6. RECENT ADVANCES IN PHYSIOLOGICAL STUDIES ON HERBICIDES

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There have been about 500 publications in this field during the last 5 years. Accordingly, only some of the more relevant papers can be referred to here, to illustrate principles relating to the absorption and translocation of herbicides, their conversion to more active substances, also their toxicity and selectivity. No reference is made to mode of action, as other speakers are contributing to that subject.

Absorption. Brief reference may be made to spray application, which is usually a preliminary to absorption. The volume of spray solution applied, its surface tension, and the size of spray drops are more important than usually thought. Thus Crafts (1956) cites work demonstrating that a large drop of 2,4-D solution gave greater plant response than smaller drops containing the same total amount of 2,4-D but covering a larger area. Hence for greatest efficiency of 2,4-D we should use coarse drops rather than fine drops. Blackman et al (1958) investigated 5 species with markedly different morphological characteristics. Differences in spray retention could be large or small depending on growth stage, volume of spray applied, also its surface tension. With 2,4-D and related poisons the total spray deposit was of primary importance in determining subsequent growth inhibition, though the positioning of the droplets was also important.

Mueller et al (1954) have studied leaf surfaces with electronmicrographs. They consider that surface wax deposits are capable of suspending small droplets of water without wetting agent so as to prevent the droplets contacting the cuticle. On the other hand they consider it doubtful if the wax deposits inhibit the action of herbicidal sprays containing appropriate wetting agents. Nevertheless the concentration of wetting agent used may not be high enough for optimal results. Thus Leonard (1958) working with 1000 p.p.m. of 2,4-D on bean plants, found

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that its effectiveness increased progressively as the concentration of Tween 20 was increased from 125 to 32,000 p.p.m.

It is generally considered that foliar penetration is better effected by molecules than by ions, and that the best penetration of weak acids such as 2,4-D is obtained at lower pH values. However the pH value of the spray solution should not be much below 3, otherwise local leaf injury impairs efficiency. Nevertheless Orgell and Weintraub (1957) have shown that in the presence of certain cations typical of weak bases (e.g. ammonium, triethanolammonium), good penetration of 2,4-D can be obtained at neutral or alkaline pH values. This finding could be important in formulation.

It is frequently claimed that leaf penetration is largely or entirely through the stomata. Nevertheless Weintraub et al (1954) claim that in intact leaves of Senecio, 2,4-D can penetrate as rapidly through the stomata-free cuticle as through the stomata-bearing cuticle. In beans, each leaf appears to pass during its expansion through a relatively brief stage of high absorbability, then the absorbability falls markedly and remains constant until the leaf yellows, when it drops further. Pretreatment of the plant may also be important as influencing cuticular penetration. Dewey et al (1957) claim that soil particles, blown about by wind or storms, can damage the cuticle to such extent that peas become susceptible to the normally safe dinoseb. Chemical pretreatment via the roots can also affect the cuticle. Thus peas planted on soil previously treated with TCA or dalapon also become susceptible to dinoseb.

Leaf penetration is largely dependent on the chemical structure of the herbicide. Davis et al (1959) have shown that almost no absorption of simazin takes place through intact leaves. For plants to take up such herbicides it is necessary to ensure that the leaves are damaged, or to apply the herbicides to the soil. Some substances normally too dangerous for leaf application on account of volatility can also be applied to the soil; thus Greenham and Robertson (unpublished data) have in pot experiments obtained complete control of the top growth of nutgrass for over three months with the methyl ester of 2,4-D applied to the soil.

It appears that with soil application, many more substances will enter the plant than by leaf absorption. Selectivity in soil application can depend on the use of innately selective herbicides, on time of application in relation to sowing, or on the positioning of the crop seed or plants. Ripper (1956) has extended the principle of positioning to covering the crop seed with a layer of material which adsorbs the herbicide. In the absence of such protection, leaching may result in the herbicide reaching and damaging deeply sown seeds. Ogle and Warren (1954) report that the readiness of leaching cannot be predicted from the solubility and molecular size of a herbicide. Nevertheless formulation or the use of additives can prevent or minimise leaching. Aldrich and Willard (1952) found that an ester of 2,4-D was not nearly as readily leached as the amine or sodium salt, and could accordingly be used for weed control in maize. When esterification is too expensive, as with NPA (naphthylphthalamic acid), other methods are available to minimise leaching, such as formulation with certain electrolytes or with water-soluble organic acids (Smith et al 1957).

Translocation. Radioactivity-tagged compounds are being used extensively in translocation studies, though caution must be used in critically interpreting the results. Thus when side-chain labelled 2,4-D is used, some of the radioactive and end-products could result from the radioactive carbon dioxide which is liberated. Moreover, as shown below, the unaltered compound is not invariably the toxic substance.

The generally accepted view is that upward translocation is in the xylem, and downward translocation is usually in the phloem; and in the phloem is invariably associated with the movement of assimilates. Some types of leaf injury result in downward movement in the xylem, and certainly the view is strongly expressed that downward movement in the phloem is lessened by local injury, which may result from too high a concentration of the herbicide. The most efficient phloem translocation results when plants are growing vigorously, also in the absence of phosphorus and potassium deficiencies (Rohbaugh and Rice, 1956).

To reach the phloem it is often necessary for a herbicide to enter and move through protoplasts. Crafts and Yamaguchi (1958) point out that low mobility may result from active accumulation by living cells, while high mobility may result from lack of active accumulation or metabolism at the point of application. Whereas 2,4-D may be restricted in movement by accumulation in living cells, amitrol moves relatively freely in the phloem, though it may leak into the system to a limited extent; maleic hydrazide moves freely in the phloem, leaks into the xylem, and circulates in the plant; on the other hand monuron moves from the point of application only to the leaf tip or edges via the xylem. Crafts (1959) has also shown similar relative mobility of herbicides holds in potato tuber parenchyma, which is devoid of specialised tissues for translocation. Comparing mobilities, 2,4-D $<$ IAA $<$ amitrol $<$ maleic hydrazide; monuron seemed to move only in the cell walls and concentrated around the edges of the slice. These differences in behaviour show how chemical structure influences mobility in the plant.

Leonard and Crafts (1956), studying a number of woody species, found that the most appropriate time for applying 2,4-D varied with the species. They also concluded that for successful results, at least four physiological factors must be favourable: (a) the herbicide must be absorbed, (b) photosynthesis must provide active assimilates for movement through the phloem, (c) translocation from actively photosynthesising leaves to the roots must be going on, and (d) root activity must involve meristematic activity and growth, not just storage. From these conclusions it appears that when poisoning green timber which is liable to sucker, one should not girdle the trunk completely before applying a poison to the axe cuts: one should space the axe cuts around the trunks, and possibly use more poison, of a type that is readily translocated.

In further studies with labelled herbicides, Crafts (1959) has shown how application of 2,4-D to the older leaves of barley results in movement to the roots, whereas application to the younger leaves results in little or no such movement. Apparently, as young barley plants become older, more and more of the assimilates are utilised in the production of new leaves and the inflorescence. However, assimilate movement from the healthy basal leaves to the roots still takes place, but at a slow rate. The associated movement of 2,4-D emphasises the importance of the source-sink relationship. As a generalisation for mature plants, including many woody species, application to the lower leaves results in movement to the roots, whereas application to the top leaves results in little or no such movement but mainly top kill.

Leonard (1958) has found that there is an inverse correlation between translocation and degree of local injury when various diluents are used for the one 2,4-D ester. He has also demonstrated that boron, originally thought to be important in translocation, had no beneficial effect on bean plants. Possibly gibberellic acid or a related compound may be used to improve translocation. Thus Ashton (1959) has obtained greater translocation of 2,4-D in bean plants pretreated with 100 p.p.m. of gibberellic acid.

Conversion in the plant to more active substances. Not infrequently the applied compound is converted to another substance before toxicity results. Thus Crafts (1960), using the labelled iso-propyl ester of 2,4-D, has shown that in barley leaves the ester is hydrolysed to the alcohol and 2,4-D. The alcohol portion moves upward, presumably in the xylem, whereas the 2,4-D moves downward in the phloem, though some is bound in active tissues. He concludes that the light alkyl esters rapidly penetrate into a leaf and decompose to cause marked local injury, which lessens the translocation of the liberated 2,4-D. Too slow penetration, as with glycol esters, results in rapid recovery of treated plants. Slow ordered penetration, as with the butoxyethanol ester or propyleneglycol-butyl ether ester (or even finely suspended 2,4-D as the acid form) results in optimum translocation and kill.

The basis of the selective toxicity of phenoxybutyric acids has been shown to depend very largely on their selective conversion to the corresponding phenoxyacetic acids by a process known as β -oxidation. Fawcett et al (1959) have made a detailed study of phenoxyalkane-carboxylic acids with varying lengths of side chain. They found that for both wheat and pea tissues, β -oxidation of the side chain with an even number of methylene groups is hindered at the propionic stage (2 methylene groups); this may result in an unusual pattern of biological activity in series where the propionic derivative contains growth-regulating properties in its own right. The breakdown of homologues with an odd number of methylene groups often results in the formation of the corresponding acetic derivative, which can be toxic; the formation of the acetic derivative happened with all compounds studied in the wheat series, however, with more than half of the compounds studied in the pea and tomato series, there is evidence that the β -oxidation is hindered at the butyric stage, resulting in no biological activity. Hindrance of β -oxidation at the butyric stage is associated with an ortho chlorine or methyl group, though the effect is largely removed by introducing a further chlorine at the para position. Nevertheless trisubstituted compounds are in a different category, and of these only the acetic acid derivative is active on peas and tomatoes. Accordingly, by selecting the appropriate side chain and ring substitution, a high degree of selectivity can be obtained.

The basis of the selective action of 2,4-dichlorophenoxy ethylamine (Ferenczy 1959) and of 2,6-dichlorobenzonitrile (Coopman and Dams 1960) may well be based on the ability or inability of plants to oxidise these substances to the corresponding carboxylic acid. Certainly Fawcett et al (1958) have proved that some plants convert -CN to -COOH more readily than others.

It is by no means invariable that herbicides are converted inside plants into more toxic substances. Thus as mentioned below, simazin can be detoxified in maize.

Toxicity and Selectivity. Toxicity is the manifestation of an interruption of some vital process, frequently an enzymatic process. The ability of a herbicide to interfere with a vital process depends on its chemical structure, often on its stereochemistry. The "lock-and key" theory or modifications of it (such as "points of attachment"), are frequently used to explain the specificity of action of a given compound.

The most detailed studies of the relationship between structure and biological activity have been made in the phenoxyacetic acid series, and benzoic acid series and the phenylacetic acid series (Pybus et al 1959a, 1959b), though the studies have been made in terms of growth activity not toxicity. However, a theory explaining activity in one series does not necessarily explain activity within another. Thus, 3,4-dichlorophenoxyacetic acid is active whereas 3,4-dichlorobenzoric acid is inactive. It may be that different mechanisms of action are involved. Doubtless further discrepancies in theories relating activity to structure would be demonstrated if studies were made in toxicity rather than in growth activity. Apparatus for determining toxicity in terms of injury is now available (de Plater and Greenham, 1960).

Recently, interest has developed in mixtures of herbicides, either with a view to killing more than one weed species with the one application, or with a view to obtaining enhanced toxicity (synergism). Strictly, synergism occurs only if the resulting kill is greater than the expected cumulative kill of the two poisons. Thus if poison A at a certain concentration kills 0.8 of the population of one species, and poison B at a certain concentration kills 0.5 of the population of the same species, the expected cumulative kill of A and B together would be

1- (1-0.8) x (1- 0.5), viz. 0.9 or 90% of the population.

If in this instance, the kill is significantly more than 90%, there is synergism, if the kill is significantly less, there is antagonism.

In some instances antagonism results from competition for a site of activity. Synergism can result from competition for a site of inactivity. Thus Veldstra and Booij (1949) suggest that only a fraction of the active substance is required for the primary action, and that the greater part of it is adsorbed elsewhere and is wasted. A synergistic substance with little activity in its own right can prevent the wasteful adsorption of the active substance, which becomes more concentrated and hence more active at its site of action. Synergism can also result from the increased injury resulting from two poisons acting at different sites.

A theory somewhat reminiscent of that of Veldstra and Booij has been proposed by Brian (1958) to explain the basis of resistance to herbicides. Finding a broad correlation between the ability of a plant to resist MCPA and the ability of a monolayer of an extract of that plant to adsorb MCPA, he suggests that the basis of resistance lies in the ability of the plant's tissues to adsorb the poison before it reaches the site of action.

Other mechanisms can be suggested for the basis of resistance to herbicides. Weintraub et al (1956) found that less 2,4-D moved out from the leaf of a resistant variety of maize than from that of a susceptible variety. Again, a species may be resistant because it has the ability to decompose the herbicide before it reaches its site of action. Thus maize is resistant to simazin, whereas wheat is susceptible (Roth, 1957). Yet isolated chloroplasts of maize are typically inhibited in the Hill reaction by the presence of simazin (Moreland et al, 1959). Roth has shown that maize sap contains a heat-sensitive substance which destroys simazin, but that wheat sap does not. Accordingly he suggests that resistance to simazin is based on the ability of the plant to detoxify it. Analogues of simazin have different selectivity, which may depend largely on the ability or inability of enzymes to detoxify them.

As more becomes known about the physiology of herbicides, so will become increased the number of crops in which selective weed control is feasible.

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