied in each repeated experiment. No difference was observed between the treatments within a period of a few hours. Nor was the photosynthesis completely inhibited after 20 hours in 2,4-D solutions. The change took place gradually rather than abruptly.

The number of the bubbles liberated in certain unit of time by the cuttings can not be depended as the measurement of photosynthesis in this experiment, since in high concentration treatments, such as in 1,000 and 2,000 ppm of 2,4-D, numerous bubbles were liberated in each minute, but the size of the bubbles was very small.

7. Plant Factors

A. Varietal Difference

Experiment I*

(1) Method and Material

One-week-old sweet pea plants were used as the experimental material. Fifteen varieties, including Sweet Alaska, Confidence, Thomas Laxton, Perfection, Mardelak, Pride, Bonneville, Cody, Multonomak, Victory Freezer, Meteor, Early Perfection, Perfected Freezer, W.R. Surprise and Oneida, were planted in 4" pots in the greenhouse, and thinned to 10 plants in each pot. Seventy plants of each variety were treated. Application of Na₂,4-D was made by spraying the plants until thoroughly wet with a concentration of 500 ppm. Since the water solution did not stick well on pea plants, a little common soap powder was added as sticker.

(2) Result

Three days after treatment, all the plants showed curvature of their stems. Two week after treatment all varieties except Sweet Alaska and Cody were dying. Finally only a few plants of Cody and more than 30 plants of Sweet Alaska survived. Sweet Alaska also showed less swelling of stem than Cody.

Experiment II**

(1) Method and Material

Four varieties, including Sweet Alaska, Thomas Laxton,

*Conducted in December, 1949.

**Conducted in December, 1949.

Perfection and Cody, were used to repeat the previous experiment. The same concentration of $\text{Na}_2,4\text{-D}$ was used, but the amount sprayed was less than in that of the previous experiment. Some plants of each variety were left untreated for comparison.

(2) Result

Treated plants of Perfection were badly injured. Thomas laxton was also injured but less seriously. Cody showed some swelling of the stems when compared with its untreated plants. Sweet Alaska showed no sign of 2,4-D symptoms (Fig. 8).

(3) Discussion

These experiments indicated that different varieties may have different tolerances to the effect of 2,4-D. It is not known whether this tolerance is inherited or not.

However, it was noted that Sweet Alaska has on its leaves, whether erect or drooped, the mid-vein prominent which seemed to prevent the clinging of sprayed drops. Also it seemed that its leaf surface and plant body were not so easily wetted as the other varieties. In this case it is possible that morphological differences between varieties have much to do with their tolerance of 2,4-D.

Plate VI

Different varieties of pea plants showed the different responses to 2,4-D salt.

Fig. 8 On the right two pots untreated and 2,4-D treated plants of Sweet Alaska showed no visible differences in their growth. On the left two pots 2,4-D treated plants of Perfection showed great inhibition of growth when compared with the untreated plants in the near-by pot.



Fig. 8

B. Strain Differences

Experiment I*

(1) Method and Material

Seeds of 56 strains of white bean, obtained from the Farm Crop Department of this college, were planted in 4" pots in the greenhouse. Each pot contained two plants and six pots of plants were used for each strain. Application of Na₂,4-D at 1,000 ppm was made by placing a drop of the solution on the base of both primary leaf blades.

(2) Result

These strains showed various degrees of tolerance to 2,4-D. No strain was highly resistant to 2,4-D. Also no strain was killed by the small application. The plants were harvested and recorded in Table 18.

Table 18

Fresh weight of white bean shoots determined 18 days after treatment with 2,4-D salt

No. of strains	No. of Plants	Average weight of shoot/plant (gm)
800	12	.95
802	"	.82
803	"	.37
804	"	.61
805	"	.90
806	"	.52
812	"	.62
813	"	.42
814	"	.42
815	"	.36
816	"	.15

*The weight represents all growth above the primary leaves.

*Conducted in August, 1949.

Continued 18

822	12	.67
823	"	.36
824	"	.41
825	"	.73
826	"	.30
832	"	.46
833	"	.64
834	"	.45
835	"	.56
836	"	.63
842	"	.50
843	"	.50
844	"	.19
845	"	.50
846	"	.31
852	"	.47
853	"	.68
854	"	.28
855	"	.91
856	"	.58
862	"	.50
863	"	.37
864	"	.46
865	"	.35
866	"	.32
872	"	.37
873	"	.33
874	"	.75
875	"	.50
876	"	.24
882	"	.43
883	"	.66
884	"	.46
885	"	.30
886	"	.50
892	"	.30
893	"	.18
894	"	.30
895	"	.37
896	"	.27
8102	"	.34
8103	"	.34
8104	"	1.10
8105	"	.77
8106	"	.16

Experiment II*

(1) Method and Material

Strains No. 800, 816 and 8104 and 8106 which were tested in previous experiment were selected as experimental material. Application of Na2,4-D at 2,000 ppm was made by placing a drop of the solution on the base of both primary leaf blades. Five percent of Geon 31X was added to the 2,4-D solution to increase the herbicidal effect during this winter time experiment.

(2) Result

A few days after treatment, the stems of all strains showed curvature. There was no visible difference between them.

However, 3 weeks after treatment, all the plants of No. 800 had recovered from the treatment, and some new growth had started. A few plants of No. 8104 and 8106 recovered; while no plant recovered in No. 816. The plants were harvested 4 weeks after treatment, and the average fresh weight above the primary leaves was recorded in Table 19. For the convenience in comparison No. 800 was taken as standard strain and regarded arbitrarily, as 100 per cent in 2,4-D tolerance.

*Conducted in January, 1950.

Table 19

Fresh weight of white bean shoots determined 28 days
after treatment with 2,4-D salt

No. of Strains	Average weight of shoot/plant (gm)	Average weight of shoots on per cent basis No. 800=100
800	.34	100
816	.03	9
8104	.32	95
8106	.20	59

*The weight represents growth above the primary leaves.
**Twenty plants were used for each treatment.

(3) Discussion

A marked difference in 2,4-D tolerance was found between strain No. 800 and 816. Both of these strains were selections from a same cross, Robust X Early prolific. Their plant vigor and appearance were almost the same; yet they responded to 2,4-D differently. From this result it is suggested that genetical constitution of the plant may influence the action of 2,4-D.

This result agreed with Rossman and Staniforth (54). They reported that certain inbred lines of corn were more susceptible than others and certain single-cross hybrids were relatively unaffected by aqueous solutions of 2,4-D.

C. Diploid and Tetraploid Plants

Experiment I

(1) Method and Material

Diploid and tetraploid rye seedlings approximately 4" tall were treated with sprays containing 5,000 ppm of $\text{Na}_2,4\text{-D}$ in January, 1950.

(2) Result

Both diploid and tetraploid plants treated with 2,4-D solution were stunted when compared with untreated plants. However, there was no difference between the diploid and tetraploid plants.

Experiment II

(1) Method and Material

Seeds of tetraploid buck wheat obtained from Ferry-Morse Seed Co., and of diploid buck wheat, from Farm Crop Department of this college were used. Application of $\text{Na}_2,4\text{-D}$ at 1,000 ppm was made by spraying method.

(2) Result

According to Ferry-Morse Seed Co. this tetraploid buckwheat is resistant to 2,4-D. But by repeated experiments no difference between these and common diploid plants was observed under greenhouse condition.

D. Observation of Toxicity Inheritance

(1) Method and Material

Red Kidney bean plants were treated with $\text{Na}_2,4\text{-D}$ at

1,000 ppm by dipping one of the primary leaves into the solution when the first trifoliate leaf was expanding. New growth of these plants was inhibited for a long time. Finally, they recovered from the treatment and bore fruit, and seeds. The seeds were harvested and stored for some days. Then, both seeds from the recovered plants and from normal untreated plants were germinated in petri-dish at room temperature. After germination they were carefully planted in 4" pots and moved to the greenhouse.

(2) Result

Seeds from recovered bean plants germinated as soon as those from untreated plants. Both epicotyle and radicle elongated normally. When they were planted in soil the treated seedlings emerged no later than the untreated one. When the trifoliate leaf expanded, no 2,4-D sign was observed and no accumulation of chlorophyll in the young leaflets. Flowering apparently was the same as untreated plants and also the date of maturity. The only difference which was noticed was the development of plant size. Plants derived from recovered parent showed comparatively dwarfed, stout, shorter internode and somewhat smaller leaves than those from untreated parent. However, this seemed due to the fact that these seedlings came from under-developed parent rather than to the toxicity of 2,4-D.

E. Doubling and shortening of Onion chromosomes induced by 2,4-D treatment

Experiment I*

(1) Method and Material

Uniform onion bulbs were rooted in clean tap water. Roots of uniform vigor were soaked in solutions containing the Na2,4-D at 1, 5, and 10 ppm concentrations. After a 24 hour treatment, they were fixed in 3 parts of alcohol and 1 part of acetic acid for 2 to 18 hours at room temperature. These roots were macerated in 1/N Hcl about 5 to 9 minutes in the oven at 60° C and, then, transferred to Feulgen stain for one half hour. The meristimatic portions of stained root-tips were placed on slides and a drop of 45% acetic acid was added. The tissue was thoroughly mashed with a glass stencil and covered with a cover glass. Permanent slides were made by the smear method.

(2) Result

After repeated examination of the slides it was found that Na2,4-D did not stop the cell division at the concentrations of 1 to 5 ppm after 24 hours treatment. Inhibition of division occurred at 10 ppm and no metaphase was found where this concentration was used.

It was also found that as low as 1 ppm of 2,4-D treatment, the chromosome became abnormally short in occasional

*Conducted in April, 1949.

cells at metaphase.

The susceptibility of the cells to 2,4-D varied from bulb to bulb, from root to root and even from cell to cell in the same root.

Experiment II*

(1) Method and Material

Onion root tips were used as in previous experiments. The same staining and cytological techniques were used. This time the methyl ester of 2,4-D was used. One CC of the methyl ester 2,4-D was dissolved in 5 CC of 95% alcohol and then made up 1,000 ppm of the ester of 2,4-D in water solution. Treated roots were fixed at intervals of 3, 6, and 20 hours. Some of the treated roots were not fixed but moved to clean water at the same intervals.

(2) Result

This experiment was repeated 3 times. In the first test, it was found that in one hour treatment both the chromosome length and cell division were not affected by the ester of 2,4-D in water solution. Contrarily it seemed that the cell division was increased. However, there was no attempt to prove this point statistically. In the three hours treatment, the length of chromosome was notably shortened, and in the six hours treatment, very short chromosomes were seen (Fig. 9 and 10). The length of chro-

*Conducted in January, 1950.

mosomes were the shortest when roots were treated more than 20 hours (Fig. 11). It was noted that although there existed a great variation of chromosome shortening from root to root and from cell to cell, but as the time of treatment increased, the degree of shortening or contraction of chromosomes increased. The stage that the chromosomes showed their maximum shortening was at metaphase. Sometimes this condition could still be distinguished at early anaphase. It was also noted that affected cells were easily broken in smear process, and the contained chromosome tended to spread out (Fig. 10 and 14). The cell division ceased as the time of treatment increased. Here, again, there was a great variation from root to root.

In the second test the same concentration of the ester of 2,4-D and the same time schedule were used. The onion bulbs used this time were larger and roots were much stronger. There was little shortening of chromosomes even if roots had been in the solution over 20 hours.

In the third test medium sized onion bulbs and medium vigorous roots were chosen. The chromosomes showed the shortening effect but less pronounced than the first test. However, there were many cells showing a disorganized anaphase and 32 chromatids scattered in one cell (Fig. 12). The disorganization of the anaphase was found after three hours treatment in the solution. Tetraploid cells which

were apparently recovered from disorganized anaphase cells were found. There were all stages from late prophase (Fig. 13) to anaphase (Fig. 14). Both tetraploid and deploid cells co-existed in the treated tissue as if in a hyperchimera condition.

It was interesting that roots treated in 1,000 ppm of methyl ester of 2,4-D did not die, and would resume growth again when transferred to tap water if this treatment was less than 6 hours. A 3 hour treatment with 1,000 ppm of sodium salt of 2,4-D killed the onion tissues even though they were subsequently placed in tap water.

Experiment III*

(1) Method and Material

The same onion root-tips and the same cytological technique were used. Instead of the ester of 2,4-D in water solution the application of it was made in a vapor form.— The rooted onion bulb was placed on the mouth of a beaker. On the bottom of each beaker, wet paper towel was placed to maintain a high humidity. A small vial full of the liquid ester of 2,4-D stood on the wet paper towel but not directly in contact with the roots.— The bulb and beaker were, then, closed in a glass jar which was air tight so that the vapor of the ester could not escape from the jar.

(2) Result

After a 24 hour treatment, the treated roots were
*Conducted in February, 1950.

fixed and examined. It was found that both doubling and shortening of chromosome had been induced, much the same as in Experiment II. The shortening was not so serious, but the frequency of doubling seemed increased, since many cells were found with disorganized anaphase. Treated roots, after transferred to tap water, could resume growth with more chances of living.

Plate VII
Shortening of the chromosomes in onion root-
tip cells caused by the ester of 2,4-D
treatment.

Fig. 9 Normal chromosomes at metaphase of mitosis in
onion root-tip cells.

Fig. 10 Notably shortened chromosomes in onion root-tip
cells resulting of 6-hour treatment with the ester
of 2,4-D. Notice also the constriction at
attachment.

Fig. 11 The shortest chromosomes and serious constriction
at attachment showed in onion root-tip cells
resulting of 2,4-D treatment over 20 hours.

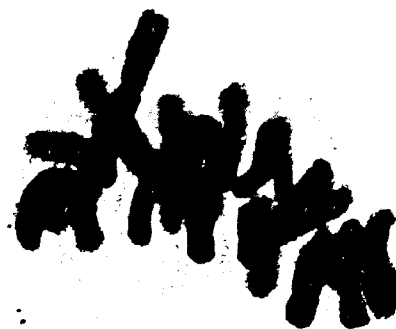


Fig. 9



Fig. 10



Fig. 11

Plate VIII
Doubling of the chromosomes in onion root-tip
cells induced by the ester of 2,4-D treatment

Fig. 12 Disorganization of anaphase in onion root-tip
cells induced by 2,4-D treatment.

Fig. 13 Induced tetraploid cell at late prophase.

Fig. 14 Induced tetraploid cell at anaphase.
(This cell was showing perfect anaphase.
Unfortunately it was broken in taking a microphoto-
graph and some chromatids with the cytoplasm
separated to the right side of this cell).



Fig. 12



Fig. 13



Fig. 14

(3) Discussion

Slight shortening of the chromosomes caused by chemical treatments has been reported by numerous authors, especially with colchecine. However, none of these effects from other chemicals were as pronounced as the results obtained with the ester of 2,4-D. It is significant that the 2,4-D shortened chromosomes could recover when subsequently transferred to tap water provided they had not been treated with this compound for more than 6 hours.

This is the first report that the ester of 2,4-D has the ability to cause doubling of chromosomes. The manner in which this compound brings about the doubling of chromosomes differs from that of the colchecine in that colchecine suppresses the spindle formation, the ester of 2,4-D disorganizes the anaphase. How frequent is the occurrence of chromosome doubling caused by the ester of 2,4-D remains unknown. But the chance is apparently much less than with colchecine, and it is also not known whether this treatment can be used in producing polyploid plants.

Although the ester of 2,4-D is seldom used to control weeds in crops, the possibility remains that the sodium salt and amine form of 2,4-D might induce chromosome doubling too. Therefore, it is questionable to use 2,4-D to control weeds in grain, sugar cane or other crop fields which are for the purpose of seed production or vegetative propagation. The

objection to the ester of 2,4-D or perhaps other forms of 2,4-D is probably not due to its herbicidal damage but due to the possibility that mutations or "bud sports" might be induced; this in turn would lead to variation within the variety.

IV Summary

- (1) These experiments deal with external and internal environmental factors which affect the action of 2,4-D.
- (2) It was found that a plastic material, Geon 31X, greatly increased the action of Na2,4-D. The exact mechanism for this increased action is not known. Perhaps the entry of 2,4-D into the plant is facilitated, and also the building up of the internal temperatures during day-time may in part account for the increased action especially in cool weather. However, many coatings or plastic materials other than Geon 31X did not show this effect.
- (3) Through two years experiments, it was shown that 2 Amino-2-Methyl-1-3-propanediol, a primary alcohol, increased the action of Na2,4-D in winter but not in summer under greenhouse conditions.
- (4) By the addition of sucrose and potassium phosphate to Na2,4-D in water solution, such as suggested by other workers, the herbicidal action was actually increased.

Here it was also found that potassium sulfate, ammonium sulfate and magnesium sulfate, when combined with sucrose, also increased the action of $\text{Na}_2,4\text{-D}$.

- (5) It was found that allyl alcohol, potassium ferrocyanide and sodium hypochlorite decrease the action of $\text{Na}_2,4\text{-D}$.
- (6) In the course of these experiments, a number of chemicals were tested and showed no definite result either in increasing or decreasing the action of $2,4\text{-D}$.
- (7) Natural factors, such as light, carbon dioxide, humidity, infra-red rays and plant temperature under conditions of the experiment did not produce any change in the mode of action of $2,4\text{-D}$. However, some undetermined or unknown seasonal factors were encountered which resulted in a variation of $2,4\text{-D}$ effect during summer and winter months under greenhouse conditions.
- (8) By the use of Elodea cutting test, the inhibition of photosynthesis caused by $\text{Na}_2,4\text{-D}$ was proven and measured numerically.
- (9) Plants showed all degrees of susceptibility to the $2,4\text{-D}$ injury from variety to variety and from strain to strain. However, deploid and tetraploid plants showed little difference in response to this compound.
- (10) When the plants recovered from $2,4\text{-D}$ treatment and, then, initiated the flower buds, the seeds from them did not carry toxicity of $2,4\text{-D}$ to the next generation.
- (11) By cytological study on onion chromosomes it showed

that $\text{Na}_2,4\text{-D}$ in as low a concentration as 1 ppm could, in some instance, shorten the chromosome length. The ester form of 2,4-D, when used in high concentration in water for a period of 6 to 20 hours, seriously shortened the length of chromosomes, and occasionally doubled the number of chromosomes. If treatment was less than 6 hours and if the treated roots subsequently were placed in tap water, they would resume growth. The manner of doubling of the chromosomes induced by the ester of 2,4-D was through disorganization of the anaphase. The vapor of the ester of 2,4-D also doubled the number of onion chromosomes.

- (12) It is suggested that since 2,4-D may produce profound changes in chromosome behavior, further study along this line may be profitable in cytogenetics and plant breeding work.

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