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The Effects of 2,4-D and Related Compounds on Plants

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THE EFFECTS OF 2,4-D AND RELATED COMPOUNDS ON PLANTS

By

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~~Armament~~ Air Proving Grounds Center
Eglin Air Force Base, Florida

FOREWORD

This report, APGC Project 8780, Task D2457, was prepared during the period July to November 1964. The information was acquired from the Agricultural Library, University of Florida and the Technical Library, Air Proving Ground Center. The report was prepared as a result of a meeting between the Air Proving Ground Center and Det 4, Research and Technology Division, in which a need for a compilation of this nature was realized. A good example of the precautions taken by the Air Force in their range efforts, this report is intended for use by the military and civilian populations. Farmers and others who spray for weed control will be particularly interested. A glossary of botanical terms is provided in this report for those who may not be familiar with the language.

This technical report has been reviewed and is approved.

J. E. Roberts
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Major General, USAF
Commander

ABSTRACT

The effects of 2,4-D and related compounds on plants and animals, including information concerning the sensitivity of plants, physiological action of the herbicides, characteristic appearance of affected plants, and methods of determining the herbicide on the plant are presented. 2,4-D being the most characteristic compound of the group is discussed in greater length. It exerts its greatest effect in the rapidly growing and differentiating plant tissues. Cotton is the most sensitive major crop in the Northwest Florida area. One ounce of 2,4-D evenly distributed over 35 acres will seriously injure a cotton crop. For this reason, extreme care should be taken during all herbicide spray operations and especially when such chemicals as 2,4-D, "Silvex" and "Falone" are applied adjacent to cotton fields. The use of mist-blower applicators should be limited to only those cases when complete meteorological data and other information pertinent to drift control are available and indicate absolute safety. Grasses being fairly tolerant to 2,4-D are not injured by a dosage of 1 lb/acre. Tolerance of other crops is given. Six methods are given for the extraction of herbicide from a sample of foliage. The characterization of the compound is then accomplished with a gas chromatograph.

CONTENTS

GLOSSARY

Apical	Of the apex, at the tip.
Atypic	Not typical.
Auxin	A plant-growth regulating substance.
Cotyledon	The first leaf of a young seed plant.
Cuticle	A thin waxy lipoid covering on the plant.
Dicotyledon	A plant type with two cotyledons, most herbs, shrubs and broadleaved trees. See Cotyledon.
Differentiation	The process whereby simple cells become specialized. Usually takes place in the meristems.
Epinasty	Twisting and curvature in plants as a result of unequal growth rates.
Lipoid	Fat-like, fatty. See lipoid system.
Lipoid System	Fatty tissues in a plant in which organic esters are found.
Meristem	The region in the plant where simple cells are found and growth occurs. See differentiation.
Monocotyledon	A plant type with one cotyledon, most grasses. See cotyledon.
Phloem	A living vascular system which commonly transports food downward in the plant. See vascular system.
Polysaccharide	A carbohydrate that can be hydrolized into two or more molecules of sugar. See saccharide.
Prophase	An early stage of cell division.
Stomates	A pore-like opening found in the leaves and stems of plants through which carbon dioxide, water vapor, and air can pass.
Saccharide	A sugar.

Surfactants	A compound which will stick to a surface and/or cause another to stick when it normally would not.
Turgid	To have turgor pressure. See turgor pressure.
Turgor Pressure	The normal water pressure in a plant which keeps it from wilting.
Vascular System	A specialized "pipe" system for the transport of fluids in the plant. See phloem, xylem.
Xylem	A dead vascular system which commonly transports water and minerals in the plant. See vascular system.

I. INTRODUCTION.

2,4-D and the related compounds MCPA; sesone; 2,4-DEP or "Falone"; 2,4,5-T; 2,4,5-TP or "Silvex" and 2,4-DB are selective herbicides against broad-leaved dicotyledonous plants. 2,4-D being probably the simplest, most effective, and most characteristic compound of the group will be discussed in greater length with only comparative remarks concerning special properties of the other related compounds listed.

II. 2,4-D AND RELATED COMPOUNDS.

The pure acid form of 2,4-D (2,4-dichlorophenoxyacetic acid) is a white crystalline material, with a molecular weight of 221.04 and a melting point of 138-140°C. Its solubility in water at 22°C is 605 ppm; at 50°C, 1490 ppm, provided by V. H. Freed, Oregon State University. The acid is insoluble in petroleum oils, fairly non-corrosive to metals, non-explosive, fire resistant, inexpensive, translocated in a plant, does not affect greatly soil microorganisms, and is non-toxic to animals within limits. 2,4-D is often formulated as salts and esters in various forms for use as a herbicide, depending on the conditions of intended usage.

The pure acid form of 2,4-D for instance is not applied as a water spray because of its low water solubility. Troublesome calcium and magnesium precipitates which are difficult to remove will form in hard water, clogging spray nozzles and filters. The ester formulations are not subject to this difficulty. The pure acid is usually applied as a granular material. The salt forms of 2,4-D are soluble in water, however, and are used when a water solution is desired. The ester formulations are oil soluble and may be used in conjunction with aromatic oils to increase the herbicidal characteristics of the mixture. Dust formulations are made from salt and ester forms but are subject to serious wind drift, hence dusts are not be applied by airplanes.

An ester formulation may be made relatively volatile or non-volatile depending on the basic alcohol used in the ester formation. Recently, the non-volatile esters, though more expensive, have come into greater use because they offer less hazard to nearby 2,4-D sensitive crops. The esters of 2,4-D are generally considered most toxic to plants because of volatility, increased lipoid-like wetting action, and compatibility with the cuticle with a resultant increased efficiency of absorption. Lower rates of application of the esters are possible with increased penetration of the woody species.

The volatility of a 2,4-D ester has been studied by Staten to determine its significance.

> Abnormal growth symptoms appeared in cotton plants around a 12 inch diameter pan containing 800 ml of a solution containing 10 ml of a 9.6% esterified 2,4-D compound, for a radius of 25 to 30 feet. The pan was in direct sunlight and 373 plants were affected by the vapor.[1]

2,4,5-T (2,4,5-trichlorophenoxyacetic acid) is very similar to 2,4-D in all respects except that it is more effective on woody species that are resistant to 2,4-D but less effective on many other plants. Mixtures of the two are often used, since in combination they affect many more species than the singular formulations.

MCPA (2-methyl, 4-chlorophenoxyacetic acid) is very similar to 2,4-D. Its effectiveness varies on different species as does the effectiveness of 2,4-D but the correlation of species is not high, so that in conjunction the two are effective on a broader range of species—similar to 2,4-D and 2,4,5-T mixtures. MCPA has been studied extensively in Europe and is used there for weed control in small grains, flax, and peas. It is a fairly expensive compound in the United States.

"Silvex" or 2-(2,4,5-trichlorophenoxy) propionic acid, also called 2,4,5-TP, controls some plants that resist both 2,4-D and 2,4,5-T, notably chickweed, henbit, wild strawberry, some oaks and maples, and a number of aquatic weeds.

4-(2,4-dichlorophenoxy) butyric acid (2,4-DB) has a low toxicity to most plants. It is converted to 2,4-D in the plant by a process known as beta oxidation. Legumes make this conversion so slowly that there is never sufficient 2,4-D to cause serious plant injury. Weed control is thus affected in legumes. Other plants make this conversion rather rapidly and are killed or damaged by 2,4-DB if subject to the effects of 2,4-D.

2,4-DEP or tris(2,4-dichlorophenoxyethyl) phosphite is sold as "Falone." It is a viscous, oily liquid with a density of 0.96, and is nearly insoluble in water. It is soluble in aromatic hydrocarbons and slightly so in alcohol. It is considered relatively non-volatile and is used where 2,4-D esters would present a hazard. 2,4-DEP is commonly used for preemergent weed control and is effective for three to seven weeks. Moisture (rain water) in the soil slowly decomposes 2,4-DEP to 2,4-dichlorophenoxyethanol and phosphorous acid. 2,4-dichlorophenoxyethanol and 2,4-D have similar effects in regard to seedling development and seed germination. Both compounds in the soil seem to be responsible for the herbicidal effect observed.

Sesone (sodium 2,4-dichlorophenoxyethyl sulfate) like "Falone" does not harm plants by direct contact but depends on soil water to hydrolize it to the active compound 2,4-dichlorophenoxyethanol, which in turn is often oxidized to 2,4-D. It is an amorphous solid and is hydrolized under acid conditions and by the soil organism Bacillus Ceres var mycoides.

III. ANIMAL TOXICITIES.

The animal toxicity of the 2,4-D compounds is fairly low. A single oral dose of 40 g of 2,4-D would be needed to produce an LD_{50} in a 180-lb man. Small doses of a gram or so have little or no effect when taken for a week or more. Different animals have been tested, with an LD_{50} in a single oral dose ranging from 300-1000 mg/kg of body weight. LD_{50}'s for the different 2,4-D like compounds fall into this range. Injury at lower doses usually consists of skin and eye irritation. At usual plant-control doses 2,4-D does not reduce the number of soil organisms. At heavier rates some are inhibited and others stimulated. Anaerobic organisms are not affected significantly and may be stimulated.

IV. 2,4-D IN THE SOIL.

2,4-D has an effect on modulation of the common bean that greatly affects the vigor of the plant. Very small amounts drastically cut the number of nodules, but 2,4-D up to rates of 200 lb/acre does not affect the Rhizobia bacteria. The response is therefore largely a plant response. See Table 1 for data on the bean plant responses to 2,4-D.

Microorganisms have a definite relation to the persistance of 2,4-D in the soil, and are of major importance in decomposition of the chemical. Low rates of 2,4-D will decompose in one to four weeks in warm wet soil; spray strength solutions lose their strength in four to seven weeks. Dry or frozen soils will not decompose the chemical quickly. Light sandy soils do not decompose 2,4-D as effectively as heavy clays with a high percentage of organic matter. There is no cumulative build-up of 2,4-D in the soil from year to year. Adaptive enzymes appear to be involved in this decomposition.[2]

At constant rates, 2,4-D is more effective on sandy soils than heavy clays. This fact is presumably dependent on the adsorptive capability of the soil.[3] Rainfall has a profound effect on the persistence of 2,4-D in the soil. A study of the persistence of the chemical in the soil was conducted using cotton plants as bio-indicators. The data is summarized in Table 2. Results indicate that 2,4-D either leaches or is inactivated in wet soil probably through decomposition and adsorption. "The application of 1 ml of a 50 ppm solution to the soil around the cotton seedlings resulted in less injury than the application of 0.1 ml of a 1 ppm solution to the cotyledons."[3] At constant rates, 2,4-D is less effective in the soil than when applied directly to the plant.

Plant roots most readily absorb the polar forms of 2,4-D whereas the leaves absorb the non-polar forms. Surfactants increase the efficiency of foliar absorption. In most cases 6-12 hours is necessary for a plant to absorb a lethal dose of 2,4-D prior to rain. The oil-like esters resist washing from the plant and are therefore fairly "rain-resistant."

V. 2,4-D IN PLANTS.

2,4-D is then translocated within the plant downward through the phloem as a food metabolite, upward through the xylem as a water-soluable ion, and transversly in the lipoid system as an ester. An excessive application of 2,4-D will kill cells locally and not injure the rest of the plant. Since translocation throughout the plant is necessary for a complete kill, smaller doses of 2,4-D are desired and ultimately more effective. Most effective treatment of plants occurs when large amounts of food are being moved to the root system for storage, in late spring or early fall. A number of low-rate applications then are more effective than are heavy applications. Soil moisture favors rapid translocation. A number of studies have been made with radioactive carbon to find translocation rates.[4] Alligator weed, for example, translocates 2,4-D through the phloem at the rate of 4.2 cm/hr. The method used to determine this rate was by measurement of the bending of the stem.

In general, all plants are susceptible at germination, but differences rapidly become apparent. Plants gain tolerance with age but generally are most susceptible during periods of rapid growth.

Regarding a mode of action for 2,4-D, the Botanical Review states that the youngest and most turgid leaves absorb the chemical best. The entry of radioactive 2,4-D into a grassy leaf (millet) and a bean leaf proceeds at the same rate. There is also no difference in plants in the metabolic rate of 2,4-D in susceptible and non-susceptible plants. A high correlation between susceptibility and translocation out of a leaf has been found: "...25% of the applied dose migrated out of bean, soybean, and cotton leaves in 24 hours, but only 1% out of oats, wheat, and rice leaves."[2] The meristem of the non-susceptible varities then is subjected to much less 2,4-D than the meristem of the susceptible species.

2,4-D in the plant, in addition to decomposition, is also rendered ineffective by forming neutral complexes with certain cell constituents. The plant apparently possesses a number of seemingly different mechanisms by which inactivation of 2,4-D can take place. It is significant that 2,4-D in the plant is much more stable than the natural auxin, indoleacetic acid.

2,4-D seems to have a remarkable persistence in plant tissues. 2,4-D injury often appears in perennial plants the following season. There seems to be particularly critical periods in a plant's development. Sometimes for instance, an application made in the fall produces a striking effect next spring. Applications made at other times, however, fail to produce any noticeable effects. It is important to distinguish between "(a) persistence of 2,4-D in plant tissue and (b) delay in visible expression of effects of 2,4-D."[5]

The auxin 2,4-D not being broken down by the plants normal metabolic system as is indoleacetic acid, causes an increase in total auxin level in the plant. It is presumed that it is this increase that is responsible for the herbicidal effect of 2,4-D. As an auxin, 2,4-D exerts its effect largely in the meristems of the plant and affects differentiation. 2,4-D has been observed to block the prophase stage in an onion root. A treated plant produces a tumorous distortion of tissues, atypic organs, and eventually dies if a high enough concentration is present. It follows that any auxin in high quantities would function as a herbicide providing it can produce a high auxin content in the plant and correspondingly, is not subject to metabolic reduction to an ineffective compound.[2]

Parenthetically, 2,4-D increases the protein and decreases the sugar content of potatoes. These changes may be significant in that an increased disease resistance has been observed. 2,4-D increases the use of both readily available and stored food in the plant so that a slow starvation takes place in conjunction with the various deformations. (See Reference 4, p. 132-135.) Auxin-treated foliage is preferred by Japanese beetles. Cane treated with 2,4-D is preferred by sugar beet bores. This indicates that the 2,4-D does indeed penetrate the foliage of even non-susceptible species. (Radioactive carbon tests also substantiate this fact.) Corn treated with 2,4-D becomes more palatable for field mice. In California, sheep ate Centaurea Solstitialis only after it was sprayed with 2,4-D, and poisonous Corium maculatum though usually avoided was eaten by cattle after it was sprayed causing the cattle to die.

Although total plant weight and total sugar content decrease rapidly, there is a slight increase in the percentage of polysaccharides about one week after treatment. This percentage increase lasts for about three weeks. It is possible that the animals prefer those aforementioned plants because of the increase in sugar content. 2,4-D slows the production of sugar; therefore, the food reserves of the plant become the sugar source with resultant starvation of the plant. The nitrogen percentage also rises above the check (or normal control plant), but most of this rise is attributed to plant weight loss and not to nitrogen increase.

Because small amounts of 2,4-D can cause such profound changes within the plant's many interrelated systems, it has long been suspected that it affects the enzyme systems. Findings indicate, however, that there is no simple explanation.

2,4-D causes the stomates of plants to close with an effect directly proportional to the dose. The subsequently reduced carbon dioxide intake and transpiration rate could account in part for the slowed food (sugar) production in the plant. A suffocation of the photosynthetic process takes place. Apparently, as a result of the closure of the stomates, the turgòr pressure increases. This increased pressure coupled with the other physiological changes observed in the plant combine to cause an unusual brittleness.

2,4-D causes plants to twist and curl as a result of different rates of growth and differentiation. Roots, stems, and leaves are often twisted and malformed. 2,4-D is found in greatest concentrations in the meristematic tissues of the plant where it exerts its greatest effect. Lateral meristems are inhibited, whereas comparatively, the apical meristems are not. The areas of greatest growth are affected the most.

VI. LOCAL CROPS.

Okaloosa County's seven most important crops are, in order of decreasing acreages: corn, soybeans, velvet beans, cotton, hay crops, wheat, and oats. Walton County statistics are comparable, with the exception of peanuts which would rate just before velvet beans in the above list. Soybeans and velvet beans seem to be increasing acreagewise in Okaloosa County.[6] These crops are all affected to some extent by 2,4-D. Particularly sensitive to 2,4-D is the cotton crop. The legumes are also generally sensitive. Oats and wheat, being monocotyledons are less affected than the others. Tomatoes are probably the most sensitive of all familiar plants, being used extensively in biodetection methods.

VII. EFFECTS OF 2,4-D ON COTTON.

In a study at the Texas Agricultural Experiment Station in 1949, using the Stoneville 2B variety of cotton, it was found that 1 oz of

2,4-D could cause serious damage to all the plants in a 35-acre area if distributed uniformly. Much lighter applications, however, resulted in much foliage distortion without an appreciable effect on the set of bolls or the seed cotton yield. Intermediate application rates produce a profound distrubance within the plant, cause chemical variations, and reduce total yield.

The quantitatively significant facts of the Texas study appear in Table 3. The table has a column showing percentage reduction in weight of the main stalk leaves. The development of leaves on the branches was not affected as greatly. The main-stalk leaves developed normally only when the plants were nearing maturity. The plants that had been treated with the 10 and 20 ppm solutions produced longer branches. The vegetative branches were affected more than the fruiting branches, perhaps a result of the fairly early treatment so that plant recovery was significant here. (See Table 4.) Leaf injury did not then necessarily lower the final yield.[7] Table 5 contains data from another related study. It can be seen from Tables 3 and 6 that amounts used in weed control, i.e., 20 to 80 oz/acre ($1\frac{1}{4}$ - 5 lb/acre) would completely destroy a cotton crop.[7]

In a Mississippi study the least susceptible stage of cotton was found to be after most of the bolls were already on the plant. With a 0.001 lb/acre application there was no appreciable effect on the yield of seed cotton even though it did produce significant injury to the leaves. (See Tables 6 and 7.) Moderate leaf malformations at the seedling stage did not lower the yield, but a decrease in stem growth did. Earlier contamination of the crop and greater severity of damage result in a greater delay of crop maturity. 2,4-D damage has been shown to be transmitted into the seed produced by affected plants.[8]

As the season advances boll weevils and worms become an important factor. Delayed maturity of a cotton crop would subject it to attack by these pests. Increased efforts in insect control would then be recommended to protect the crop.

A study at the State College of Mississippi regarding the relative effects of 2,4-D, 2,4,5-T and MCPA, showed that on the Coker 100 wilt variety of cotton, 2,4-D had the greatest effect on reducing the yield. In the first part of the season up till the blossom stage 2,4-D damage will affect the yield of seed cotton. During the blossom and boll stages, the damage appears in progeny seedlings. See Table 4 for data on these subjects.[8]

The appearance of 2,4-D damaged cotton leaves varies, of course, with many factors, viz., dosage, length of time since treated, meteorological conditions, etc. The leaves first begin to ruffle and roll at the margins. Progressively more severe damage results in an epinastic effect. Leaves that grow on the plant after treatment are narrow, deeply lobed, and closely and thickly veined—an effect of the inhibition of the lateral meristem in the leaf. The stems swell and form galls as the cortex splits. Secondary roots are often formed. Lateral stems forming later are less affected.[10] (See Table 6 for treatment rates and their effects.)

VIII. ATMOSPHERIC DRIFT EFFECTS OF 2,4-D.

In recent years there have been instances that involved 2,4-D drift from places of application to neighboring crops, notably cotton. In Tennessee, cotton was injured by a fine liquid spray 2½ mi away. In a report from Texas in the summer of 1947, 2,4-D injured cotton fields were found 15 to 20 mi from fields dusted with airplanes. The airplanes may have passed closer than this to those affected fields but the damage over the fields was uniform, indicating that the dust must have traveled a distance of at least 10 mi.[11]

According to a table in Phytopathology,[6] water droplets about five microns in diameter can drift over three mi in falling 10 ft when the velocity of the wind is 3 mph. The dust particles used on the rice in Texas ranged from 4 to 16 microns in size and could have been released from the leaky container into the air at altitudes of 100 to 200 ft while the airplane was turning. With winds possibly up to 10 mph the dust could have drifted even further than the 15 to 20-mi distance mentioned. See Reference 12 for further meteorological data regarding deposition rates of small particles in different velocity winds. It is interesting to note that one of the weeds, Caperonia Palustria found in the rice fields, was also often found unaffected among badly damaged cotton plants.

IX. EFFECTS ON SELECTED CROPS.

Legume crops while not as sensitive as cotton are generally subject to damage by 2,4-D. Application rates of 1/2 lb/acre generally control weeds in such species as ladino and white clover and lespedeza with only slight vegetative injury. Rates up to 4 lb/acre however will kill these legumes.

The tolerance of peanuts to 2,4-D is generally 1½ lb/acre although slight damage will occur with no reduction in yield. The hydrolizable compounds "Sesone" and "Falone" are often applied to peanut crops with good results. By the time the peanut plant appears above the ground, an extensive root system has already developed. The 2,4-D type herbicide (see earlier discussion concerning "Falone") that is formed by the hydrolization then will not leach deeply enough into the soil to kill the peanuts, but adequate weed control is affected.

Common bean plants are sensitive to 2,4-D and are generally damaged at a 1/2 lb/acre concentration. A more sensitive reaction to 2,4-D by common beans can be measured by observation of the nodule formation. Concentrations of 0.07 lb/acre totally inhibit nodule formation. The nitrogen-fixing Rhizobium bacteria found in nodules can tolerate rates of 200 lb/acre and are not affected but do not live in the plant depriving it of an important nitrogen source. See Table 1 for data regarding beans and 2,4-D.

Pea plants react to 2,4-D in much the same way as the beans do. Compared quantitatively, however, the vegetative reaction of peas is more severe and the nodule reduction is less severe at identical rates of 2,4-D.

Clover and alfalfa react in a similar manner. 0.03 lb/acre gave slight injury to clover but reduced the nodulation percentage to zero. 0.003 lb/acre reduced the nodulation of alfalfa by 30%. Another legume, soybeans, seems to be tolerant to 2,4-D up to rates of 2 lb/acre.

The grasses and monocotyledonous plants in general are quite resistant to 2,4-D for specific reasons mentioned earlier. Corn for example is generally tolerant to 2,4-D at rates up to $1\frac{1}{2}$ lb/acre. At this rate injury to corn on sandy soil has occurred. If applied for pre-emergence treatment on sandy soil heavy rains may result in injury. Varietal differences in tolerance have been observed but are quite small. Better herbicides than 2,4-D are available for controlling weeds in corn.

One-half pound per acre of 2,4-D applied in early spring increased wheat yields due to weed kill in these varieties: Coastal, Knox, Atlas 66, Atlas 50, Redhart, and Thorne. Premergent applications on oats at the rate of 0.6 lb/acre increased yields due to weed kill in the following varieties: Appler, Delta Red, and Carolina Red. Similarly, a barley crop is benefitted by 3/4 lb/acre post-emergent applications; rice tolerates 1/2 lb/acre applications except at flowering time; pasture plants tolerate 1 lb/acre applications; lawns tolerate 1 1/2 lb/acre of 2,4-D; cane tolerates 1 3/4 lb/acre; sorghum is tolerant to 1/3 lb/acre when the plant is 4-12 inches high but is more susceptible in other stages of more rapid growth.

Certain garden vegetables are fairly sensitive to 2,4-D. 0.15 lb/acre caused permanent damage to beets; 0.2 lb/acre seriously damaged spinach; 0.1 lb/acre seriously damaged turnips. Although 0.3 lb/acre causes noticeable damage on onions, it takes 2.0 lb/acre to cause severe damage. Tomatoes are more sensitive than cotton.

X. METHODS FOR DETERMINATION OF 2,4-D.

A method for detecting 2,4-D compounds on foliage by either chemical or physical means is of interest. Analytical procedures are given in Appendix I. Chromatography and infrared spectroscopy would seem to be the best methods to use. Table 8 gives R_f values of 2,4-D and 2,4,5-T with different solvent systems for paper chromatography. The R_f value is the fractional distance a compound will move up a paper with the solvent's distance taken as the whole.

XI. SUMMARY.

2,4-D and a number of related compounds are systemic herbicides capable of causing extensive damage to susceptible plants at fairly low application rates. The auxin-like characteristics of the compounds make this low-application high-damage effect possible. 2,4-D being an auxin affects most greatly meristematic tissue in which growth and differentiation are taking place. The resultant appearance of the 2,4-D affected plant may be twisted, have ruffled leaves, seriously malformed leaves, split and malformed stems, or may die, depending on the dosage received.

Dicotyledonous plants in general are quite susceptible to 2,4-D. Tomatoes, cotton, and some legumes are very susceptible to even trace quantities of the chemical. Monocotyledonous plants are generally non-susceptible to 2,4-D at rates of 1 lb/acre or more. 2,4-D generally does not affect other living organisms at this dosage rate except for some bacteria.

When plant injury occurs, considerable interest is generated in determining the cause of the injury. The methods given enable one to characterize the 2,4-D and related compounds present in and on the plant.

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APPENDIX I

TABLES

TABLE 1. EFFECTS OF 2,4-D ON COMMON BEANS WHEN GROWN IN TREATED SOIL.

2,4-D lb/acre	Average No. Nodules/Plant	General Plant Reactions
36.6	0	Seed failed to grow
18.3	0	Seed failed to grow
9.1	0	Seed failed to grow
4.6	0	Seed grew, plant dwarfed
2.3	0	Secondary roots, cotyledons malformed
1.1	0	Secondary roots, cotyledons malformed
0.6	0	6 in. tall, secondary roots, first true leaves
0.15	0	6 in. tall, secondary roots, first true leaves
0.075	0	11 in. tall, leaves normal
0.037	1.0	11 in. tall, leaves normal
0.018	2.4	11 in. tall, leaves normal
0.009	5.3	11 in. tall, leaves normal
0.0	24.03	11 in. tall, leaves normal

Reference 13

TABLE 2. COTTON AND 2,4-D DUST, LEACHING IN SOIL BY RAINWATER.

2,4-D Rate lb/acre	Appearance of Plants After	
	1 in. of Rain	2 in. of Rain
1	Malformed	Normal
0.1	Normal	Normal
0.01	Normal	Normal

Reference 3

TABLE 3. 2,4-D APPLIED AS AN AMINE SALT TO STONEVILLE 2B VARIETY COTTON.

Applied as ppm H_2O solutions by dipping one leaf	Calculated amount of pure acid equivalent of 2,4-D applied per plant (mg)	Injury	Reduction in weight of main stalk leaves (%)	Increased Height (%)	1 oz dosage per X acres*
0	0.0	None	0	0	—
1	0.002	None	—	—	700
5	0.01	Mild	27	—	140
10	0.02	Significant	—	—	70
20	0.04	Severe	77	38	35

*Assuming 20,000 plants per acre

Reference 7

TABLE 4. THE YIELD OF SEED COTTON FROM PLOTS TREATED WITH 0.1 LB/ACRE OF 2,4-D OR 2,4,5-T AT ONE OF FOUR STAGES OF PLANT DEVELOPMENT EXPRESSED AS A PERCENTAGE OF THE AVERAGE OF THE UNTREATED CHECKS.

Stage	2,4-D (%)	2,4,5-T (%)
Seedling	16	64
Square	53	91
Early Blossom	32	83
Boll	79	82

Reference 9

TABLE 5. THE EFFECTS OF 2,4-D ON COTTON IN A STUDY AT BATON ROUGE.

0.1 ml of ppm solutions	Quantity 2,4-D applied per plant (microgram)	Effects (Injury)
0.01	0.001	None
0.1	0.01	None
0.5	0.05	2-3 leaves, normal in 4 weeks
1.0	0.1	4-5 leaves, recovery in 8 weeks
10.0	1.0	Deformed, no recovery in 8 weeks
25.0	2.5	Deformed, no recovery in 8 weeks
50.0	5.0	Deformed, no recovery in 8 weeks

Reference 3

TABLE 6. RESPONSES OF COTTON TO 2,4-D.

Treatment rates of pure acid equivalent of 2,4-D applied (lb/acre)	Effect
0.1	Severe epinastic response in one day, stunted plants for several weeks.
0.01	Similar to above, not as severe, branched profusely, many malformations.
0.001	Very mild symptoms, complete recovery in one or two weeks.

Reference 14

TABLE 7. A COMPARISON IN THE YIELD OF SEED COTTON FROM PLOTS TREATED IN THE SEEDLING STAGE WITH THREE RATES OF 2,4-D EXPRESSED AS A PERCENTAGE OF THE UNTREATED CHECK.

Rate lb/acre	Yield %
0.1	16
0.01	68
0.001	99

Reference 9

TABLE 8. R_f VALUES FOR 2,4-D AND 2,4,5-T IN THREE SOLVENT SYSTEMS FOR PAPER CHROMATOGRAPHY.

Compound	Phenol, Water	Butanol, Proponic Acid, Water	Isopropanol, Ammonia, Water
2,4-D	0.83	0.91	0.67
2,4,5-T	0.76	0.94	0.80

Reference 15

APPENDIX II

DETECTION OF 2,4-D ON COTTON USING A GAS CHROMATOGRAPH

Cassil's Method

De Vries' Method

The Acetonitrile Method

U.S. Department of Agriculture's Method

A Method for Obtaining 2,4-D Esters From Plants

The Gutenmann and Lisk Method

THE CASSIL METHOD

In this (method) 100 gm of chopped foliage is extracted with ... of 2:1 benzene/isopropanol solution by tumbling for 1/2-1 hr. ... is filtered and the alcohol is removed by water extraction in a ... 10 ml of this extract is dried with anhydrous sodium sulfate and ... Nuchar Attaclay added and swirled for 30 sec. The solution is ... once and injected.

DE VRIES' METHOD

100 grams of finely chopped material is extracted in a high-... Waring blender with 400 ml of 3:1 hexane/isopropanol for 3 min. ... mixture is poured from the blender cup into a separatory funnel ... about 50 ml of distilled water through a funnel containing a glass ... plug. When no further isopropanol can be smelled (six 100-ml wash ... water), the solution is concentrated to a final volume of 100 ml. ... is passed through a 60/100 mesh Florisil column, with the sample ... washed then with an additional 40 ml of hexane. 10 ml aliquots are analyzed by electron capture.

THE ACETONITRILE METHOD

The hexane extract referred to in the method by De Vries is ...tioned into acetonitrile by successively washing the hexane solu... with acetonitrile in a separatory funnel. Four 10-ml portions ...tonitrile are used for a 25-ml aliquot of sample. The pooled ac... extracts are then dried over sodium sulfate and evaporated to ... a stream of warm air. The residue is taken up in 3 ml of a me... trifluoride solution. This solution is warmed in a boiling wat... 2 min continuously swirling. The methanol is evaporated and the ... is taken up in hexane and the methyl esters injected into the gas ...matograph.

U.S. DEPARTMENT OF AGRICULTURE'S METHOD

This method consists of blending 100 gm of chopped leaves in a Waring blender for 3 min with 20 ml of 10% sulfuric acid in ethanol, 150 ml diethyl ether, and 50 ml of petroleum ether. The solution was decanted and the leaf mass extracted 3 times with 100 ml of 3:1 diethyl ether/petroleum ether. These combined extracts are washed with 100 ml of aqueous 4% sodium bicarbonate. The aqueous layer containing the herbicide is extracted 2 times with 50 ml of chloroform, the pooled extracts are then taken to dryness. The residue is methylated with methanol-boron trifluoride solution as previously described, the methyl esters taken to dryness, taken up in petroleum ether and injected for analysis.

A METHOD FOR OBTAINING 2,4-D ESTERS FROM PLANTS

For obtaining the esters an aliquot of the ester extracts of the foliage (from the U.S. Department of Agriculture's method) is concentrated to near dryness. It is then put through a Florisil column using petroleum ether to wash the sample in. The pesticide is leached with 100 ml of 15% ethyl ether in petroleum ether. The leachate is concentrated to 25 ml and washed by shaking in a separatory funnel four times with 25 ml portions of 80% acetonitrile in water. The washings are combined, 100 ml of water added, and this aqueous solution back-extracted into petroleum ether using 3 teaspoonsful of sodium chloride. The petroleum ether layer is then dried in sodium sulfate and again put through a Florisil column. The pesticide is leached from the column with 100 ml of 10% ethyl ether in petroleum ether, concentrated to dryness, taken up in a small volume of petroleum ether and injected into the gas chromatograph.

THE GUTENMANN AND LISK METHOD

In this method, 25 grams of chopped foliage is transferred to a Waring jar, 1 ml of 85% orthophosphoric acid is added and 80 ml of acetone. The sample was blended for 2 min, filtered through a coarse sintered glass funnel stopped with a glass wool plug. The glass wool stopper serves to catch the plant solids. The filtered (residue) is rinsed with two 20 ml portions of acetone, each time compressing the sample with the bottom of a 50 ml beaker to squeeze out the remaining acetone. The volume of the filtrate is reduced to approximately 75 ml and transferred to a 100 ml volumetric flask. The...(beaker) sides are washed down with acetone and these washings are added to the 100 ml volumetric flask. The final volume is adjusted to 100 ml with acetone.

One ml of the acetone solution is transferred to a 10 ml volumetric flask and evaporated in an air stream. 3 ml of methanol-boron trifluoride reagent is added and the flask held in a boiling water bath for 2 min with frequent swirling. The flask is cooled, 1 ml of hexane is added and solution made up to volume with 2% aqueous sodium sulfate at which time the flask is shaken vigorously for 30 sec. The hexane layer (is) injected for analysis.

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The effects of 2,4-D and related compounds on plants and animals, including information concerning the sensitivity of plants, physiological action of the herbicides, characteristic appearance of affected plants, and methods of determining the herbicide on the plant are presented. 2,4-D being the most characteristic compound of the group is discussed in greater length. It exerts its greatest effect in the rapidly growing and differentiating plant tissues. Cotton is the most sensitive major crop in the Northwest Florida area. One ounce of 2,4-D evenly distributed over 35 acres will seriously injure a cotton crop. For this reason, extreme care should be taken during all herbicide spray operations and especially when such chemicals as 2,4-D, "Silvex" and "Falone" are applied adjacent to cotton fields. The use of mist-blower applicators should be limited to only those cases when complete meteorological data and other information pertinent to drift control are available and indicate absolute safety. Grasses being fairly tolerant to 2,4-D are not injured by a dosage of 1 lb/acre. Tolerance of other crops is given. Six methods are given for the extraction of herbicide from a sample of foliage. The characterization of the compound is then accomplished with a gas chromatograph.

14. KEY WORDS	LINK A ROLE	LINK A WT	LINK B ROLE	LINK B WT	LINK C ROLE	LINK C WT
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