Insecticide Inhibition of Herbicide Metabolism in Leaf Tissues

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The effects of eight insecticides on the metabolism of nine herbicides were investigated in isolated leaf tissues of plants. About half of the 72 herbicide–insecticide combinations showed some degree of interaction. The metabolism of 3,6-dichloro-*o*-anisic acid (dicamba), isopropyl *m*-chlorocarbanilate (chlorpropham), and 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (linuron) in wheat, bean, and plantain, respectively, was commonly inhibited by organophosphate insecticides. 3′,4′-Dichloropropionanilide (propanil) metabolism in tomato was strongly inhibited by all the insecticides examined, especially the carbamates. The metabolism of 5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone (pyrazon) in red beet and *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide (Kerb) in lettuce was inhibited by some of the carbamates and some of the organophosphates. No insecticides significantly inhibited the metabolism of 3-amino-2,5-dichlorobenzoic acid (chloramben), 3-amino-*s*-triazole (amitrole), or 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB) in bean.

Several interactions have been noted for pesticide combinations (Kaufman *et al.*, 1970). Hacskaylo *et al.* (1964), for example, found that severe injury or death of cotton seedlings occurred when systemic phosphate insecticides were applied to the soil with the herbicide monuron or diuron. Nash (1967, 1968) also observed that combinations of diuron with some phosphate insecticides showed synergistic phytotoxicity to oats or corn. Swanson and Swanson (1968) found that certain carbamate insecticides inhibited the degradation of monuron in cotton leaf discs and thus enhanced the inhibition of photosynthesis by monuron in the leaf tissue.

Similar interactions have been found to exist with the herbicide propanil and certain phosphate or carbamate insecticides in rice plants (Bowling and Hudgins, 1966). Matsunaka (1968) and Yih *et al.* (1968) observed that the insecticides which increased the phytotoxicity of propanil also inhibited the metabolism of this herbicide. Certain organophosphate and carbamate insecticides have also been found to inhibit the microbial degradation of several amide, carbamate, or urea herbicides (Kaufman *et al.*, 1970; Kaufman and Miller, 1970). While the most frequently observed interactions are synergistic in terms of phytotoxicity, some antagonistic effects have also been noted in herbicide–insecticide combinations (Hamill and Penner, 1970; Smith, 1970).

Most of the research on pesticide interactions in crop plants has been directed toward explaining toxic effects already observed in the field. The present paper represents a somewhat different approach to the problem in that the influence of carbamate and organophosphate insecticides on the metabolic fate of several "chemically different" herbicides in plants is examined in an *in vitro* system. Hopefully, the results can be used to predict the insecticide–herbicide combinations that may be synergistically phytotoxic to plants.

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MATERIALS AND METHODS

Nine herbicides, eight insecticides, and six plant species were used in the experiments. The radio-labelled herbicides and technical grade insecticides are listed in Table I. The radio-labelled herbicides had a purity of at least 99%.

The plants used were bean (*Phaseolus vulgaris* L. cv. Red Kidney), lettuce (*Lactuca sativa* L. cv. Grand Rapids), plantain (*Plantago major* L.), red beets (*Beta vulgaris* L. cv. Detroit Dark Red), tomato (*Lycopersicon esculentum* Mill. cv. John Baer), and wheat (*Triticum vulgare* L. cv. Opal). The plants were grown in soil in a controlled environment chamber (16-hr light period at 21° C and 8-hr dark period at 18° C). With the exception of wheat, leaf discs 11 mm in diameter were cut from leaves of the same age and size just before treatment. For wheat, 2-cm leaf segments were used. For most species 15 leaf discs were used for each treatment observation. For plantain and wheat, 10 and 20 leaf discs were utilized, respectively.

Stock solutions of the herbicides were prepared in acetone, ethanol, or methanol. Aliquots of the stock solutions were evaporated to dryness and then dissolved in 0.35 *M* mannitol to provide the desired concentrations of 10^{-5} to 10^{-4} *M*. The insecticides were dissolved in ethanol and then added to the herbicide solutions to give concentrations of 10^{-6}, 10^{-5}, and 10^{-4} *M*. The final concentration of ethanol in all treatment and control solutions was 0.1%.

The excised leaf tissues were placed in the solutions and were vacuum infiltrated with a water aspirator (Stephenson *et al.*, 1971). After infiltration, the leaf tissues were removed from the treatment solution, blotted dry with tissue, transferred into Petri dishes lined with wet filter paper, and incubated in a growth chamber at 25° C. The light intensity was 12,000 lux. The incubation period varied from 2 to 20 hr, depending on the rate at which the individual herbicides were metabolized in the leaf tissue. Preliminary time-course studies were carried out for each herbicide with the selected leaf tissues to obtain an indication of the metabolism rate. At the end of the incubation period, the leaf tissues were freeze-

Table I. Herbicides and Insecticides Used

Compound	Chemical name	Specific activity (mCi/mmol)	Source
Herbicides			
Amitrole-5-^{14}C	3-Amino-*s*-triazole	4.89	New England Nuclear Corp.
Chloramben carboxyl-^{14}C	3-Amino-2,5-dichlorobenzoic acid	2.19	Amchem Products Inc.
Chlorpropham ring-^{14}C	Isopropyl *m*-chlorocarbanilate	4.3	PPG Industries, Inc.
2,4-DB ring-^{14}C	4-(2,4-Dichlorophenoxy)butyric acid	0.82	May & Baker, Ltd.
Dicamba carboxyl-^{14}C	3,6-Dichloro-*o*-anisic acid	1.89	Velsicol Chem. Corp.
Kerb[a] carbonyl-^{14}C	*N*-(1,1-Dimethylpropynyl)-3,5-dichlorobenzamide	2.56	Rohm & Haas Co.
Linuron carbonyl-^{14}C	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea	1.70	E. I. du Pont de Nemours & Co.
Propanil ring-^{14}C	3′,4′-Dichloropropionanilide	0.48	Rohm & Haas Co.
Pyrazon phenyl ring-^{3}H	5-Amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone	38.65	Badische Anilin- & Soda-Fabrik Corp.
Insecticides			
Carbaryl	1-Naphthyl methylcarbamate		Union Carbide
Carbofuran	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate		Niagara Chemicals
Chlorfenvinphos	2-Chloro-1-(2,4-dichlorophenyl)-vinyl diethyl phosphate		Shell Chemical
Disulfoton	*O*,*O*-Diethyl *S*-2-(ethylthio)ethyl phosphorodithioate		Chemagro Ltd.
Dyfonate[a]	*O*-Ethyl *S*-phenyl ethylphosphonodithioate		Stauffer Chem. Co.
Fensulfothion	*O*,*O*-Diethyl *O*-*p*-(methylsulfinyl)phenyl phosphorothioate		Chemagro Ltd.
Malathion	Diethyl mercaptosuccinate, S ester with *O*,*O*-dimethyl phosphorodithioate		American Cyanamid Co.
PCMC	-Chlorophenyl *N*-methylcarbamate		PPG Industries, Inc.

[a] Trade name.

killed with Dry Ice and stored in a freezer for no more than 1 week prior to extraction and analysis.

The treated leaf tissues were homogenized in 1 ml of 95% ethanol. After grinding, the homogenates were centrifuged at 1000 × *g* for 4 min. The radio-labelled compounds in the supernatants were then separated by thin-layer chromatography, with 0.50 mm cellulose for amitrole, 0.50 mm silica gel G/Kieselguhr G (4:6) for 2,4-DB, and 0.25 mm silica gel G for the other herbicides. The radioactive spots were located on the chromatograms by exposing the plates to X-ray film. Once located, the spots were scraped off the plate and the radioactivity in each spot was measured by liquid scintillation counting. In experiments with ^{3}H-pyrazon, the location and quantity of the radioactivity were determined by counting 1-cm sections of the chromatograms.

To determine if any radio-labelled derivatives were artifacts of the experimental procedures, radio-labelled herbicides were added to the untreated leaf tissue during the extraction procedure. Also, after infiltration, the remaining treatment solutions were incubated for the same periods of time as the tissues and then examined for radio-labelled derivatives due to microbial, chemical, or photolytic degradation. These checks of the procedures did not yield any products differing from the parent compounds.

No attempt was made to account for nonextractable radioactivity in the residue or for possible losses of radioactivity as $^{14}CO_2$. However, these appeared to be minimal since there was very little variation in the radioactivity recovered in the ethanol extracts of tissue with different incubation times. Only one herbicide and one plant tissue was used with the eight insecticides in any one experiment. Duplicate samples were used in all experiments and each experiment was conducted at least twice.

RESULTS

The relative amounts and the chromatographic properties of the radio-labelled herbicides and metabolites in the control treatments (herbicides only) are presented in Table II. The overall rate of metabolism in the control treatments was taken as 100% and the percent inhibition of this metabolism by the insecticides is shown in Figure 1.

In approximately one-half of the 72 herbicide–insecticide combinations tested, there was some degree of inhibition of herbicide metabolism (Fig. 1). The metabolism of propanil in tomato leaf discs was markedly inhibited by all the insecticides tested. The carbamate insecticides, *i.e.*, carbaryl, carbofuran, and PCMC, were the most inhibitory and showed at least 55% inhibition at 10^{-6} *M*, the lowest concentration used. The metabolism of linuron, chlorpropham, and dicamba in plantain, bean, and wheat, respectively, was inhibited by the organophosphate insecticides, Dyfonate, chlorfenvinphos, malathion, fensulfothion, and disulfoton. The carbamates had very little effect on the metabolism of linuron, chlorpropham, or dicamba. Pyrazon metabolism in red beet and Kerb metabolism in lettuce was moderately inhibited by some carbamate and some organophosphate insecticides. The metabolism of chloramben, amitrole, and 2,4-DB in bean was not significantly influenced by any of the insecticides examined. No insecticide stimulated the metabolism of the herbicides.

Generally, when the metabolism of all the herbicides was considered, the organophosphate insecticides were more inhibitory than the carbamate insecticides. Among the organophosphates, Dyfonate, fensulfothion, and disulfoton appeared to be more inhibitory than chlorfenvinphos and malathion. At a concentration of 10^{-4} *M*, Dyfonate almost completely blocked the metabolism of chlorpropham, linuron,

Table II. Relative Amounts and Chromatographic Properties of Radio-Labelled Herbicides and Metabolites

Herbicide	Plant	Incubation time, hr	Tlc solvent[a]	% Radioactivity (R_f values in parentheses) Parent compd	Metabolite I	Metabolite II	Metabolite III	Metabolite IV
Chloramben	Bean	2.7	A	29(0.58)	71(0.30)			
Amitrol	Bean	3.5	B	27(0.75)	7(0.40)	66(0.16)		
Chlorpropham	Bean	18.0	C	85(0.26)	15(0.00)			
Dicamba	Wheat	18.0	D	77(0.50)	23(0.00)			
2,4-DB	Bean	4.5	E	53(0.46)	24(0.27)	23(0.00)		
Linuron	Plantain	10.0	F	67(0.89)	5(0.69)	2(0.49)	2(0.05)	24(0.00)
Propanil	Tomato	16.0	G	67(0.47)	2(0.56)	11(0.23)	20(0.00)	
Pyrazon	Beets	4.0	H	34(0.54)	66(0.27)			
Kerb	Lettuce	16.0	I	86(0.51)	3(0.12)	11(0.00)		

[a] Solvent systems: A. 2-propanol:ammonium hydroxide (28%):water (8:1:1); B. 2-butanone:methanol:water (1:2:1); C. hexane:ether (9:1); D. benzene:acetic acid:water (2:2:1); E. liquid paraffin:benzene:acetic acid:cyclohexane (5:15:10:100); F. acetone:benzene (1:2); G. acetone:benzene (15:85); H. benzene:ethanol (75:25); I. acetone:benzene (5:95).

and propanil. At this same concentration disulfoton inhibited the metabolism of pyrazon, Kerb, propanil, and chlorpropham by 50 to 75% and fensulfothion inhibited the metabolism of linuron, Kerb, dicamba, propanil, and chlorpropham by 50 to 60%.

DISCUSSION

With the exception of chloramben, amitrole, and 2,4-DB, the metabolism of all the herbicides was strongly or moderately inhibited by some of the insecticides. Since the metabolic alteration of herbicide molecules often represents a detoxification mechanism, inhibition of herbicide metabolism by insecticides could increase the effectiveness of the herbicides for weed control. On the other hand, these interactions may result in damage to the crops if selectivity is based on the differential metabolism of the herbicide.

Metabolism of linuron, a substituted urea herbicide, was inhibited by the organophosphate insecticides. These results suggest that the synergistic phytotoxicity of organophosphate insecticides and substituted urea herbicides observed by Hacskaylo *et al.* (1964) and Nash (1967, 1968) may be due to inhibition of the herbicide degradation by the insecticides. Linuron metabolism in plantain leaf tissue was not significantly influenced by carbamate insecticides. This was surprising since Swanson and Swanson (1968) reported that the metabolism of monuron in cotton was inhibited by carbamate insecticides. It is possible that the different plant species used and the slightly different chemical properties of linuron may account for the differential effect of carbamate insecticides on the metabolism of these two herbicides.

Chlorpropham metabolism in bean leaf discs was strongly inhibited by some of the organophosphates tested, but inhibition by the carbamates was very slight. However, Kaufman *et al.* (1970) and Kaufman and Miller (1970) were able to show that chlorpropham degradation by soil microbes was strongly inhibited by several methylcarbamate insecticides including carbaryl and PCMC. The reasons for the different effects of the carbamate insecticides upon microbial degradation of chlorpropham in soil and on the metabolism of chlorpropham in bean leaf tissues is not clear. It is possible that different enzymes and metabolic pathways may be involved in the transformation of chlorpropham in the two systems.

In wheat plants, dicamba is metabolized mainly to 5-OH-3,6-dichloro-*o*-anisic acid, which exists in the form of a sugar conjugate (Broadhurst *et al.*, 1966; Chang and Vanden Born, 1969). This metabolism was significantly inhibited by all the phosphate insecticides tested. Since differences in metabolism of dicamba between cereal crops and weeds is an important factor in its selectivity (Chang and Vanden Born, 1971), combinations of this herbicide with organophosphate insecticides may increase the herbicide phytotoxicity against wheat and reduce the margin of safety.

Kerb is a new herbicide which shows promise for the control of certain grasses in lettuce and legume crops. In lettuce leaf discs, it was metabolized to two compounds of higher polarity. This metabolism was inhibited by a number of insecticides tested. The significance of this inhibiting effect on Kerb's performance in weed control is not clear, because the mechanism of its selective action is not yet known. However, the metabolism of Kerb in the leaf discs of lettuce, a resistant species, was much faster than in leaf tissues of wheat, a susceptible species, suggesting that differential metabolism of this herbicide may play a role in its selectivity.

The herbicides pyrazon and chloramben are both metabo-

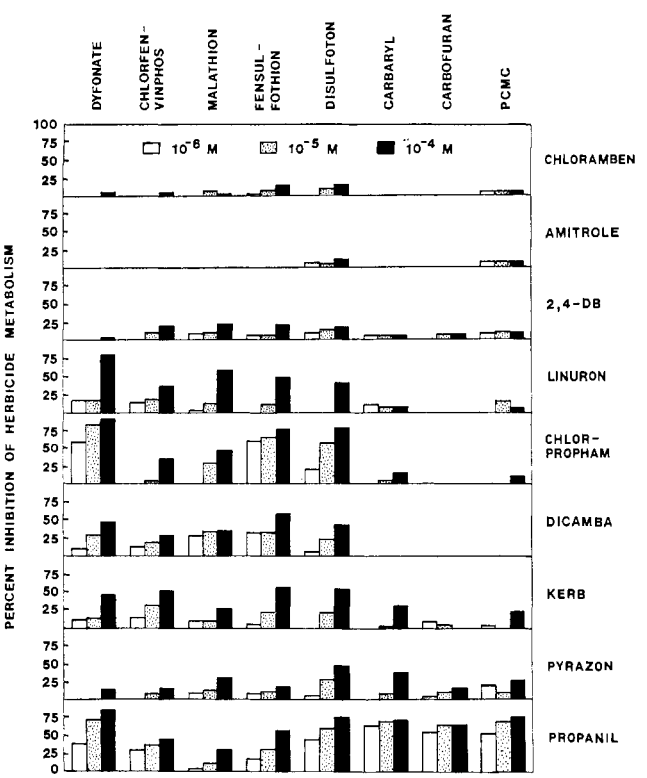

Figure 1. Effects of insecticides on the metabolism of herbicides in isolated leaf tissues. Coefficients of variability for these experiments ranged from 5.4% to 11.0%

lized directly to *N*-glucosides in beets and beans, respectively (Ries *et al.*, 1968; Swanson *et al.*, 1966). However, despite the similarity in metabolic pathways, the metabolism of these herbicides was affected differently by some of the insecticides. Pyrazon metabolism in red beet was strongly or moderately inhibited by disulfoton, carbaryl, malathion, and PCMC, whereas the metabolism of chloramben in bean was not significantly influenced by any of these insecticides. Similar results were observed when the metabolism of chloramben and pyrazon was examined in the same species, red beet. It is expected, therefore, that a synergistic interaction may exist between the above mentioned insecticides and pyrazon, but not with chloramben.

The herbicide propanil is not phytotoxic in rice because it is degraded to nontoxic 3,4-dichloroaniline and propionic acid (Yih *et al.*, 1968). In paddy fields the combination of several insecticides with propanil has resulted in injury to rice plants (Bowling and Hudgins, 1966). This effect is due to an inhibition of the propanil-hydrolyzing enzyme by the insecticides. The results in this study establish that several organophosphate and carbamate insecticides inhibited propanil degradation in leaf tissues of tomato plants. Although all the insecticides included in the study inhibited the metabolism of propanil, the carbamates appeared to be more inhibitory than most of the organophosphates. Malathion was the least inhibitory to propanil metabolism of all insecticides tested. These observations are in agreement with Frear and Still (1968), who found that the hydrolysis of propanil by an enzyme preparation from rice seedlings was only slightly inhibited by malathion at 10^{-6} *M*, whereas carbaryl inhibited 90% of the enzyme activity. Bowling and Hudgins (1966) have also noted that malathion is a less effective synergist with propanil than other phosphate insecticides.

It should be emphasized that these experiments were conducted under strictly artificial conditions and, therefore, the results cannot be extrapolated directly to field operations. On the other hand, the data cover a wide range of chemical groups and they do point out the possible influences of the insecticides upon the metabolism of the herbicides which may be manifested in lower crop tolerance or more persistent residues when these pesticides are simultaneously present in the plant tissues.

ACKNOWLEDGMENT

We thank Jane McLeod, Susan Gardner, and Prasanta C. Bhowmik for their technical assistance throughout the course of this investigation. We also thank the various companies for supplying the radio-labelled herbicides and technical grade insecticides.

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Received for review February 19, 1971. Accepted May 6, 1971. This investigation was supported by grants from National Research Council of Canada. Presented at Weed Sci. Soc. Amer. 11th Meeting, Dallas, Texas, February 1971.