

Herbicidal Selectivity through Specific Action of Plants on Compounds Applied

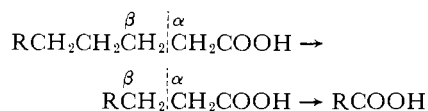
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Reviewing the evidence at present available, it would seem that the principle underlying the use of γ -(2-methyl-4-chlorophenoxy)butyric acid, γ -(2,4-dichlorophenoxy)butyric acid, and other aryloxybutyric, caproic, and octanoic acids represents a new advance in the field of selective weed control, a development which well illustrates the value of fundamental investigations in furthering the progress of applied research.

CHEMICAL ASPECTS of plant growth-regulating activity have received considerable attention here in recent years (7). One line of investigation has been concerned with the breakdown of ω -phenoxyalkylcarboxylic acids within plant tissues, as Synerholm and Zimmerman (4) have suggested that the aliphatic side chain in such compounds might be degraded within the tomato plant by a β -oxidation mechanism. This suggestion arose from their finding that in the first seven members of a series of 2,4-D homologs, $\text{Cl}_2\text{C}_6\text{H}_3\text{O}(\text{CH}_2)_n\text{COOH}$, an alternation in activity was exhibited in the tomato leaf epinasty test, the acetic, butyric, caproic, and octanoic derivatives ($n = 1, 3, 5, 7$) being active and the propionic, valeric, and heptanoic acids ($n = 2, 4, 6$), inactive [see also Grace (2)]. Such alternation is fully consistent with β -oxidation of the side chain leading to the production of either 2,4-D [(2,4-dichlorophenoxy)acetic acid] or 2,4-dichlorophenol, depending on whether an odd or an even number of methylene groups is present (4).

It is now 50 years since Knoop, as a result of classical experiments in which he fed ω -phenylalkylcarboxylic acids to dogs, put forward the suggestion that fatty acids can be oxidized in the β -position to the carboxyl group (3).



Since that time, overwhelming evidence has been obtained supporting the idea that β -oxidation is a means by which these acids can be metabolized in the animal body, and a number of enzymes, which can act only on coenzyme A derivatives of these acids, are now thought to be involved (5).

Although so much attention has been given to studies on β -oxidation in the animal, it is only within recent years that evidence has appeared indicating that this type of oxidative breakdown may operate in plants. The biological evidence of Synerholm and Zimmerman has been supported by the results of investigation carried out in the writer's laboratory, in which a homologous series of ten ω -phenoxyalkylcarboxylic acids, $\text{C}_6\text{H}_5\text{O}(\text{CH}_2)_n\text{COOH}$, was supplied to flax seedlings through their roots (7). The results were explicable on the β -oxidation hypothesis, for it was shown that appreciable quantities of phenol, which was estimated colorimetrically, were produced only in those plants treated with acids containing an even number of side chain methylene groups ($n = 2, 4, 6, 8, 10$). In addition to this

chemical evidence, studies on the plant growth-regulating activity of a number of substituted ω -phenoxyalkylcarboxylic acids has provided further support for the idea that β -oxidation can occur within plant tissues (9).

In assessing the growth-regulating activity of new compounds, the author has always considered it necessary to use a number of tests, each employing a different type of plant tissue (7); in investigating these homologous series of phenoxy acids, each compound was examined in the wheat cylinder elongation test, the pea curvature test, and the tomato leaf epinasty test (9). Not less than the first six members of each homologous series of substituted ω -phenoxyalkylcarboxylic acids were synthesized for these investigations. In most cases—e.g., with the 4-chloro-, 2,4-dichloro-, 2-methyl-4-chloro-, 3,4-dichloro-, and 3-methyl-4-chloro- derivatives—the alternation in activity expected from β -oxidation considerations was exhibited in all three tests. However, in other series—e.g., the 2,4,5-trichloro- and the 2,4-dichloro-5-methyl—although alternation was shown in the wheat cylinder test, all homologs higher than the acetic derivative were inactive in the pea curvature and tomato epinasty tests. Synerholm and Zimmerman, using the tomato leaf epinasty test, had previously reported

Table I. Activity of Substituted ω -Phenoxyalkylcarboxylic Acids in Three Tests

Derivative	No. of Methylene Groups in Side Chain	4-Chloro- Series			2,4,5-Trichloro- Series		
		Cylinder test	Curvature test	Epinasty test	Cylinder test	Curvature test	Epinasty test
Acetic	1	+	+	+	+	+	+
Propionic	2	—	—	—	—	—	—
Butyric	3	+	+	+	+	—	—
Valeric	4	—	—	—	—	—	—
Caproic	5	+	+	+	+	—	—
Heptanoic	6	—	—	—	—	—	—
Octanoic	7	+	+	+	+	—	—

+ Active.
— Inactive.

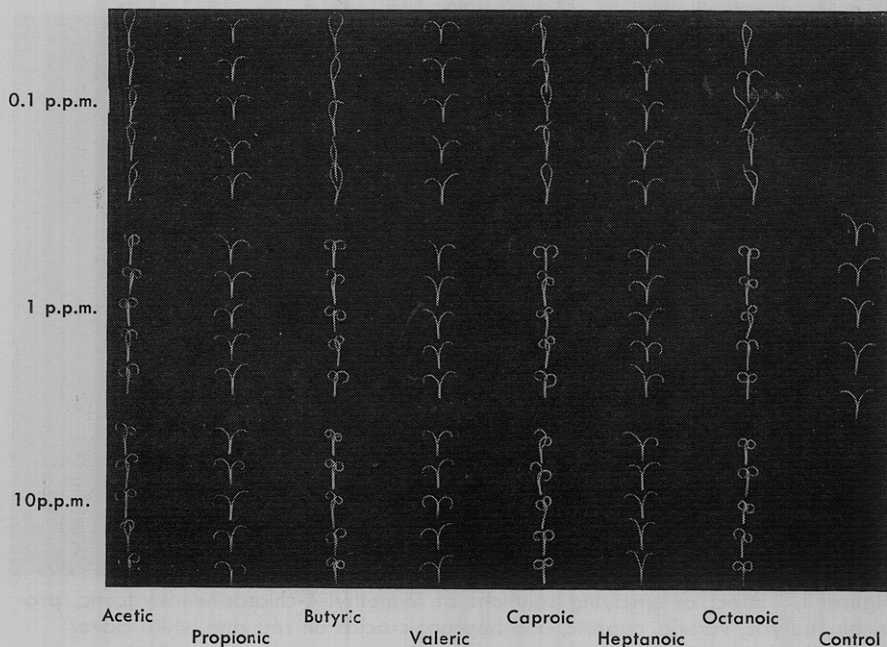


Figure 1. Pea curvature test

Responses obtained by treatment with ω -(4-chlorophenoxy)alkylcarboxylic acids. Water controls indicated at right

γ - (2, 4, 5-trichlorophenoxy) butyric acid to be inactive as a growth substance (4).

The two types of behavior are shown with reference to the 4-chloro- and 2,4,5-trichloro- series in Table I and in Figures 1 and 2.

As alternation is shown with the 4-chloro- acids in all three tests, it is reasonable to suppose that a β -oxidizing enzyme system is present in all these three types of plant tissue. It would appear, however, that the enzymes present in pea and tomato tissue cannot operate when the 2,4,5-trichlorophenyl ring system is present, although those present in wheat tissue can operate. Evidence of this was obtained by direct experiment. Solutions of the 2,4,5-trichloro- homologs were exposed to wheat cylinder tissue and then examined in the pea curvature test. They now showed the typical alternation in activity, indicating that the enzyme system present in the wheat tissue had degraded alternate homologs to the acetic derivative, active in the pea test (9). Further evidence that the enzymes in the wheat tissue were effecting a β -oxidation of the side chain was obtained by treating wheat coleoptile tissue with solutions of γ -(2, 4, 5 - trichlorophenoxy) butyric acid and demonstrating, by chromatographic methods, that conversion to the corresponding acetic acid had taken place (9). Results obtained with a homologous series of ω -(1-naphthyl)- acids and studies on the effect on activity of substituting alkyl groups in the side chain of some of these compounds were also found to be explicable on the β -oxidation hypothesis. These investigations have been discussed (9). The important point to consider here is the indication from the

experiments outlined above, that specific β -oxidase enzyme systems capable of degrading the side chain of a particular aryloxy acid may be present in the tissues of one species of plant and not in another.

To the writer, this seemed of far-reaching importance, for clearly, if the response to treatment of intact plants was dependent on such factors, here was a logical approach to selective weed control based upon the plant's own enzyme make-up. In other words, one species of plant when treated with an aryloxy-butyric, caproic, or octanoic acid might

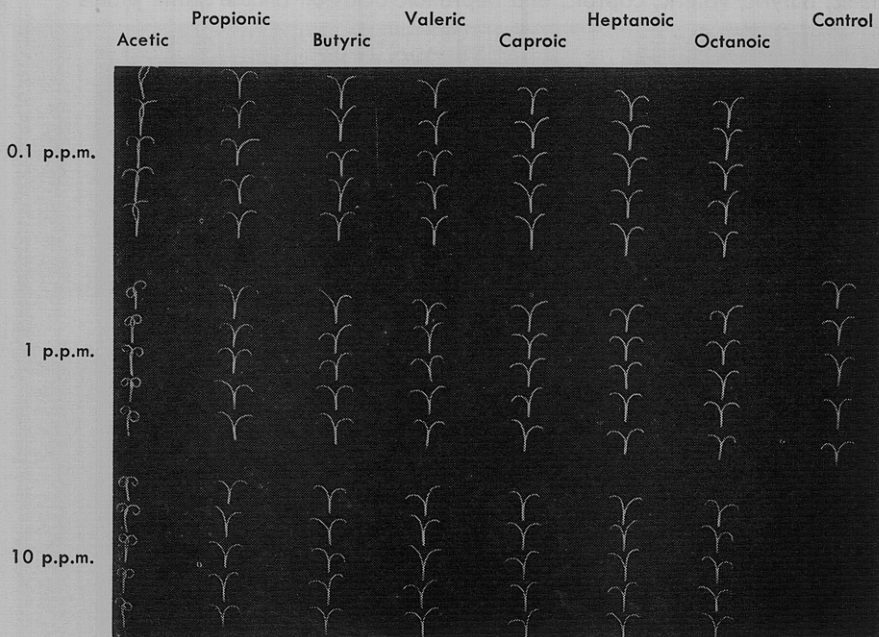
be expected to degrade the compound to the highly active acetic derivative and thus succumb to herbicidal action. Another plant species not possessing the specific enzymes necessary to effect the breakdown of these particular aryloxy acids, would remain unharmed. Such selective activity could be independent of differences in morphology and habit of growth between crop and weed, factors upon which the selective herbicidal action of sulfuric acid, dinitro compounds, and copper salts is largely based.

Experiments designed to investigate the above possibilities have been carried out this season and some striking results have been obtained (6). Thus, when certain plant species—e.g., annual nettle (*Urtica urens*) or creeping thistle (*Cirsium arvense*)—are sprayed with solutions of ω -(2-methyl-4-chlorophenoxy)alkylcarboxylic acids, the plants treated with the acetic derivative and its alternate homologs are destroyed, whereas the plants receiving the other members of the series remain unharmed (Figure 3). On the other hand, when celery or clover plants are so treated, only the acetic derivative is active (Figure 4). Such results are fully consistent with the concept of β -oxidation and indicate the substrate specificity of the enzymes involved.

It has also been shown this season that various important weeds in addition to the two named above—e.g., fumitory (*Fumaria officinalis*), fat hen (*Polygonum aviculare*), and charlock (*Sinapsis arvensis*)—can be destroyed by one application of these new materials at rates as low as 2 pounds per acre, whereas a number of crop plants are but little affected by such treatment. The most promising compounds so far investigated are γ -(2-

Figure 2. Pea curvature test

Responses obtained by treatment with ω -(2,4,5-trichlorophenoxy)alkylcarboxylic acids. Water controls at right



methyl-4-chlorophenoxy) butyric acid (MCPB) and γ -(2,4-dichlorophenoxy)-butyric acid (2,4-DB), both of which control the above and other weeds. The corresponding 2,4,5-trichloro- derivative, although safe to use, for example, on peas, is less effective as a weed killer than γ -(2-methyl-4-chlorophenoxy)butyric acid and γ -(2,4-dichlorophenoxy)butyric acid. Other compounds studied this season include the 4-chloro-, 3,4-dichloro-, 2,5-dichloro-, 2,3,4-trichloro-, and 3-methyl-4-chloro- derivatives of γ -phenoxybutyric acid. Preliminary results obtained by Frank Wightman on potted plants, usually 2 to 4 inches high, sprayed with 0.1% solutions of these compounds are available (8). A number of small scale field experiments have also been carried out this season. In one such experiment, a striking control of annual nettle growing as the dominant weed with celery was obtained by one application of γ -(2-methyl-4-chlorophenoxy)butyric acid or γ -(2,4-dichlorophenoxy)butyric acid at 2 pounds per acre. No damage to the crop occurred, though (4-chloro-*o*-tolylxy) acetic acid and 2,4-D applied at a corresponding rate killed the plants within 3 weeks.

One of the most important properties of γ -(2-methyl-4-chlorophenoxy)butyric acid and γ -(2,4-dichlorophenoxy)butyric acid from the agricultural viewpoint is that they may be used with safety on red and white clover. A number of noxious weeds can be controlled with these compounds with practically no effect on this valuable legume crop. The acetic derivatives, on the other hand, applied at the same rate produced disastrous effects.

Other crop plants with which γ -(2-methyl-4-chlorophenoxy) butyric acid might be used include carrot, parsnip, and flax. Some damage may arise in



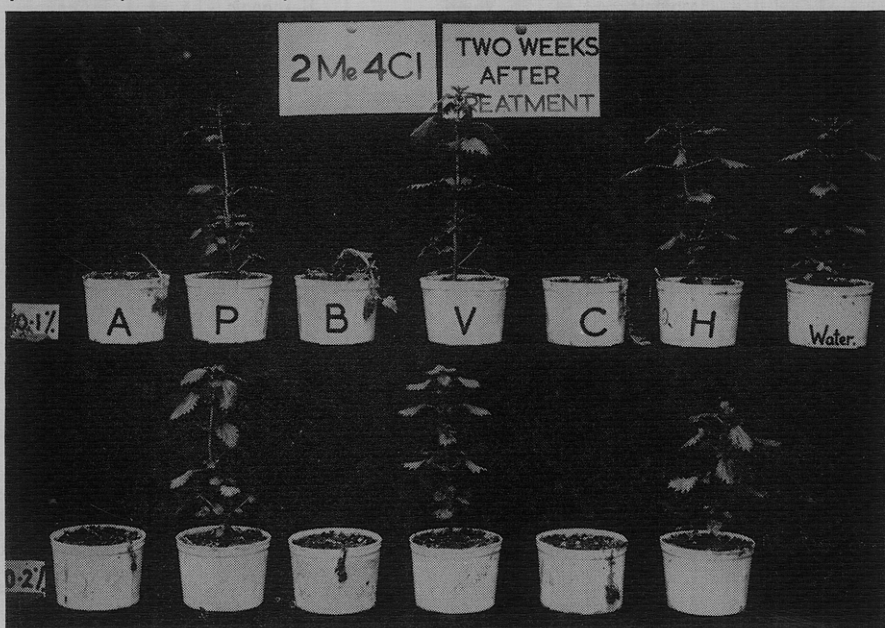
Figure 4. Effect of spraying solutions of 2-methyl-4-chlorophenoxyacetic, propionic, butyric, valeric, caproic, and heptanoic acids on red and white clover

these crops following treatment, but this is slight compared with that arising from (4-chloro-*o*-tolylxy)acetic acid or 2,4-D. γ -(2,4-Dichlorophenoxy)butyric acid also produces more damage with these crops than γ -(2-methyl-4-chlorophenoxy)butyric acid. On the other hand, with alfalfa, γ -(2,4-dichlorophenoxy)butyric acid appears to be much safer to use than γ -(2-methyl-4-chlorophenoxy) butyric acid, which may cause severe damage.

The above observations are based on only a small number of experiments. Many crop plants and weeds have not yet been investigated, and much remains to be determined in other cases regarding performance at different stages of growth. These compounds do not control as wide a range of weeds as (4-chloro-*o*-tolylxy)acetic acid and 2,4-D,

for all the author's work indicates that their herbicidal action is due to the production of these acetic acids, which in turn is assumed to depend on the presence of a specific enzyme in the tissues of the plant. The question also arises whether γ -(2-methyl-4-chlorophenoxy)-butyric acid or γ -(2,4-dichlorophenoxy)-butyric acid reaching the soil might be converted there by the activities of soil microorganisms to the corresponding acetic derivative and be absorbed as such through the roots of the crop plants. Although soil perfusion experiments indicate that side chain degradation of these compounds can occur in contact with soil, with low volume applications, little damage from this cause has been found in the field.

Figure 3. Effect of spraying solutions of 2-methyl-4-chlorophenoxyacetic, propionic, butyric, valeric, caproic, and heptanoic acids on annual nettle plants



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