Mechanism, Formulation, and Residues Are All Part of Herbicides Technology

2,4-D
Mechanisms of Action

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Although there exists a considerable body of observations relating to the morphological, physiological, and biochemical effects of 2,4-D on plants, it has not been possible to integrate this information into a unified picture of mechanism of action. The present paper summarizes a portion of this knowledge and discusses the relationship between activity and chemical structure of related growth regulators, of the interactions between 2,4-D and endogenous growth regulators, and of in vivo transformations of 2,4-D which may be expected to contribute to final solution of the problem.

HERBICIDE SYMPOSIUM

THE REMARKABLE INCREASE in the use of 2,4-D (2,4-dichlorophenoxyacetic acid) and related compounds as selective herbicides during the past few years has been based almost wholly on empirical experience. Still further advances are to be anticipated as understanding is gained of the mechanism of herbicidal action, the nature of species and varietal selectivity of response, the specificity of action of diverse compounds, and means of potentiating or suppressing particular biological effects.

The processes of penetration into and movement within the plant undoubtedly play a very important role in these connections. In studies of action mechanisms it has not always been possible to evaluate fully the role of entry and transport, and interpretation of results is frequently complicated thereby.

The mechanism of action of 2,4-D is very incompletely and imperfectly understood. The bits of information now available may be likened to the pieces of a jigsaw puzzle, all of which must ultimately be fitted together to provide a unified picture. There is already a considerable number of such pieces; indeed, it sometimes seems that there are too many, and that a variety of pictures, rather than a single one, are taking shape.

Auxin Action

The basic fact concerning action of 2,4-D is that it closely resembles in many respects the action of the endogenous hormones, generically known as auxins, which presumably regulate plant growth and development. The best known of the auxins is 3-indoleacetic acid. Unfortunately, the term "auxin" has been employed with a variety of meanings; whether or not 2,4-D is designated an auxin is a matter of semantics devolving upon the particular definition which one accepts. Among the developmental processes that can be influenced by application of indoleacetic acid, and in the normal plant may be presumed to be regulated by auxins, are increase in size of cells responsible for polarized growth and growth curvatures, initiation of roots, activation of the cambium, stimulation of callus, correlative inhibition of lateral buds, stimulation of fruit development and ripening, and abscission of organs. All these processes are influenced also by 2,4-D in very much the same fashion as by indoleacetic acid; in these cases 2,4-D behaves as an auxin.

Although the mechanisms of action of 2,4-D in all these processes are of both intrinsic and practical interest, the mechanism of herbicidal action is the primary concern here. Inasmuch as 2,4-D is a powerful herbicide, whereas indoleacetic acid is relatively impotent in this respect, effects of 2,4-D must be sought which are not common to auxins in general. The thesis (*36*) that the difference between 2,4-D and indoleacetic acid is merely quantitative does not appear convincing.

By definition the end result of herbicidal action is death, but death in plants, being a much less dramatic and less easily demonstrable phenomenon than in animals, is not readily amenable to precise, quantitative study. The ability of plants to exist for prolonged periods in a dormant state and their remarkable powers of regeneration often make it impossible to determine whether an individual is dead or alive.

Sequence of 2,4-D Action

In general, two approaches have been employed in efforts to trace the chain of events which leads from application of 2,4-D to death. The first seeks to catalog the abnormalities of treated plants, in the hope of following the cause-and-effect sequence backward in time. The other approach attempts to discover the initial effects of 2,4-D and to trace their consequences.

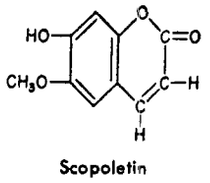
Introduction of 2,4-D into the plant is followed by disturbances of various biochemical, physiological, and developmental processes. Whether any of the hitherto demonstrated derangements are the proximate cause of death is not established. It seems probable that, in many instances at least, death is not directly due to some specific internal cause but rather is brought about by the invasion of saprophytic and parasitic microorganisms, favored by the compositional,

physiological, and morphological abnormalities of the deranged plant. A contributing cause of the debility is very likely the impaired movement of metabolites through the plant due to disruption of the phloem strands by the abnormal proliferation of phloem parenchyma (*5*). Diminished translocation of sugars from the leaves in which they are formed has been noted (*37*). Concurrently, photosynthesis may be decreased and respiration very appreciably increased in treated plants (*29*). All these effects would be expected to favor a diminution in the content of respirable sugars, as has been observed in a number of studies; it seems doubtful, however, that starvation is the ultimate cause of death.

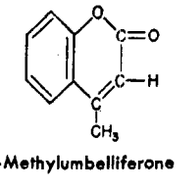
A number of other physiological processes appear to be retarded in 2,4-D treated plants; among these are the uptake of water, of nitrate, and of potassium, and the loss of water by transpiration (*9, 26, 29, 32*). Effects on stomatal movement have been noted and presumably are associated with the diminished transpiration and photosynthesis (*2*).

Treatment with 2,4-D results also in marked alterations in the quantitative chemical composition of the plant with respect to sugars, polysaccharides, amino acids, proteins, and other nitrogenous constituents, lipides, organic acids, vitamins, and various pigments (*1, 9, 11, 19, 29, 30, 43*). The pattern of such changes has not been completely and systematically worked out, however, and appears to vary with the species, the mode of treatment, environmental conditions, etc. To what extent such changes in composition are related to the altered pattern of growth of treated plants and to concomitant effects on translocation, rather than to more specific metabolic disturbances, is not known.

The increase in content of certain unsaturated lactones related to coumarin has been thought by some workers to be of particular importance, inasmuch as several compounds of this class possess high biological toxicity. Scopoletin and β-methylumbelliferone have been mentioned particularly (*10, 36*). However, when applied to plants, neither these compounds nor a variety of other coumarin derivatives are capable of eliciting



Scopoletin



β-Methylumbelliferone

the responses characteristic of 2,4-D (*42*).

Much of the information on chemical composition derives from analyses made after considerable intervals following application of 2,4-D on plants which are grossly abnormal in structure or even moribund. Little success has been had in fitting these observations into a sequential chain and thus far they have made a negligible contribution to understanding of the initial events induced by 2,4-D.

Effect on Enzyme Activity

A very potent drug with widespread effects might reasonably be expected to act through an influence upon activity of some key enzyme system. Careful studies of the influence of 2,4-D on in vivo enzyme activity have been made in very few cases and the findings have not been in agreement. Treated plants exhibit apparently increased activity of phosphatase and pectin methoxylase and decreased phosphorylase (*27, 31*). Catalase, peroxidase, and amyloclastic enzymes may be either increased or decreased according to the tissue investigated and the experimental conditions (*4, 7, 12, 28, 33, 44*). Whether such effects are due to 2,4-D itself or to secondary causes, and whether to effects directly on the enzyme or upon inhibitors or other detrimental factors of the milieu, have not been distinguished in the studies with crude extracts from affected plants.

A number of attempts have been made to distinguish among these possibilities by investigating the action of 2,4-D on more or less purified enzymes in vitro. The results of such studies have usually been less conflicting, principally because they have been largely negative. Concentrations in the range 10^{-4} to 10^{-2} *M* have relatively little effect on amylase, ascorbic acid oxidase, catalase, cytochrome oxidase, glycolic acid oxidase, α-hydroxyacid oxidase, invertase, peroxidase, phenolase, or starch phosphorylase (*8, 15, 23, 38, 42*).

More interesting effects have been reported with two other enzymes, however. One of these is lipase, the activity of which is claimed to be markedly diminished in the presence of 2,4-D (*15*). An intriguing aspect of this study was the finding that lipase from wheat, a species relatively resistant to 2,4-D was much less sensitive than lipase from castor bean, a susceptible species (*20*). Unfortunately, this comparison was made on the basis of separate studies employing different techniques; corroboration is greatly to be desired. The other enzyme, which has been reported to be activated by 2,4-D, is indoleacetic acid oxidase (*13*).

Only a small sampling of the enzymes known to be of importance in plant metabolism has yet been studied and it is to be hoped that many others will be investigated.

The study of influences of 2,4-D on enzyme activity in vitro may be regarded as an example of the second of the two general approaches mentioned earlier. In this case an attempt is made to simplify the system under investigation, recognizing that current knowledge of plant function is still much too superficial to cope with the plant as a whole. Otherwise stated, the philosophy here is to subdivide the whole problem into a series of partial problems.

It seems a reasonable premise that the effects which ensue from application of 2,4-D originate in an interaction between the exogenous reagent and some cellular constituent which plays an important role in metabolism. For convenience this interaction may be termed the "primary reaction."

Structure-Activity Relations

A knowledge of the structural requirements, or architectural specificity, of the exogenous reagent might assist in identifying the cellular constituent with which it reacts. 2,4-D elicits a considerable variety of physiological and developmental end results. It is not certain whether these all arise from a single primary reaction, or master reaction, as it has sometimes been termed, or whether independent primary reactions between 2,4-D and various cellular constituents are involved. It is clear, however, that the structural requirements for activity in certain responses may be quite unlike those for other responses.

In studying the structural requirements for herbicidal action, the death response itself proves rather inconvenient and a much more useful tool is found in the formative effect. This response is manifested in developing leaves as a macroscopically characteristic malformation due to replacement of the normal mesophyll by tightly packed parenchymalike cells; the resulting leaf blade is much narrower and thicker than the normal. The formative response has the advantage of being capable of fairly precise quantitative evaluation (*3*). Survey of a considerable number of compounds has indicated that, on the one hand, there is a high degree of correlation between formative activity and herbicidal activity while, on the other hand, the majority of the compounds which are most active as auxins possess little or no formative activity. With respect to formative activity, 2,4-D does not seem to behave as an auxin.

Although formative activity is known to be exhibited by representatives of a few other classes of compounds, by far the most potent substances are certain relatives of phenoxyacetic acid. This compound is itself inactive but becomes activated by appropriate substitution.

The molecule may be thought of as having four sites of specificity: the ring, the ether oxygen, the methylene group, and the carboxyl group.

Substitution of halogen atoms in suitable positions of the ring confers activity. Ring substituents other than halogen have little or no activating influence. Among the halogens, fluorine and chlorine are about equal in activating effect, bromine is less effective, and iodine is still less so. The positions which lead to highest activity on halogen substitution are 4, 2,4, and 2,3,4.

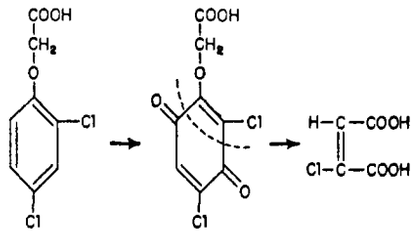
Replacement of the ether oxygen of 2,4-D by nitrogen reduces the activity to about 1%, and replacement by sulfur abolishes it altogether.

Activity is not necessarily diminished, and indeed may be appreciably enhanced, by conversion of the carboxyl group to certain functional derivatives, such as ester, amide, anilide, ureide, hydrazide, nitrile, and acid chloride. Such derivatives presumably may be readily hydrolyzed in vitro with regeneration of the free carboxyl. Other functional derivatives which could be converted to the acid only by oxidative reactions generally exhibit much lower activity. These relations suggest that a free carboxyl group may be required for the primary reaction, a view which seems to find support in recent studies of the optical enantiomorphs of a series of *N*-(2,4-dichlorophenoxyacetyl)-amino acids (*42*). In general the L-, or natural, isomers possess activity of the same order as 2,4-D, whereas the D-isomers have little or no activity; the DL-derivatives are approximately half as active as the L-compounds. This finding may be interpreted as indicating that the amide bond of the natural amino acids is readily hydrolyzed by cellular hydrolytic enzymes, whereas the unnatural forms are much more resistant to attack.

Replacement of one of the methylene hydrogen atoms by a methyl group has virtually no influence on the activity of 2,4-D. Replacement by ethyl or larger groups results in very marked diminution of activity. Replacement of both hydrogen atoms by methyl groups brings about complete inactivation. These facts suggest that the presence of an alpha-hydrogen atom is requisite to activity of the molecule, although it is not excluded that the inactivating influence of a substituent on the methylene carbon may be due to steric hindrance of reaction of the carboxyl group.

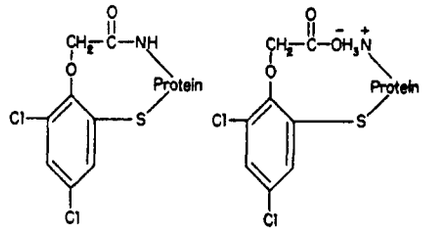
Studies of structure-activity relations of phenoxy acids for other types of biological responses have led to a number of suggestions as to the nature of the primary reaction and it is of interest to examine how far these may be applicable also to the formative response.

Tests of inhibition of growth of lupine roots by a series of mono-, di-, and trichlorophenoxyacetic acids led to the conclusion (*27*) that the active compounds were those in which the ring has two unsubstituted positions para to each other. A suggested explanation of this requirement was that the potentially active compounds may be converted to quinonoid derivatives, which would then either enter into some oxidation-reduction system of the plant with deleterious



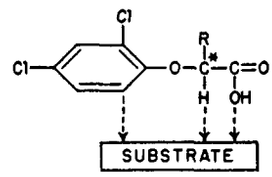
consequence, or be further degraded to chloromaleic acid, this compound then constituting the primary reagent. However, several observations suggest that this hypothesis may not be directly applicable to the formative effect. In the first place, appreciable formative activity is shown by a number of halophenoxyacetic acids which lack unsubstituted para positions. Such compounds include 2,3,4-trichloro-, 3,4,5-trichloro-, 4-chloro-3,5-dimethyl-, 2,3,6-trichloro-, 2,4,6-trichloro-, 2,3-dichloro-, and 2,6-dichlorophenoxyacetic acids (*42*). Furthermore, no formative potency is exhibited by a number of quinones which have been tested; however, the particular quinone derivable from 2,4-D has not been examined. Finally, no formative activity is shown by chloromaleic anhydride or chloromaleic hydrazide; chloromaleic acid itself has not yet been tested.

A second suggestion has been advanced on the basis of observations (*16, 25*) that cell elongation was stimulated by a series of halogenated phenoxyacetic acids in which at least one of the positions ortho to the side chain was open, whereas the auxin activity of those having both ortho positions occupied was nil, or at most very slight (*35*). Two possibilities were suggested to explain the inactivating influence of the substituent in the ortho position: It might block a reaction at the ortho position itself, or it might sterically hinder a reaction of the side chain. Muir and Hansch (*24, 25*) favor the first possibility and advance the hypothesis that a chemical reaction occurs at the ortho position. They believe also that the carboxyl group functions as a second point of attachment. According to this hypothesis, the first step would be formation of either a salt or an amide link with an amino group of a protein. Following such attachment, reaction at the ortho position with another group on the protein—e.g., sulfhydryl—would result in cyclic products such as

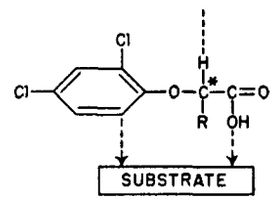


It is doubtful that the proposed mechanism can be applied without modification to the formative response, since, although it is true that the diortho-halogenated phenoxyacetic acids themselves—e.g., 2,4,6-trichlorophenoxyacetic acid—have low formative activity, certain of their functional derivatives possess relatively high activity (*42*).

The apparent necessity of an alpha-hydrogen atom has been mentioned. Replacement of one of the alpha-hydrogens by a methyl group gives rise to an asymmetric carbon in the methylene position. Studies of several series of these isomeric pairs have shown that for various types of response one of the isomers is much more active than the other. This has led to the suggestion that a three-point attachment of the growth regulator is involved (*34*).



Active



Inactive

Such optical enantiomorphs have not yet been evaluated for formative activity.

These hypotheses are very useful in guiding further experimentation, although they cannot yet be said to have provided definitive information on the nature of the primary reaction. The suggestion of a multipoint attachment of reagent to substrate is of considerable interest, however.

In Vivo Fate of 2,4-D

Another approach to the study of the primary reaction is to trace the metabolic fate of the 2,4-D molecule. A beginning has been made in this direction by the use of 2,4-D labeled in various positions with carbon-14 (*6, 18, 39*). It has been found that bean plants are

capable of bringing about extensive transformations of the 2,4-D molecule, although there appears to be a definite limitation of the amount of 2,4-D that can be metabolized by the plant. Within a few days after application of small quantities of 2,4-D to buds, both side-chain carbon atoms can be found distributed among a wide variety of plant fractions, including acids, sugar, dextrins, starch, pectin, protein, and cell wall materials (*41*). As none of these labeled substances has been isolated in a state of chemical purity, it is uncertain to what extent the 2,4-D molecule may have been degraded. In addition to these crude fractions, several other tagged compounds have been isolated in apparently pure form but none of these has been identified as yet. One of these substances is a water-soluble, dialyzable material that is hydrolyzable to an organic acid; Holley (*17*) has suggested that this substance may be a ring-hydroxylated derivative of 2,4-D.

Inasmuch as certain of the tagged transformation products can be demonstrated in the plant within a few hours or even within some minutes after application of 2,4-D (*42*), there is reason to expect that their eventual identification will shed considerable light on the course of the initial transformations.

Relationship between 2,4-D And Endogenous Auxin

Another experimental approach is the study of the relationship of 2,4-D to the endogenous auxin of the plant. A primary question is whether the endogenous auxin content is affected by application of 2,4-D. Inasmuch as 2,4-D is virtually inactive by the classical *Avena* coleoptile test, it is possible to perform a bioassay for the native auxin in the presence of 2,4-D. Such experiments have shown that the auxin content of treated bean buds is very much smaller than that of the normal buds (*22*). The difference is greater the greater the dose of 2,4-D and can be demonstrated at least as early as one or two days after treatment—that is, before there is any detectable macroscopic effect.

Whether the lower auxin content is due to a diminished rate of production or to an augmented rate of destruction has not been ascertained. Inasmuch as the auxin of bean plants may be assumed provisionally to be 3-indoleacetic acid, the reported stimulation of the indoleacetic acid–inactivating enzyme by 2,4-D (*13*) would appear to be a possible mechanism. However, reinvestigation of this phenomenon has indicated that the observed activation of indoleacetic acid oxidase by 2,4-D was due not to the 2,4-D per se but rather to traces of 2,4-dichlorophenol present as impurity (*14*). While this may seem to be a surprisingly fortuitous circumstance, there appears to be no evidence that activation of indoleacetic acid oxidase by 2,4-dichlorophenol has any relation to the action of 2,4-D, although the possibility that 2,4-dichlorophenol is an in vivo metabolic product of 2,4-D is not excluded. At present, the mechanism by which 2,4-D brings about a diminished content of extractable auxin is unknown.

Regardless of the mechanism involved, there is indicated a correlation between the formative response and the lowered auxin content. If the formative response to 2,4-D is caused by diminished auxin, it might be expected to be preventable by artificially increasing the auxin content. This proves to be the case. The formative response to low doses of 2,4-D can be prevented completely by concurrent application to the plant of indoleacetic acid (*40*). A quantitative study of this antagonism suggests that it is of the competitive type and that approximately 80 molecules of indoleacetic acid are required to suppress completely the action of 1 molecule of 2,4-D. The existence of such an antagonism emphasizes the difference in action of 2,4-D and the classical auxin. Indeed, if one wished to use the term, it would be appropriate to speak of 2,4-D as an antiauxin in this case.

The mechanism of the formative effect of 2,4-D thus appears to be intimately related to the mechanism of action of the endogenous auxin. Consideration of the histological basis of the formative response may be informative. One of the striking features of leaf development is the stratified character of the various meristematic layers in which the cell divisions occur predominantly at right angles to the leaf surface and so produce a thin expanded blade. The number of layers in the mature normal leaf is the same as in the embryonic leaf. 2,4-D disrupts this orderly process, causing an early cessation of the anticlinal divisions while permitting the cells to divide periclinally. This results in the much narrower and thicker blade characteristic of the formative response. Antagonism of the 2,4-D effect by indoleacetic acid suggests that endogenous auxin may play a role in maintaining the normal polarity of division, perhaps by inhibiting nonpolarized or random divisions.

Working Hypothesis

It is now possible to assemble some of the above facts into a working hypothesis. According to this hypothesis the development of the growing point into normal leaves and stems depends on polarized division of meristematic cells. Polarity is controlled by endogenous auxin which, acting perhaps as a coenzyme, becomes fixed to one or more cellular entities by a multipoint attachment. Structurally related compounds, such as 2,4-D, compete with auxin for these substrates and bring about a deficiency of the auxin complex which is essential for normal growth. Mild deficiency is manifested as a formative malformation of the leaves, more severe deficiency as proliferation and galling of leaf and stem. The effectiveness of the competition depends in part upon the relative affinities of antagonist and auxin for the substrate and is reflected in the observed structural specificity of herbicidal growth regulators. A consequence of the multipoint attachment is that excessive concentrations of auxin itself also lead to decrease in the auxin complex, since some of the auxin molecules will attach by one reactive site while others attach by another site and mutually prevent each other from completing the requisite multiple unions. The abnormal growth pattern engendered by the unpolarized divisions leads to widespread derangement of metabolic and physiological processes and ultimately to death, in many cases probably because of reduced resistance to disease.

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COMMERCIAL HERBICIDES

Present Methods of Formulation

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Herbicides in wide use today include 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-chloro-o-toloxyacetic acid (MCP), dinitro-o-sec-butylphenol (DNOSBP), and pentachlorophenol. This paper discusses some of the methods used to formulate these chemicals into usable commercial products, as well as problems relating to the choice of amines as solubilizers for 2,4-D and 2,4,5-T formulations. Many types of emulsifiable oil solutions of 2,4-D and 2,4,5-T esters are in commercial use in formulations containing 1 to 4 pounds of the acid equivalent per gallon.

HERBICIDE SYMPOSIUM

IN PROVIDING usable commercial formulations of herbicides, the prime objectives are to put the particular herbicidal chemical into a form handy for the user and his equipment and to supply it in the most effective form, as cheaply as possible.

A formulation is necessary with most herbicides because of the physical nature of the chemical. Some chemicals are water-insoluble, some are oil-insoluble, others are solid lumps. Frequently in their original form they are practically useless to the consumer. For example, a water solution of a chemical simplifies its application when dosages as low as 4 ounces per acre are involved, for it is nearly impossible to distribute 4 ounces of a solid over an acre in a uniform manner. Because herbicides of the plant growth regulator type are effective in such small amounts, their proper formulating is essential to their most effective and economical use.

This paper discusses some of the practices encountered in the formulation of herbicides such as 2,4-D, 2,4,5-T, pentachlorophenol, and the dinitro compounds.

Water-soluble salts of 2,4-dichlorophenoxyacetic acid are currently enjoying wide usage in the United States and elsewhere. Such forms of 2,4-D include the sodium, ammonium, and amine salts. The first two forms find little usage today whereas the amine salts are widely used.

Among the amines that are being used in commercial quantities in 2,4-D herbicides the following can be listed: triethanolamine, diethanolamine, 2-propanol amine, alkanolamine mixtures, dimethylamine, trimethylamine, triethylamine, and isopropylamine.

Current large scale practice with the amine salts of 2,4-D consists of formulating these salts as a water solution containing 4 pounds of 2,4-D acid equivalent per gallon. Lesser amounts are formulated at lower or higher quantities of 2,4-D acid per gallon. Where less than about 2 pounds of 2,4-D acid per gallon is used, it is generally necessary to use an antifreeze in the formulation in addition to the water.

For purposes of discussion a typical amine formulation may be considered to have the following general formula:

2,4-D acid	4 pounds per gallon
Amine	.....
Sequestering agent	.....
Water	To make 1 gallon

Some properties of certain of the amines useful in 2,4-D formulation work are listed in Table I.

As these amine salts of 2,4-D have nearly equal weed-killing properties and all can be used to provide formulations having good storage properties,