Degradation of 4-(2,4-Dichlorophenoxy)butyric Acid (2,4-DB) by *Phytophthora megasperma*

A. E. Smith* and D. V. Phillips

The objective of our research was to investigate the efficacy of *Phytophthora megasperma* var. *sojae* in metabolizing (β-oxidizing) 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid] releasing 2,4-D [(2,4-dichlorophenoxy)acetic acid]. Results indicate that the cultured fungus degraded ca. 45% of the 2,4-DB included in the treatments over a 21-day assay period. However, 2,4-D was not detected in the nutrient medium or fungus mycelium throughout the experimental period. In similar experiments using 2,4-D, this fungus was found incapable of degrading 2,4-D. These data suggest that the initial step in 2,4-DB metabolism by *P. megasperma* does not include β-oxidation of 2,4-DB. Additionally, 3–5 times more 2,4-DB than 2,4-D was absorbed and accumulated by this fungus.

Synerholm and Zimmerman (1947) first discovered that the phenoxy herbicides with an even number of carbon atoms in the side chain were active growth regulators while those with an odd number of carbon atoms were inactive. The use of 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid] to control weeds in several leguminous crops that are susceptible to the phenoxyacetic acid herbicides was proposed by Wain (1955a,b). He attributed the inherent tolerance of certain legumes to 2,4-DB to their inability to β-oxidize 2,4-DB to phytotoxic 2,4-D [(2,4-dichlorophenoxy)acetic acid]. Recently, Walters and Caviness (1968) reported that soybean varieties susceptible to *Phytophthora megasperma*, the causal organism of phytophthora rot, were additionally injured by 2,4-DB applications while infected by the organism. However, varieties tolerant to the organism were not affected as severely by the herbicide applications. The coincidence of these reports would suggest that the organism *P. megasperma* may serve to β-oxidize 2,4-DB releasing 2,4-D to the infected plant.

Based on this thesis, the purpose of our research was to investigate the degradation of 2,4-DB and 2,4-D by *P. megasperma*.

METHODS AND MATERIALS

Aqueous solutions of technical grade 2,4-D and 2,4-DB, previously filtered through a sterile 0.22 μm membrane filter, giving a final herbicide concentration of 10 ppm, were added to separate 250-ml flasks containing 100 ml of autoclaved nutrient media. The medium contained the necessary mineral elements (Phillips, 1973) and 20% V-8 juice. Following the addition of 0.1 ml of fungus suspension to all flasks except standard solutions, the flasks were shaken on a laboratory shaker at a rate of 200 excursions/min and 22°C. Standard solutions were included to correct data for herbicide lost by volatility and photodegradation. Four flasks from each treatment were periodically removed from the shaker following initiation of the treatments. The flask contents were filtered through a 0.8-μm membrane filter and aliquots of the filtrate were analyzed for 2,4-D and 2,4-DB. Fungus mycelium retained on the filter was freeze-dried and weighed, and the herbicide extracted by grinding the membrane filter and mycelium in 30 ml of acidified acetone (1% HCl) for 5 min in an Omni mixer at maximum speed. The mixture was centrifuged for 10 min at 2700*g* and the filtrate was evaporated at 70°C. The residue was dissolved in diethyl ether and 0.1 N HCl for partition extraction of the herbicide in preparation for quantification. Fungi cultured in flasks of media not containing the herbicide treatments and retained on an 0.8-μm membrane were used for mycelium moisture content determinations.

Herbicide, in 10-ml aliquots of nutrient medium, was extracted twice with 35 ml of diethyl ether following acidification with 1 ml of 1 N HCl. Following ether evaporation, the extracted herbicides from mycelium and nutrient medium were methylated with 6 ml of BF_3–methanol (125 g/l.) (Merkle and Davis, 1966). Methyl esters of 2,4-D and 2,4-DB were taken up, quantitatively, in Skellysolve F and determined using an F&M 700 gas chromatograph equipped with a nickel-63 electron capture detector. A 1.8-m spiral glass column packed with 80–100 mesh Chromosorb W coated with 3% SE-30 was used. The flow rate of argon–methane (95%/5%) carrier gas was 40 ml/min. Column, injection port, and detector temperatures were 195, 250, and 260°C, respectively. Retention times for 2,4-D and 2,4-DB were 70 and 126 s, respectively.

The experiment was repeated once and all data were combined and statistically evaluated and mean differences determined by Duncan's multiple range test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The capacity of the fungus to β-oxidize 2,4-DB, releasing 2,4-D, was determined by assaying the fungus and nutrient media, initially containing 2,4-DB, for 2,4-D. We were unable to detect 2,4-D in the nutrient media or fungus mycelium cultured in the presence of 2,4-DB for the duration of the experiment. However, approximately 45% of the 2,4-DB was degraded by the fungus over the 21-day experimental period (Table I). Gas chromatograph analyses were allowed to proceed for up to 10 min post-sample injection and the presence of electron capturing compounds, in addition to 2,4-DB, was not detected. These data indicate that the fungus, *P. megasperma*, is capable of degrading 2,4-DB. Since an accumulation of 2,4-D was not detected it was apparent that either: (1) the 2,4-D resulting from 2,4-DB metabolism was subsequently degraded or (2) the mode of 2,4-DB degradation did not include β-oxidation. The former thesis was checked by determining the rate of 2,4-D metabolism by *P. megasperma* while replacing 2,4-DB with 2,4-D in a similar type experiment. No significant degradation of 2,4-D occurred over the 21-day experimental period (Table II). These data

Departments of Agronomy and Plant Pathology, University of Georgia, College of Agriculture Experiment Stations, Georgia Station, Experiment, Georgia 30212.

Table I. 2,4-DB Accumulation and Metabolism by *P. megasperma* Cultured in Nutrient Broth

Days after treatment	Mycelium dry wt, mg	μg of 2,4-DB/g of mycelium (dry wt)	2,4-DB concn in mycelium based on mycelium moisture content,[b] mg/l.	2,4-DB concn in nutrient media, mg/l.	2,4-DB recovered, %
3	84e[a]	892a	51.0	9.8	100
5	193d	840a	48.0	8.6	100
7	266bc	683ab	39.0	7.8	95
10	309a	715ab	40.9	7.1	92
12	326a	551bc	31.5	7.1	89
14	323a	442c	25.3	6.0	74
17	294ab	514bc	29.4	5.8	73
19	256bc	327c	18.7	5.3	61
21	235c	512bc	29.3	4.4	56

[a] Means in the same column followed by the same letter are not different ($P = 0.05$). [b] Data computed on the basis of mycelium containing 94.6% moisture following filtration.

Table II. 2,4-D Accumulation and Metabolism by *P. megasperma* Cultured in Nutrient Broth

Days after treatment	Mycelium dry wt, mg	μg of 2,4-D/g of mycelium (dry wt)	2,4-D concn in mycelium based on mycelium moisture content,[b] mg/l.	2,4-D concn in nutrient media, mg/l.	2,4-D recovered, %
3	103g[a]	199a	11.4	9.7	99
5	152f	246a	14.1	8.7	99
7	215e	242a	13.8	9.4	99
10	388a	237a	13.5	9.0	99
12	366abc	117b	6.7	9.7	100
14	383ab	72b	4.1	9.3	96
17	329cd	72b	4.1	9.7	99
19	321d	52b	3.0	9.2	93
21	343bcd	59b	3.4	9.4	95

[a] Means in a column followed by the same letter are not different ($P = 0.05$). [b] Data computed on the basis of mycelium containing 94.6% moisture following filtration.

would indicate that β-oxidation is probably not the initial reaction for 2,4-DB degradation by *P. megasperma*.

The difference in the metabolism rate for 2,4-D and 2,4-DB was not completely resolved in our experiments. However, data (Tables I and II) indicate that considerably more 2,4-DB than 2,4-D was accumulated by the fungus at all sample periods. The accumulated 2,4-DB ranged from 3 to 5 times greater than 2,4-D for the same sampling periods. The general trend for mycelium accumulation of these herbicides was inverse to the changes in mycelium weight throughout the experimental periods. When the herbicide concentrations within the mycelium were computed on a mycelium moisture content basis, data indicate that 2,4-DB was accumulated against a concentration gradient. The concentration of 2,4-DB within the fungus was 300–500% higher than the herbicide concentration in the nutrient media for all sample periods, whereas the concentration of 2,4-D within the fungus was only 30–140% as high as the herbicide concentration in the nutrient media. Previous research has indicated that certain chlorinated phenoxy herbicides accumulate in treated parenchyma tissue to concentrations many times higher than the external solution (Blackman, 1955).

The thesis that β-oxidation totally explains differential plant tolerance to 2,4-DB has been questioned by other researchers (Fertig et al., 1964; Hawf and Behrens, 1974; Linscott et al., 1968). Hawf and Behrens (1974) found that certain 2,4-DB tolerant species were as effective as some 2,4-DB susceptible plants in β-oxidizing 2,4-DB. They concluded that the differential tolerance was a response to differences in spray retention, absorption and translocation, and rate of degradation of 2,4-D resulting from β-oxidation of 2,4-DB. Linscott et al. (1968) offer another possible explanation for the differential susceptibility of plants to 2,4-DB. In plants treated with 2,4-DB, they detected 2,4-dichlorophenoxycaproic and 2,4-dichlorophenoxydecanoic acid as well as 2,4-D. They attributed the 2,4-DB tolerance of alfalfa to its ability to add methylene units to the side chain of a substantial amount of 2,4-DB. Therefore, less 2,4-DB was available for the formation of the phytotoxic 2,4-D molecule by β-oxidation. Results of our study show that the pathogenic fungus, *P. megasperma*, will not function as a mechanism for β-oxidizing 2,4-DB releasing 2,4-D to the nutrient media. The differential tolerance of soybean varieties to 2,4-DB reported by Walters and Caviness (1968) is probably due to some other factor(s). We have not eliminated the possibility that *P. megasperma* is indirectly involved through its influence on host metabolism or that the soybean host has an influence on fungal metabolism.

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Interaction of Pesticide Chemicals. Effect of Eptam and Its Antidote on the Uptake and Metabolism of [^{14}C]Phorate in Corn Plants

Ken R. Schulz, Tom W. Fuhremann, and E. Paul Lichtenstein*

The effects of soil treatments with the herbicide Eptam (EPTC) and its corn antidote (Stauffer, R-25788) on the translocation and metabolism of [^{14}C]phorate in corn seedlings were investigated. While at concentrations of 5 ppm of Eptam in a [^{14}C]phorate treated (2 ppm) Plainfield sand no visible damage to the growing corn plants was noticeable, the amounts of ^{14}C compounds in corn greens from herbicide-treated soil were significantly increased by a factor of 1.8. In particular, the amount of unextractable ^{14}C-labeled residues was higher in plants grown in [^{14}C]phorate plus Eptam-treated soil. After 18 days soils contained phorate, phorate sulfoxide, and phorate sulfone, while in greens only the metabolites phorate sulfoxide, phorate sulfone, and phoratoxon sulfoxide could be detected. Due to the presence of Eptam in the [^{14}C]phorate-treated soil, the amounts of both phorate sulfoxide and phorate sulfone were significantly increased by a factor of 1.6 and those of phoratoxon sulfoxide by a factor of 2.4. Addition of the antidote (at 1 ppm) to [^{14}C]phorate-treated soil had no effect on the translocation and metabolism of insecticide in corn greens, yet counteracted the effects of Eptam. Thus, analyses of corn greens grown in [^{14}C]phorate-treated soil containing also Eptam and its antidote gave results similar to those grown in soil which had only been treated with [^{14}C]phorate. These findings further point to the problem of potential interactions of environmental chemicals in biological systems.

Modern agricultural practices have made extensive use of insecticides and plant growth regulators to increase food production. As a consequence, many soils contain residues of the various chemicals and/or their degradation products. The effects of these residues on subsequent pesticide applications as well as the effects of simultaneous applications of mixtures of agricultural chemicals on their metabolism in soil and their potential uptake by plants must be investigated. While the fate of individually applied agricultural chemicals has been extensively studied, relatively few reports on the interaction of herbicides and insecticides are available. Arle (1968), Hacskaylo et al. (1964), Nash (1967), and Parks et al. (1972) have all reported that phorate and other systemic insecticides increase the uptake of various herbicides and can alter their toxicity toward plants. However, the number of reports concerning the effects of herbicides on insecticides is relatively small. This laboratory has previously reported on the interactions and synergistic action of herbicides in combination with insecticides (Lichtenstein et al., 1973b; Liang and Lichtenstein, 1974). Krueger and Mason (1974) tested 45 plant growth regulators for their effects on the uptake and metabolism of phorate and aldicarb in soybeans but they found no significant differences. Chang et al. (1971) demonstrated that certain herbicides may alter the metabolism of carbaryl, dyfonate, and malathion in bean and tomato leaves.

This study was initiated to investigate the effects of the herbicide Eptam (*S*-ethyl dipropylthiocarbamate) on the persistence and metabolism of phorate in soil and on its uptake by corn seedlings. Eptam is an effective preemergence herbicide commonly used for the control of grasses and broadleafed weeds but its phytotoxicity toward certain varieties of corn has necessitated the introduction of antidotes to minimize that effect. The compound *N*,*N*-diallyl-2,2-dichloroacetamide (Stauffer R-25788) is one of these antidotes. Lay et al. (1975) have shown that the antidote raises the glutathione (GSH) and GSH *S*-transferase levels in corn, resulting in rapid detoxication of the translocated Eptam sulfoxide, which is the presumed active growth regulator (Casida et al. 1974).

EXPERIMENTAL SECTION

Materials. Phorate, [S-methylene-^{14}C]phorate (sp act. 9.7 mCi/mmol), phorate sulfoxide and sulfone, and phoratoxon and its sulfoxide and sulfone were obtained through the courtesy of the American Cyanamid Co. Eptam and its corn antidote (R-25788) were provided by the Stauffer Chemical Co. The solvents used were anhydrous methanol, redistilled acetone, benzene, and acetonitrile, and reagent grades nitromethane and toluene.

Preliminary experiments in this laboratory demonstrated that the growth of corn seedlings was greatly stunted when 75 ml of water containing 10 mg of Eptam was applied to the surface of 1 kg of a Plainfield sand previously planted with germinated corn seeds. No reduction in growth of corn plants occurred when 75 ml of water containing 10 mg of Eptam plus 1 mg of the antidote had been applied as described. The concentrations of Eptam and its antidote in the 1 kg of soil would have been 10 and 1 ppm, respectively, if the chemicals had been mixed throughout the soil. However, the effective concentration in the soil surrounding the developing corn roots was probably considerably larger, since the herbicide had

Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706.