A Survey of Herbicides for their Effect upon Protein Synthesis[1, 2, 3]

Jay D. Mann, Lowell S. Jordan, and Boysie E. Day
Department of Horticultural Science, University of California, Riverside

The means by which many herbicides kill susceptible plants are presently unknown (1, 3). Although it is not essential to know the mode of action in order to develop and use a herbicide efficiently, the screening processes by which herbicides are discovered are based upon comparative biochemistry. Selective herbicides with low mammalian toxicity (2) must take advantage of differences in metabolism between plants and animals, and between plant species, just as therapeutically effective antibiotics take advantage of differences between bacterial and mammalian biochemistry. Knowledge of herbicide effects may prove useful for other areas within plant physiology.

A screening technique has been used in the present study to determine the effect of various herbicides on the incorporation of leucine into protein. The results show differences between those herbicides which, more or less directly, inhibit protein synthesis, and those which act upon quite unrelated pathways. This approach is designed to indicate the areas that should be studied more intensively for each herbicide.

Methods and Materials

The general technique has been reported previously (4). In brief, seedlings of Betzes barley and of *Sesbania exaltata* were grown in the dark for 3 to 4 days. Ten 1-cm segments of barley coleoptile (with primary leaf inside) or of *Sesbania* hypocotyls were preincubated for 1 hour in darkness in 1 % sucrose containing 2 and 5 ppm concentrations of each herbicide (stock solutions of herbicides were prepared in 8 % ethanol at 100 ppm). L-Leucine-1-C^{14} (0.1 μc/vial giving 125,000 cpm) was added, and incubation was continued for an additional 2 hours. The tissue was then extracted twice with hot 80 % (v/v) ethanol, and residual C^{14} was assayed by liquid scintillation counting. Previous studies (4) have shown that at least 84 % of this radioactivity can be extracted by subsequent incubation with proteolytic enzymes.

Controls included tissue incubated with sucrose and buffers in the absence of herbicide, and tissue incubated with a volume of 8 % (v/v) ethanol equal to that added in herbicide-containing vials. Preliminary tests showed that incorporation of leucine into protein was much more sensitive to ethanol than was methionine incorporation.

Uptake of amino acid was measured with DL-α-amino-*n*-butyric acid-3-C^{14}. Tissue segments were preincubated for 1 hour in sucrose-phosphate buffer containing herbicides. Then 0.1 μc of this amino acid, which is not used for protein synthesis, was added. An hour later, the tissue was washed once with distilled water for 15 minutes, and then added to a water-dioxane liquid phosphor.

Common and chemical names of the herbicides tested are given in table I.

Results

Table II shows the percentage inhibition of protein synthesis by the herbicides tested on both barley coleoptiles and stems of *Sesbania* (a small-seeded legume). Out of 23 herbicides, only 5 caused severe inhibition of leucine incorporation into protein. A number of herbicides inhibited this process to a

Table I. *Common and Chemical Names of Herbicides Tested*

Common name	Chemical name
Amiben	3-Amino-2,5-dichlorobenzoic acid
Amitrole	3-Amino-1,2,4-triazole
Atrazine	2,Chloro-4-ethylamino-6-isopropylamino-*s*-triazine
CDAA	2-Chloro-*N*,*N*-diallylacetamide
CDEC	2-Chloroallyl diethyldithiocarbamate
CIPC	Isopropyl *N*-(3-chlorophenyl) carbamate
Dacthal	Dimethyl-tetrachloroterephthalic acid
Dalapon	2,2-Dichloropropionic acid
DATC	2,3-Dichloroallyl diisopropylthiolcarbamate
2,4-D	2,4-Dichlorophenoxyacetic acid
Dichlobenil	2,6-Dichlorobenzonitrile
Diphenamide	*N*,*N*-Dimethyl-2,2-diphenylacetamide
Endothal	3,6-Endoxohexahydrophthalic acid
EPTC	Ethyl *N*,*N*-di-*N*-propylthiolcarbamate
Hadacidin	*N*-Hydroxy-*N*-formyl sodium glycinate
Ioxynil	3,5-Diiodo-4-hydroxybenzonitrile
Monuron	3-(*p*-chlorophenyl) 1,1-dimethylurea
NPA	*N*-1-naphthylphthalamic acid
PCP	Pentachlorophenol
Propanil	3,4-Dichloropropionanilide
Pyrazon	5-Amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Trifluralin	2,6-Dinitro-*N*,*N*-di-*n*-propyl-a,a,a-trifluoro-*p*-toluidine

[1] Received February 23, 1965.
[2] Paper No. 1612, University of California Citrus Research Center and Agricultural Experiment Station, Riverside, California.
[3] Supported in part by Public Health Service Grant GM12664-01.

Table II. *Inhibition by Various Herbicides of Leucine-1-C^{14} Incorporation into Protein*

Herbicide	Barley			*Sesbania*		
	2 ppm	5 ppm	Importance of inhibition	2 ppm	5 ppm	Importance of inhibition
None	0*	0*		0**	0**	
Ethanol	−1	8		−6	23	
Amiben	−20	...	−	4	22	−
Amitrole	−13	−4	−	19	33	−
Atrazine	7	11	−	37	32	−
CDAA	51	70***	+	58	87***	+
CDEC	25	29	−	7	0	−
CIPC	26	84	+	32	98	+
Dacthal	18	34	−	15	21	−
Dalapon	7	8	−	4	8	−
DATC	34	18***	−	22	8***	
2,4-D	15	...	−	4	...	−
Dichlobenil	26	0	−	24	14	−
Diphenamid	21	27	−	17	22	−
Endothal	21	24	−	63	72	+
EPTC	38	22	−	14	11	−
Hadacidin	12	15	−	18	1	−
Ioxynil	44	70	+	82	88	+
Maleic hydrazide	6	−1	−	0	−3	−
Monuron	32	19	−	24	24	−
NPA	−20	...	−	20	12	−
PCP	13	62	+	42	65	+
Propanil	...	8	−	−7	14	−
Pyrazon	25	29	−	35	26	−
Trifluralin	8	3	−	13	29	−

* Control values of protein synthesis by barley ranged, in 6 experiments, from 4405 to 5785 cpm, with a mean of 4850 cpm; total uptake of leucine was approximately 4-fold greater than incorporation into protein.

** Control values of protein synthesis by *Sesbania* ranged, in 5 experiments, from 17,785 to 24,150 cpm, with a mean of 19,980 cpm, compared with total leucine uptake of 80,000 cpm.

*** For reasons of solubility, 4 ppm rather than 5 ppm used.

moderate extent at 2 ppm, but further inhibition did not occur at 5 ppm.

Maleic hydrazide and dalapon are used in field work at high rates. These were tested, in experiments not reported here, at 100 ppm, again with no more meaningful effect upon leucine incorporation than at 5 ppm.

A detailed comparison of ioxynil effects upon barley and *Sesbania* is presented in table III. The dicotyledonous plant was at least 5 times more sensitive to ioxynil than was the cereal.

Various herbicides were tested for their effects upon uptake of α-amino-*n*-butyric acid (table IV).

Table III. *Effect of Different Ioxynil Levels upon Leucine Incorporation by Barley Coleoptiles and* Sesbania *Hypocotyls*

Conc (M)	% Inhibition	
	Barley	*Sesbania*
1×10^{-6}	9	50
2×10^{-6}	8	74
5×10^{-6}	44	82
1×10^{-5}	62	83
2×10^{-5}	73	88

Inhibition of uptake due to CIPC, CDAA, and endothal (with *Sesbania*) was much less than the inhibition of amino acid incorporation caused by these compounds. On the other hand, ioxynil and PCP inhibited both amino acid uptake and amino acid incorporation.

Since the assay with α-amino-*n*-butyric acid measured the ability of tissue both to concentrate this amino acid from a dilute solution, and to retain the amino acid during a 15-minute wash in distilled water, the leaching rate was studied separately. *Sesbania* stem segments were incubated with α-amino-*n*-butyric acid-3-C^{14} for 1 hour in the presence and in the absence of 2×10^{-6} M ioxynil. Replicate samples were taken after 1, 5, and 15 minutes of washing in distilled water. Control samples lost 16 % of their C^{14} content between minutes 1 and 15 (32,731 cpm per 10 segments decreasing to 27,379 cpm). Ioxynil-treated samples lost 23 % of their C^{14} under the same conditions (6151 cpm decreasing to 4734 cpm). Thus, inhibition of uptake during incubation with amino acid, rather than excessive loss of amino acid during washing, was responsible for the inhibitions shown in table IV.

Table IV. *Effect of Various Herbicides upon Uptake of* DL-α-*Amino-n-Butyric Acid-1-*C^{14}

Uptake of the amino acids (0.1 μcurie; 125,000 cpm) was assessed after a 1-hour incubation, following 1-hour preincubation with herbicides. Values in parentheses give the percentages inhibition of protein synthesis, repeated here for comparison.

Herbicide	Barley			*Sesbania*		
	Uptake (cpm)	% Inhibition Uptake	Synthesis	Uptake (cpm)	% Inhibition Uptake	Synthesis
None*	5160	...	...	36,615	...	...
None	11,190	0		60,775	0	
Amitrole, 5 ppm	10,670	5	(−4)	42,730	30	(33)
Atrazine, 5 ppm	10,350	7	(11)	39,490	35	(32)
CDAA, 4 ppm	7115	36	(70)	**	50	(87)
CIPC, 5 ppm	8645	23	(84)	64,940	−7	(98)
DATC, 4 ppm	9015	19	(18)	...	...	...
Endothal, 5 ppm	7915	29	(24)	37,035	39	(72)
Ioxynil, 2×10^{-6} M	9730	30	(8)	**	78	(74)
Ioxynil, 1×10^{-5} M	...	...		6465	89	(83)
Ioxynil, 2×10^{-5} M	5115	54	73)	**	89	(88)
Monuron, 5 ppm	11,235	0	(19)	**	−2	(24)
PCP, 5 ppm	7545	33	(62)	**	63	(65)
Pyrazon, 5 ppm	8445	25	(29)	**	21	(26)

* 30 Minute incubation, to demonstrate linearity of uptake.

** Percentage inhibition for this compound was calculated from other experiments in which control values were slightly different.

Discussion

Experimental conditions were designed to minimize the likelihood of detecting secondary effects of herbicides. Short incubation periods and low herbicide concentrations were used. (These concentrations are comparable to field levels). The hour-long preincubation was found necessary to permit diffusion of some herbicides into the tissue. Nevertheless, quite a number of herbicides when tested at 2 ppm inhibited leucine incorporation by 10 to 30 %. The problem was to distinguish between secondary effects, as opposed to direct effects. Two assumptions were made. First, that a given herbicide, used near its lowest effective concentration, affects only 1 or 2 metabolic reactions; this is conveniently described as that herbicide's target area. Second, that for most of the herbicides tested, the appropriate target areas had been severely inhibited at a concentration of 2 ppm.

If the target area of a given herbicide is not directly concerned with protein biosynthesis, but rather with some energy-yielding reactions, leucine incorporation may be diminished somewhat due to lowered ATP levels. Further inhibition of the target area by higher herbicide concentration should not cause much further reduction in the rate of leucine incorporation. On the other hand, if some reaction directly involved in protein biosynthesis is the target area of a given herbicide, then higher herbicide level should lead to greater inhibition of leucine incorporation.

Therefore, for each herbicide, comparisons should be made between the 2 and 5 ppm rates. Table II shows that only 5 out of the 23 herbicides seriously affected leucine incorporation.

Another means for distinguishing between the actions of the 5 effective herbicides is provided by the data of table IV. The stem and coleoptile segments used have the ability to concentrate amino acids from a dilute external medium, and to retain these amino acids during a 15-minute wash with distilled water. If active uptake of the C^{14}-labeled leucine is blocked, clearly its incorporation into protein will be greatly reduced. In preliminary experiments, the effect of CIPC upon both leucine uptake and incorporation was determined. Numerically similar inhibitions were found, but since only 20 % of the leucine absorbed was used for protein synthesis, percentages of inhibition varied. The uptake date reported here were obtained with α-amino-*n*-butyric acid, a synthetic compound that is actively and rapidly concentrated by these tissues but is not utilized for protein synthesis (5).

Ioxynil and PCP prevented uptake of α-amino-*n*-butyric acid. Several of the herbicides tested (table IV) moderately inhibited amino acid uptake, with corresponding moderate inhibition of leucine incorporation. On the other hand, CIPC and CDAA inhibited protein synthesis much more than amino acid uptake.

Since it is impossible to discuss here each herbicide tested, this discussion will be restricted to those compounds which had important effects upon amino acid uptake or protein synthesis.

CIPC is representative of a small class of economically important herbicides. These have been studied more intensively in an earlier publication (4).

It should be noted that these herbicides inhibited not only protein synthesis, but also synthesis of a polymer tentatively identified as lignin. In unpublished experiments we have noted that carbamate herbicides inhibit the incorporation of orotic acid-6-C^{14} into RNA. In contrast to all but one of the other herbicides tested, CIPC did not inhibit uptake of α-amino-*n*-butyric acid.

CDAA is a subsituted acetamide herbicide. Inhibition of protein synthesis by this compound is probably due to a more fundamental action, since even uptake of amino acid was somewhat inhibited.

Endothal is unlike any other currently used herbicide. Only NPA bears even a remote resemblance to this compound. In field usage, endothal is much more toxic to grasses than to dicotyledonous plants. The opposite results were obtained in the experiments shown in table II. This suggests that roots, rather than shoots, should be the material of choice for study of this compound. Although endothal moderately inhibited amino acid uptake, protein synthesis in *Sesbania* was more severely inhibited than can be explained on this basis alone.

Ioxynil is extremely potent in killing broadleaved seedlings in cereal fields; one-fourth to one-half lb per acre rates are employed. At 2 lb per acre, cereals are also killed. (The agricultural interest in this compound stems from its possible use in killing weeds resistant to 2,4-D). Table III emphasizes the taxonomic selectivity of ioxynil. However, inhibition of protein synthesis by ioxynil can be ascribed to inhibition of amino acid uptake (table IV). Thus, it is unlikely that protein synthesis is directly affected by ioxynil. Rather, a process (or processes) required for both amino acid uptake and peptide-bond formation is blocked.

Pentachlorophenol (PCP) is a contact herbicide believed to kill plant tissue by disruption of oxidative phosphorylation in a fashion similar to 2,4-dinitrophenol (6). Clearly, total lack of ATP and resulting membrane disorganization would account for the inhibition of amino acid uptake and protein synthesis observed in the present study.

Our studies suggest that endothal, CDAA, and CIPC (plus related carbamates), inhibit protein synthesis at points after the transport of amino acids across the cell membrane. Whether any one of the steps directly involved in polypeptide formation, from amino acid activation to eventual release of completed protein from ribosomes, is affected, or whether the supply of necessary coreactants (ATP, messenger RNA, etc.) is lowered, cannot be determined from the data now available. Protein synthesis is probably not the only process affected. Cell-free studies are obviously required.

On the other hand, several herbicides prevented active uptake of an amino acid. This may be physiologically significant, since cells of an intact plant must be capable of concentrating their required nutrients from rather dilute phloem and xylem contents. However, our data suggest that effects on the energy balance should be investigated.

Summary

The effect of herbicides upon protein synthesis and amino acid uptake, in segments of barley and *Sesbania* seedlings, has been tested. Of 23 herbicides used, 5 depressed protein synthesis from leucine-1-C^{14}. In 2 cases (pentachlorophenol and 3,5-diiodo-4-hydroxybenzonitrile), uptake of α-amino-*n*-butyric acid was inhibited. But amino acid incorporation, in addition to uptake, was inhibited by isopropyl-*N*-(3-chlorophenyl)-carbamate (CIPC), 2-chloro-*N*,*N*-diallylacetamide (CDAA), and disodium 3,6-endoxohexahydrophthalate (endothal).

Acknowledgments

Purified, solvent-free samples of the herbicides tested were generously supplied by each manufacturer. The skillful assistance of Miss Heidi Haid is gratefully acknowledged.

Literature Cited

1. Crafts, A. S. 1961. The Chemistry and Mode of Action of Herbicides. Interscience Publishers, Inc., New York. 269 pp.
2. Dalgaard-Mikkelson, S. and E. Poulsen. 1962. Toxicity of herbicides. Pharmacol. Rev. 14: 225–50.
3. Hilton, J. L., L. L. Jansen, and H. M. Hull. 1963. Mechanisms of herbicide action. Ann. Rev. Plant Physiol. 14: 353–84.
4. Mann, J. D., L. S. Jordan, and B. E. Day. 1965. Effects of carbamate herbicides on polymer synthesis. Weeds 13: 63–66.
5. Mothes, K. and L. Englebrecht. 1961. Kinetin-induced directed transport of substances in excised leaves in the dark. Phytochemistry I: 58–62.
6. Van Overbeek, J. 1964. Survey of mechanisms of herbicide action. In: The Physiology and Biochemistry of Herbicides. L. J. Audus, ed. Academic Press, New York.