ABSORPTION, DISTRIBUTION AND METABOLISM OF BIFENOX

by

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Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Plant Physiology

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December, 1975

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ACKNOWLEDGMENTS

The author expresses sincere appreciation to his major professor, , Department of Plant Pathology and Physiology, for his guidance in the research and writing of this dissertation. Special thanks go to , , and for serving on the author's graduate committee.

The support of Mobil Chemical Company, Richmond, Va. is fully appreciated both for financial and technical assistance.

Special appreciation goes to for his support and help in obtaining educational leave from the Department of Army to pursue these studies and for his comments during the writing of this dissertation.

Thanks go to for his assistance in preparing the graphic artwork, and for her excellent typing to complete this dissertation.

The author expresses sincere gratitude to his wife and children for their understanding and patience during his graduate studies.

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I. INTRODUCTION

Bifenox [methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate] is a recently introduced herbicide belonging to the class of substituted diphenylethers (12). The structure of bifenox is shown in Figure I-1. It shows promising control of a wide range of broadleaf and grass weeds at low rates of application. The best methods of application are as a preemergence spray or a directed spray. Registration with the Environmental Protection Agency is now complete for application in soybeans (*Glycine max* L.). Tests are presently underway for registration in corn (*Zea mays* L.) and rice (*Oryza sativa* L.).

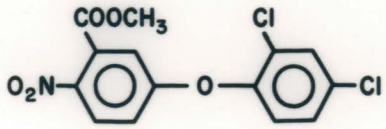
The published studies with bifenox are limited to those necessary for registration (5, 6, 12), crop yield data (1, 2, 4, 9, 11, 13, 14), and metabolism (3, 7, 8, 10). Before there is widespread use of this herbicide in crop plants, particularly those utilized for food, studies concerning the persistence in soil and absorption by plants are needed. In addition, the degradation products must be known to ascertain the possibility of toxic products being formed which may be environmentally harmful. Finally, to expand the usage to other methods of application and for use in different crops, the mode of action and basis of selectivity must be delineated. Therefore, the objectives of this investigation were as follows:

(1) To determine the absorption and accumulation of bifenox in selected plant species, and to chart the persistence of residues in plant tissue and soil.

Figure I-1. The chemical structure of bifenox [methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate]. Mobil Chemical Co. #MC 4379. Tradename MODOWN.

BIFENOX

METHYL 5-(2,4-DICHLOROPHENOXY)-2-NITROBENZOATE



(2) To determine if differential absorption and distribution of bifenox could account for its selectivity, and to assess the absorption by different plant organs.

(3) To elucidate, in greenhouse and laboratory studies of soil and plant reactions, the degradative pathway of bifenox.

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II. UPTAKE AND DISTRIBUTION OF ^{14}C-BIFENOX IN CROP AND WEED SPECIES.

Abstract. The uptake of ^{14}C-bifenox by plants was dependent upon application rate in the range of 1.7 to 4.5 kg/ha preemergence. Velvetleaf (*Abutilon theophrasti* Medic.) absorbed and translocated more ^{14}C-bifenox during early seedling growth than did corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). Crop plants grown to maturity had less ^{14}C residue on a ppm (w/w) basis, indicating little absorption and movement after the seedling stage. The presence of a low level of ^{14}C residue in the seed of mature crop plants (101 to 117 days after treatment) showed that some translocation did take place. Autoradiographs of seedlings harvested 14 days after treatment, showed the ^{14}C to be in (or on) those areas of the crop plant in contact with the treated soil. Velvetleaf translocated ^{14}C residue throughout the shoot. The uptake of phenoxy-ring-labeled compound was greater than that of the benzene-ring-labeled compound at equal rates of application.

INTRODUCTION

Bifenox [methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate] is a recently introduced diphenylether herbicide[1] showing promising weed control in corn, soybean, and rice (*Oryza sativa* L.). It is of particular interest because of its selectivity to both broadleaf and grass weeds while being utilized in crops with the same morphological characteristics.

The most susceptible broadleaf weeds are: velvetleaf, smartweed (*Polygonum pensylvanicum* L.), jimsonweed (*Datura stramonium* L.), lambsquarters (*Chenopodium album* L.), and pigweed (*Amaranthus retroflexus* L.). Susceptible grass weeds include: barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), red sprangletop (*Leptochloa filiformis* (Lam.) Beauv.), and mexican sprangletop (*Leptochloa uninervia* (Presl.) Hitchc. and Chase).

Prior to registration and widespread use of a new herbicide, exhaustive studies concerning its persistence in soil and absorption by plants are needed. These studies are of particular importance when the herbicide is to be used in food crops.

The objectives of this study were to determine the absorption and accumulation of bifenox in selected plant species and to chart the persistence of residues in plant tissue and soil.

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[1]Mobil Chemical, Technical Bull. MODOWN™ Herbicide, 4 Feb 1974.

MATERIALS AND METHODS

Radiochemicals. In these studies, parallel experiments were performed using ^{14}C-bifenox randomly labeled in either the benzene ring (specific activity 9.4 µCi/mg) or phenoxy ring (specific activity 2.42 µCi/mg).[2]

Seedling uptake. Galvanized metal flats, 30 by 20 by 8 cm, were filled with a soil mixture of loam, peat moss and sand in a 2:1:1 (v/v/v) ratio. Each flat was seeded by row with corn (Dekalb XL66), soybean (York), and velvetleaf. The soil surface was then sprayed (preemergence application) using a DeVilbiss atomizer with ^{14}C-bifenox at the rate of 1.7 kg/ha. The flats were maintained under greenhouse conditions utilizing subirrigation during the experimental period.

Crop plants were harvested (roots included) on day 7, 14, and 28 after seeding. Velvetleaf was harvested on day 7, 14, and 60. After harvest, the plants were dried in an oven at 60° C. The dried plants were then ground in a Wiley mill fitted with a number 20 mesh screen, thoroughly mixed, and a 300 to 500 mg sample removed for combustion.

Sample combustion was by the method of Peterson (6), utilizing an OXYMAT™ sample oxidizer to collect the $^{14}CO_2$. The collected samples were assayed by liquid scintillation counting and the results expressed as ppm (w/w).

Duplicate samples harvested on day 14 were dried, pressed, and placed in contact with GAF No-Screen X-ray film for a period of four weeks. The resulting autoradiographs were then surveyed for areas of

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[2] Donated by Mobil Chemical Company, Edison, New Jersey.

^{14}C accumulation.

Residue in mature plants. Large plastic pots, with a capacity of 11.4 L, were filled with unsterilized field soil (clay loam) and seeded with corn or soybeans. The soil surface was then sprayed (preemergence application) by DeVilbiss atomizer with either benzene or phenoxy-ring-labeled bifenox at 2.2 and 4.5 kg/ha. The pots were maintained under greenhouse conditions with surface watering on a daily basis. Because labeled material was limited, there was no duplication of treatments.

The plants were harvested on days 21, 42, and at maturity (day 101 - soybeans; day 117 - corn). Volunteer weeds were included in the sampling at the final crop harvest. Sample preparation and counting were as described in the seedling uptake study.

Crop rotation. The pots of soil used in the mature crop study were allowed to remain fallow after crop harvest and with regular watering until day 250 from the time of ^{14}C-bifenox application. Volunteer weeds were then harvested as before, the soil tilled to a depth of 12 cm, and seeded the following day with oat (*Avena sativa* L. Clinton) and velvetleaf (30 oat and 15 velvetleaf seeds) in combination to simulate crop rotation. After 60 days (day 311 from treatment) the crop and weeds were harvested. At this time the oats were in the soft-dough-stage. Sample preparation and counting were as previously described.

All residue data are expressed as ppm (w/w) on a dry-weight basis.

RESULTS AND DISCUSSION

Uptake and distribution in seedlings. The results, as tabulated from combustion data of seedlings, are shown by species in Tables II-1 to II-3. In general, the crop plants showed a substantial accumulation of ^{14}C-residue in the roots over a 28-day period. These results are in agreement with uptake studies of other diphenylether herbicides as previously reported (1, 2, 3, 4, 5, 7, 8). This accumulation in the roots appeared to be a result of rapid conjugation of the bifenox and/or its metabolites which prevented their translocation to the shoots.

The residue in the above-ground portion of the crop plants declined from day 7 to day 28. This decline indicated an initial rapid absorption (or adsorption) of chemical during emergence and little translocation to the shoots after day 7. The results are best explained as a dilution effect due to an increase in dry matter over the growing period.

Velvetleaf, as a weed species susceptible to the herbicide, showed an accumulation of ^{14}C in the roots and a constant ratio in the shoots. In contrast to the crop plants, velvetleaf appeared to absorb and translocate ^{14}C-compound(s) to a greater extent over the growing period, a possible basis for the selectivity of bifenox. Although the rate utilized in this experiment was sublethal to velvetleaf, the plants were stunted but flowered by day 60.

The differential in residue accumulation of the phenoxy-ring-labeled compound over that of the benzene-ring-labeled compound in all plants,

Table II-1. Total ^{14}C uptake by corn seedlings growing in a greenhouse soil mixture following preemergence application of ^{14}C-bifenox at the rate of 1.7 kg/ha.[a]

Day	Benzene-ring		Phenoxy-ring	
	Root	Shoot	Root	Shoot
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
7	1.02	2.79	1.64	10.35
14	13.06	3.83	12.72	3.86
28	10.94	3.11	36.79	2.18

[a] Results are the average of two plants from each treatment and expressed as ppmw bifenox or its equivalent.

Table II-2. Total ^{14}C uptake by soybean seedlings growing in a greenhouse soil mixture following preemergence application of ^{14}C-bifenox at the rate of 1.7 kg/ha.[a]

Day	Benzene-ring		Phenoxy-ring	
	Root	Shoot	Root	Shoot
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
7	1.27	2.39	1.97	4.57
14	3.59	1.68	3.56	6.12
28	26.98	2.09	21.21	4.72

[a] Results are the average of two plants from each treatment and expressed as ppmw bifenox or its equivalent.

Table II-3. Total ^{14}C uptake by velvetleaf plants growing in a greenhouse soil mixture following preemergence application of ^{14}C-bifenox at the rate of 1.7 kg/ha.[a]

Day	Benzene-ring		Phenoxy-ring	
	Root	Shoot	Root	Shoot
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
7	0.49	7.08	1.03	12.30
14	3.49	5.89	9.47	15.95
60	9.29	5.96	27.89	13.47

[a] Results are the average of two plants from each treatment and expressed as ppmw bifenox or its equivalent.

may have been a result of cleavage between the rings which allowed a more easily translocated metabolite to accumulate. This point is discussed further in sections III and IV of this dissertation.

Autoradiography. A comparison of the autoradiographs in Figure II-1, showed the ^{14}C-label to be associated with the coleoptile of corn and the cotyledons of soybean which were the parts of the plants in contact with the treated soil during emergence. Velvetleaf, in contrast, showed some accumulation in the primary as well as the cotyledonary leaves. There was no difference in the patterns of absorption between the benzene and phenoxy-ring-labeled bifenox treatments. It could not be determined from this study, whether the site of absorption was at the root or cotyledonary tissue.

Uptake in crop plants through maturity. The pattern of ^{14}C-residue accumulation in this study (Tables II-4 to II-9) paralleled that of the previous experiment, that is, a dilution effect was noted over the growing period in the shoots and there was an accumulation in the roots. Another similarity was the differential between the uptake of the phenoxy and benzene-ring-labeled compounds.

The results also showed that the uptake was rate dependent within the range used. An exception was noted in root accumulation of the day 42 harvest of phenoxy-ring treated plants (Tables II-5 and II-8). This exception can best be explained on the premise that cleavage of the ether linkage occurred and there was conjugation of the compounds within the roots.

Figure II-1. Autoradiographs of seedlings growing in a greenhouse soil mixture and harvested 14 days after preemergence application of ^{14}C-bifenox at the rate of 1.7 kg/ha. (A) Corn. (B) Soybean. (C) Velvetleaf.


A
B
C

Table II-4. Total ^{14}C uptake by corn plants harvested 21 days after preemergence application of ^{14}C-bifenox to unsterilized Frederick clay loam soil under greenhouse conditions.[a]

Rate	Benzene-ring		Phenoxy-ring	
	Root	Shoot	Root	Shoot
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
2.2 kg/ha	5.11	1.74	7.37	3.21
4.5 kg/ha	4.27	2.25	22.87	11.25

[a] Results are the average of two plants from each treatment and expressed as ppmw bifenox or its equivalent.

Table II-5. Total ^{14}C uptake by corn plants harvested 42 days after preemergence application of ^{14}C-bifenox to unsterilized Frederick clay loam soil under greenhouse conditions.[a]

Rate	Benzene-ring		Phenoxy-ring	
	Root	Shoot	Root	Shoot
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
2.2 kg/ha	7.30	1.17	58.23	8.78
4.5 kg/ha	25.71	4.22	62.84	12.28

[a] Results are the average of two plants from each treatment and expressed as ppmw bifenox or its equivalent.

Table II-6. Total ^{14}C uptake by corn plants harvested 117 days after preemergence application of ^{14}C-bifenox to unsterilized Frederick clay loam soil under greenhouse conditions.[a]

Rate	Benzene-ring		Phenoxy-ring	
	Ear	Shoot	Ear	Shoot
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
2.2 kg/ha	0.11	0.93	0.33	1.26
4.5 kg/ha	0.60	1.20	0.76	2.87

[a] Results are the average of two plants from each treatment and expressed as ppmw bifenox or its equivalent.

Table II-7. Total ^{14}C uptake by soybean plants harvested 21 days after preemergence application of ^{14}C-bifenox to unsterilized Frederick clay loam soil under greenhouse conditions.[a]

Rate	Benzene-ring		Phenoxy-ring	
	Root	Shoot	Root	Shoot
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
2.2 kg/ha	1.17	5.18	10.36	9.98
4.5 kg/ha	13.01	7.93	32.54	13.21

[a] Results are the average of two plants from each treatment and expressed as ppmw bifenox or its equivalent.

Table II-8. Total ^{14}C uptake by soybean plants harvested 42 days after preemergence application of ^{14}C-bifenox to unsterilized Frederick clay loam soil under greenhouse conditions.[a]

Rate	Benzene-ring		Phenoxy-ring	
	Root	Shoot	Root	Shoot
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
2.2 kg/ha	--	--	60.72	4.50
4.5 kg/ha	40.39	4.12	54.27	4.90

[a] Results are the average of two plants from each treatment and expressed as ppmw bifenox or its equivalent.

Table II-9. Total ^{14}C uptake by soybean plants harvested 101 days after preemergence application of ^{14}C-bifenox to unsterilized Frederick clay loam soil under greenhouse conditions.[a]

Rate	Benzene-ring		Phenoxy-ring	
	Bean	Shoot	Bean	Shoot
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
2.2 kg/ha	0.79	0.76	0.45	2.49
4.5 kg/ha	0.31	2.38	0.98	5.61

[a] Results are the average of two plants from each treatment and expressed as ppmw bifenox or its equivalent.

That some translocation of ^{14}C-compound(s) in crop plants took place was indicated by the amount of residue found in the seed parts of both corn and soybean (Tables II-6 and II-9). Again, the uptake was rate dependent and there was a greater accumulation of the phenoxy-ring-labeled compound(s).

Rotation crop. There was no difference in yield of plant dry matter of treated oat and velvetleaf when compared to that of controls. The results indicated that the residue found in velvetleaf (Table II-10) was either: (1) an accumulation of metabolites; (2) an amount of active ingredient below the inhibitory level; or (3) a combination of these. The amount of ^{14}C-residue in the top 12 cm of the soil prior to planting the rotation crop was not appreciably reduced from that present on day 21 and was high in metabolites.[3] In addition, the oat plants had a higher level of accumulation than did velvetleaf. These results indicated that the residues in the rotation plants were alterations of the bifenox, such as metabolites and/or conjugation products.

Residue in volunteer weed species. The amount of residue in volunteer weeds harvested on day 117 (Table II-11) and day 250 (Table II-12) after treatment varied according to species. Common chickweed (*Stellaria media* (L.) Cyrillo) and yellow woodsorrel (*Oxalis stricta* L.) had the highest amounts of residue among the weeds. The prostrate nature of these two species allowed for greater contact area between

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[3] See Section IV - Metabolism of ^{14}C-Bifenox in Plants and Soil.

Table II-10. Total ^{14}C residue in oat and velvetleaf seeded as a rotation crop 250 days after treatment of the Frederick clay loam soil with ^{14}C-bifenox. Plants were harvested 60 days after seeding or 310 days after soil treatment.[a]

Sample	Benzene-ring		Phenoxy-ring	
	2.2 kg/ha	4.5 kg/ha	2.2 kg/ha	4.5 kg/ha
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
Oat shoot	0.47	1.72	0.83	2.18
Oat seed head	0.06	0.15	0.14	0.25
Velvetleaf shoot	1.35	0.24	0.21	0.44

[a] Results are the average of duplicate samples consisting of the total harvest (25 to 30 oat plants; 11 to 15 velvetleaf plants) and expressed as ppmw bifenox or its equivalent.

Table II-11. Total ^{14}C residue in the shoots of volunteer weeds harvested 117 days after preemergence application of ^{14}C-bifenox to Frederick clay loam soil.[a]

Weed Species	Benzene-ring		Phenoxy-ring	
	2.2 kg/ha	4.5 kg/ha	2.2 kg/ha	4.5 kg/ha
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
Common chickweed (*Stellaria media* (L.) Cyrillo)	6.32	6.97	4.03	21.20
Yellow woodsorrel (*Oxalis stricta* L.)[b]	1.10	--	--	21.14
Buckhorn plantain (*Plantago lanceolata* L.)	0.43	--	--	--
White clover (*Trifolium repens* L.)	0.52	--	--	--
Quackgrass (*Agropyron repens* (L.) Beauv.)	--	--	5.71	--
Broadleaf dock (*Rumex obtusifolius* L.)	--	1.27	--	--
Large crabgrass (*Digitaria sanguinalis* (L.) Scop.)[b]	--	1.10	--	--
Burdock (*Arctium minus* (Hill) Bernh.)	--	--	1.19	--
Perennial sowthistle (*Sonchus arvensis* L.)	5.42	--	--	--

[a] Results are based on one sample for each treatment and expressed as ppmw bifenox or its equivalent. Samples consisted of several plants when available.

[b] Weed species susceptible to bifenox.

Table II-12. Total ^{14}C residue in the shoots of volunteer weeds harvested 250 days after preemergence application of ^{14}C-bifenox to Frederick clay loam soil.[a]

Weed Species	Benzene-ring		Phenoxy-ring	
	2.2 kg/ha	4.5 kg/ha	2.2 kg/ha	4.5 kg/ha
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
Large crabgrass (*Digitaria sanguinalis* (L.) Scop.)[b]	--	0.40	--	--
Yellow woodsorrel (*Oxalis stricta* L.)[b]	1.48	0.96	--	12.52
Buckhorn plantain (*Plantago lanceolata* L.)	0.45	--	--	--
Common chickweed (*Stellaria media* (L.) Cyrillo)	--	3.14	2.62	--
Lambsquarters (*Chenopodium album* L.)[b]	--	0.40	0.25	--
Yellow rocket (*Barbarea vulgaris* R. Br.)	1.28	--	--	--
Burdock (*Arctium minus* (Hill) Bernh.)	--	--	2.13	--
Wild carrot (*Daucus carota* L.)	0.41	0.45	--	--
Broadleaf dock (*Rumex obtusifolius* L.)	0.18	1.91	--	--
Fall panicum (*Panicum dichotomiflorum* Michx.)[b]	0.24	--	--	1.38
Prickly sida (*Sida spinosa* L.)[b]	--	--	--	1.00

[a] Results are based on one sample for each treatment and expressed as ppmw bifenox or its equivalent. Samples consisted of several plants when available.

[b] Weed species susceptible to bifenox.

the tissue and treated soil which probably accounted for the higher levels of residue accumulation. Where the availability of plants permitted analysis, there was a higher residue accumulation at the 4.5 kg/ha application rate than at 2.2 kg/ha.

In summary, the susceptible weed used in these studies (velvetleaf), had a greater residue accumulation in the shoots than did the crop plants during early periods of growth after application of bifenox. This pattern was altered with time from application and indicated a loss of active ingredient by metabolism or other means.

The greatest amount of residue in the crop plants (corn and soybean) was found immediately after emergence and decreased with an increase in plant dry matter through maturity. The residue found in the seed of corn and soybean at maturity indicated that some translocation of bifenox or its metabolites occurred in the resistant crop plants.

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III. DIFFERENTIAL ABSORPTION AND DISTRIBUTION AS A BASIS FOR THE SELECTIVITY OF BIFENOX.

Abstract. The patterns of absorption and distribution of ^{14}C-bifenox [methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate], were different among corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.) and velvetleaf (*Abutilon theophrasti* Medic.). Absorption of ^{14}C-compound(s) from treated nutrient solution accumulated in the roots of the three species but to a greater extent in soybean. There was no difference in the total transport to the shoots. However, in corn and soybean the ^{14}C-compound(s) was confined to the primary and secondary leaf veins while velvetleaf showed a general distribution throughout the leaf tissue. Velvetleaf absorbed and translocated bifenox from shoot zones to a greater extent than the crop plants. Some acropetal movement was noted following leaf application but movement was only 3% of applied ^{14}C from the treated leaf. No movement was detected in soybean.

INTRODUCTION

The herbicide methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate (bifenox) is presently registered for preemergence use in soybeans (*Glycine max* (L.) Merr.) and shows good control of a wide range of broadleaf and grass weeds. It also shows selective control in corn (*Zea mays* L.) and rice (*Oryza sativa* L.) and registration is expected for these crops.

Information concerning the basis of selectivity is needed to expand the use of bifenox in other crops and is of interest since this selectivity is not based on taxonomic differences. There are no reports on the mechanism of selectivity of bifenox and, indeed, little on the class of diphenylether herbicides. In general, selectivity of this class appears to be associated with the rate of metabolism (6, 7), extent of adsorption or conjugation (8, 17), and the rate of translocation (1, 3, 5, 11, 14, 17).

Several investigators (12, 13) have stressed the importance of shoot entry for the uptake of preemergence herbicides. However, the reports dealing with the selectivity of diphenylethers do not consider this as an alternative to root absorption.

The objective of this study was to determine by radiochemical techniques if differential absorption and distribution of bifenox could account for its selectivity, and to assess the absorption by different plant organs.

MATERIALS AND METHODS

Differential uptake. Seeds of corn (Dekalb XL66), soybean (York), and velvetleaf were germinated in horticultural grade vermiculite at 25 ± 2 C and 40% relative humidity (RH) under a 16-hr photoperiod. When the seedlings reached a height of 8 cm they were transferred to 1-liter plastic pots containing aerated 0.5 strength Hoagland and Arnon's (10) nutrient solution. The plants were maintained in a growth chamber at 25° C, 68% RH, and a 16-hr photoperiod of 5,900 lux provided by a combination of cool white fluorescent and incandescent lamps. Because of the different growth rates among the plant species, the transfer and treatment was made at different ages according to Table III-1.

Treatment of each species was as follows: Three pots containing two plants each were treated with ^{14}C-bifenox randomly labeled in the benzene ring (specific activity 17.25 μCi/mg), and three pots with randomly labeled phenoxy-ring compound (specific activity 2.42 μCi/mg),[1] by addition to the nutrient solution. Final concentration in each pot was 50 ppm (w/v). Aeration of the solution surrounding the roots was stopped during the 72-hr treatment period.

At harvest, one plant from each pot was placed in running distilled water for 10 min, quick-frozen in dry ice, and placed in a freeze-dry apparatus. After drying, the plants were prepared for autoradiography according to the method described by Crafts and Yamaguchi (4).

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[1] Donated by Mobil Chemical Company, Edison, New Jersey.

Table III-1. Plant age from day of seeding to various stages of treatment.

Plant	Transferred to nutrient culture	Treated with ^{14}C-bifenox	Harvest
	(days)	(days)	(days)
Corn	7	14	17
Soybean	10	17	20
Velvetleaf	13	31	34

The duplicate plant from each pot was severed at the root-stem transition region and the root and shoot dried in an oven at 60° C. The dried plants were then ground in a Wiley mill, fitted with a number 20 mesh screen, thoroughly mixed and a 300 to 500 mg sample removed for combustion.

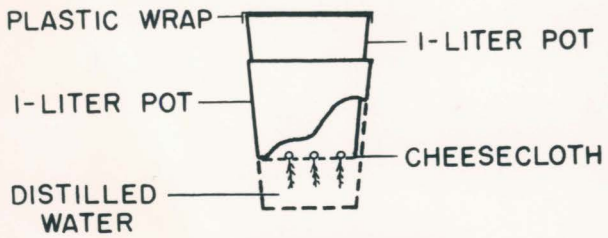
Sample oxidation was by the method of Peterson (15). The collected samples were assayed by liquid scintillation counting and the results expressed as ppm (w/w) on a dry-weight basis.

Soil placement. This study was designed to measure the uptake of ^{14}C-bifenox by different organs of corn, soybean, and velvetleaf exposed to treated soil (1 ppmw). Three organs were distinguished: (1) root, (2) cotyledon(s), and (3) coleoptile of corn or hypocotyl of soybean and velvetleaf.

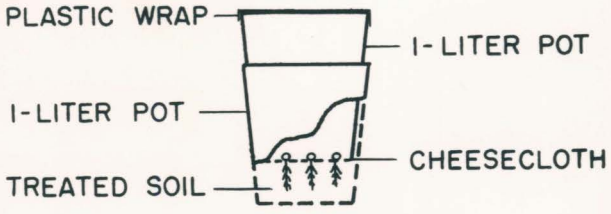
The procedure for treatment (Fig. III-1) was a modification of the methods described by several investigators (2, 16). To treat the roots only, the bases of 1-liter plastic pots were replaced with open-weave cheesecloth. The seeds were placed on the cloth and the pots placed inside solid-base pots of equal size with the distilled water height brought to the point of wetting the cloth. Plastic wrap was placed over the pots to prevent desiccation and germination allowed to proceed in the dark. When the radicles reached approximately 1 cm, the water was replaced with treated soil and the plants allowed to grow under a 12-hr photoperiod at 25° C for a period of 10 days.

Treatment of the cotyledonary zone was accomplished by placing a

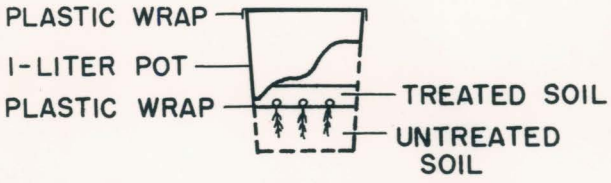
Figure III-1. Experimental procedure used to expose different organs of corn, soybean, and velvetleaf to soil treated with 1 ppmw ^{14}C-bifenox.



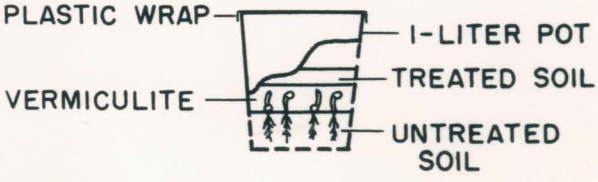
GERMINATION



ROOT TREATMENT



COTYLEDON TREATMENT



COLEOPTILE OR HYPOCOTYL TREATMENT

two-ply layer of plastic wrap over untreated soil, puncturing holes with a micropipet, and the radicles placed into the soil so that the cotyledon(s) rested on the plastic. Treated soil to a 1-2 cm depth (depending on species) was then placed over the cotyledons. The treated plants were allowed to grow under the conditions described for root treatment.

For the third treatment, seeds were germinated in a vermiculite layer over untreated soil. When the coleoptiles of corn or hypocotyls of soybean and velvetleaf reached a height of 2 cm, vermiculite was added in a layer to the top of the seedling and a 1-cm layer of treated soil placed over the vermiculite. The pots were then placed in a chamber at 25° C and 12-hr photoperiod until harvest, 10 days after treatment.

Each soil layer was brought to near field capacity prior to placement in the pots. Plastic wrap was placed over the pots to prevent desiccation during the treatment period.

The treatments consisted of three replications of six plants per pot. At harvest, one plant was removed from each replicate and prepared for autoradiography as described previously. The remaining five plants (roots not included) in each pot were combined, dried, and ground as one sample for combustion.

Foliar application. Seeds of corn, soybean, and velvetleaf were germinated in vermiculite at 25 $\pm$ 2 C and 40% RH under a 16-hr photoperiod of 5,900 lux. The seedlings were allowed to grow under the same conditions until treatment by subirrigation with 0.5 strength Hoagland and

Arnon's nutrient solution.

Treatment of corn seedlings was by a single 10 µl droplet containing 0.01 µCi of ^{14}C-bifenox (benzene-ring-labeled) and 0.5% Tween 20 [polyoxyethylene (20) sorbitan monolaurate], a nonionic surfactant. At the time of treatment, the corn was in the three-leaf stage. Soybean and velvetleaf were treated by placement of three-10 µl droplets (0.03 µCi) on a single primary leaf. The soybean plants were in the first trifoliate leaf stage and the velvetleaf in the three-leaf stage. A total of 10 plants of each species was treated.

Duplicate plants of each species were harvested at 8, 16, 24, 48, and 72 hr after treatment and freeze-dried for one week. After drying, one plant was pressed and used for autoradiography. The duplicate plant was sectioned, oxidized and the ^{14}C assayed by liquid scintillation counting.

Chromatography. Extracted plant samples were assayed by comparative and co-chromatography with 18 synthesized possible metabolites and ^{14}C-bifenox on silica gel plates (Eastman Chromagram with fluorescent indicator). Areas of ^{14}C were detected by strip scanning and/or autoradiography. One-dimensional solvent systems included: chloroform-methanol-acetic acid (75:20:5), benzene-acetic acid (50:4), and benzene-chloroform (8:2).

RESULTS AND DISCUSSION

^{14}C Uptake from nutrient solution. The total ^{14}C uptake by soybean, corn, and velvetleaf is shown in Table III-2. There was no difference among the species in the amount of ^{14}C translocated to the shoots. In agreement with investigations of other diphenylether herbicides (17, 18), there was a significant accumulation of ^{14}C-compound(s) associated with the roots of soybean over that of the other species tested. The root accumulation appeared to be the result of rapid metabolism by soybean of the bifenox to nitrofen (2,4-dichlorophenyl 4-nitrophenyl ether) which may have been bound to root substances (9).

Thin layer chromatography (TLC) of methanol-water (80% v/v) extracts from the roots of soybeans, resulted in spots (in order of intensity by autoradiography) corresponding to nitrofen, bifenox, and the hydrolysis product of bifenox; 5-(2,4-dichlorophenoxy)-2-nitrobenzoic acid. There were two compounds in the soybean shoot extract: bifenox and nitrofen; the latter was present in a greater amount as shown by spot intensity. There was some hydrolysis of bifenox to its acid in corn plants but the amount was less than 1% as measured by liquid scintillation counting of the area.

In extracts of velvetleaf roots, unaltered bifenox was the major compound with less than 1% of the acid appearing. The shoots, however, contained the acid as the major compound with nitrofen and bifenox at low concentrations. There was no breakdown of bifenox in the nutrient solution.

Table III-2. Total ^{14}C in plants grown in nutrient solution containing 50 ppm (w/v) radiolabeled bifenox for a period of 72 hr before harvest.[a]

Plant	Benzene-ring		Phenoxy-ring	
	Root	Shoot	Root	Shoot
	(ppmw)[b]	(ppmw)	(ppmw)	(ppmw)
Soybean	114.30a	0.89a	223.83a	3.21a
Corn	19.23b	0.88a	63.19b	5.12a
Velvetleaf	24.12b	1.27a	61.66b	2.84a

[a] Values not followed by the same letter in each column are significantly different at 0.05% level by Duncan's multiple range test.

[b] Results are expressed as ppmw bifenox or its equivalent on a dry-weight-basis.

As mentioned, there was no difference in acropetal translocation of total ^{14}C among the species. However, the pattern of accumulation as seen by autoradiography in Figure III-2 was different in velvetleaf from that of corn and soybean. The label was confined to the stem and primary leaf veins of soybean shoots (A). In corn, the label was apparent in the culm and the primary and secondary leaf veins (B). Velvetleaf autoradiographs showed ^{14}C throughout the leaf and stem tissue (C). The lack of ^{14}C-label in the primary and secondary veins of the older leaves was indicative of rapid transport from the conducting tissue to the surrounding tissue. The patterns of ^{14}C distribution were not different between the benzene and phenoxy-ring-labeled bifenox.

Soil placement. As determined by sample oxidation and liquid scintillation counting, the total ^{14}C-bifenox in above-ground portions of the plants was greater in velvetleaf than in corn or soybean (Table III-3). The results showed that velvetleaf absorbed greater amounts of ^{14}C-bifenox at all zones and was capable of translocating the chemical after root absorption. The slightly elevated values for soybean treated at the cotyledonary and coleoptile/hypocotyl zones were attributed to the absorption or adsorption of the cotyledons (included in the sample) with a greater surface area in contact with the treated soil.

Autoradiographs of the treated plants showed the label to be located in (or on) those portions of the crop plants which were in

Figure III-2. Autoradiographs of plants grown in nutrient solution after 72 hr root exposure to 50 ppm (w/v) of benzene-ring-labeled ^{14}C-bifenox. (A) Corn. (B) Soybean. (C) Velvetleaf.


A
B
C

Table III-3. Total ^{14}C in the shoots of emerging seedlings having different organs exposed to ^{14}C-bifenox treated soil (1 ppmw) for a period of 10 days.[a]

Treatment zone	Corn	Soybean	Velvetleaf
	(ppmw)	(ppmw)	(ppmw)
Root	0.02 ± 0.01	0.01 ± 0.00	0.32 ± 0.00
Cotyledon(s)	0.02 ± 0.00	0.13 ± 0.03	0.26 ± 0.13
Coleoptile/hypocotyl	0.03 ± 0.01	0.03 ± 0.02	0.11 ± 0.00

[a] Results are ppmw of bifenox or its equivalent on a dry-weight-basis, results also show the error of the mean based on three replications.

contact with the treated soil. The low amount of ^{14}C in the plants prevented good resolution of the autoradiographs and therefore descriptive results are listed in Table III-4. The results showed a differential absorption and translocation of ^{14}C-compound(s) from all tissue zones among the crop and weed species studied.

Leaf application of ^{14}C-bifenox. Autoradiographs of the plants harvested 72 hr after treatment (Figure III-3), showed absorption of the ^{14}C-bifenox with slight acropetal movement for all species. Results of earlier harvests showed a progressive amount of absorption over the 48-hr time period. Visual observation of the treated leaf gave no indication of tissue damage even at the site of application.

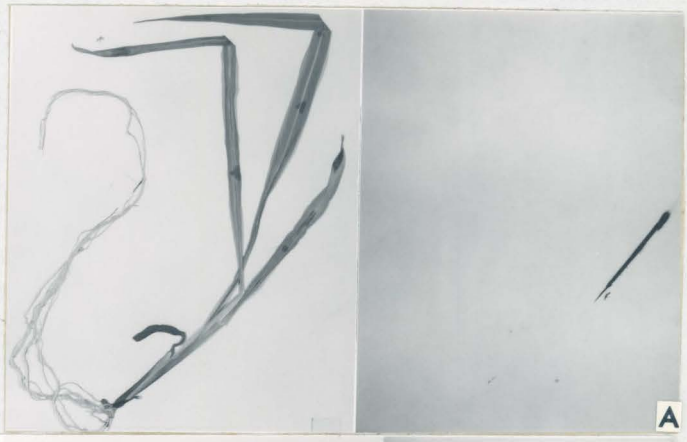
Quantitative analysis of the duplicate plants by sample oxidation, showed movement of a low level of ^{14}C from the treated leaf in corn and velvetleaf. In corn, 2% of the total ^{14}C applied was found in the culm and younger leaves. The velvetleaf translocated 3% of the total and this was found to be in the stem and leaves above the treated leaf. No movement was detected in the soybean.

The results of these studies showed that velvetleaf, as the susceptible weed species to bifenox, absorbed and translocated a greater amount of chemical from those areas of emerging seedlings which were in contact with treated soil. The pattern of translocation (Figure III-2) after absorption, i.e., the movement of chemical from the transporting vessels into the surrounding tissue and hence to the site of action, could account for the susceptibility of velvetleaf to the

Table III-4. Descriptive results of the autoradiography of plants having different organs exposed to ^{14}C-bifenox for a period of 10 days.

Treatment zone	Location of ^{14}C-label		
	Corn	Soybean	Velvetleaf
Root	culm, leaves	hypocotyl	throughout
Cotyledon(s)	culm, coleoptile	hypocotyl, cotyledons	throughout
Coleoptile/hypocotyl	coleoptile	hypocotyl, cotyledons	throughout

Figure III-3. Autoradiographs of plants harvested 72 hr after leaf application of benzene-ring-labeled ^{14}C-bifenox. (A) Corn. (B) Soybean. (C) Velvetleaf.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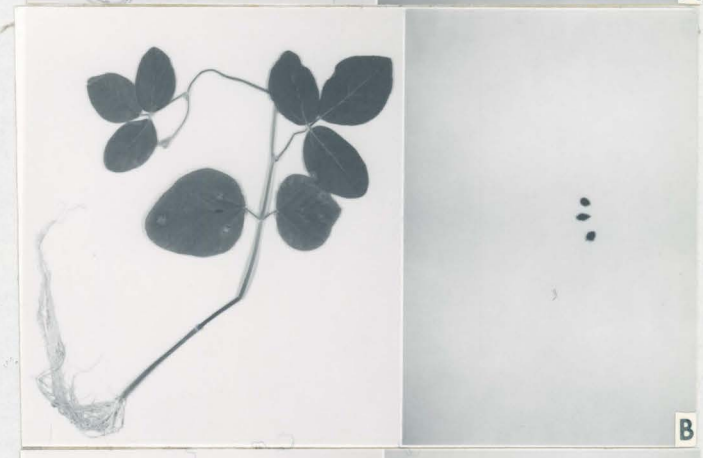
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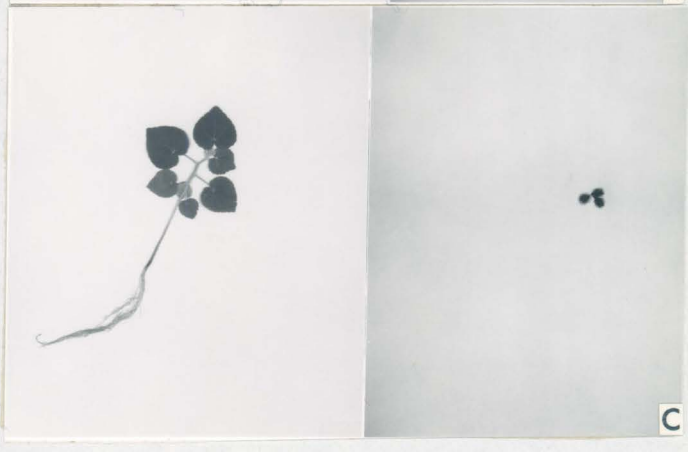
C

herbicide.

The resistant crop plant showed a minimal translocation of the chemical from the treated zones (Tables III-3 and III-4) and, as shown by utilizing high rates in nutrient culture, the movement was confined to the transport vessels possibly as a result of metabolism and/or conjugation.

It is concluded that the selectivity of bifenox is based on the differential uptake and movement of the herbicide to the site of action among species.

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IV. METABOLISM OF ^{14}C-BIFENOX IN SOIL AND PLANTS

ABSTRACT

The herbicide, methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate (bifenox), had a half-life of 3 to 7 days after preemergence application to soil. Metabolites identified included: 5-(2,4-dichlorophenoxy)-2-nitrobenzoic acid, 2,4-dichlorophenyl 4-nitrophenyl ether (nitrofen), and 5-(2,4-dichlorophenoxy) anthranilic acid over a 313-day sampling period. Comparison of the total ^{14}C in the soil to that extractable by methanol showed an increase in the proportion of bound material. The major metabolite eluted from a soil column was identified as the acid of bifenox and its mobility was associated with the short half-life of bifenox in soil. *In vitro* studies with shoot-tissue macerates showed that bifenox was not degraded by corn (*Zea mays* L.) and soybeans (*Glycine max* (L.) Merr.), and to less than 1% by velvetleaf (*Abutilon theophrasti* Medic.).

INTRODUCTION

The herbicide, methyl 5-(2,4-dichlorophenoxy)-4-nitrobenzoate (bifenox) is a recently introduced product in the class of substituted diphenylethers. This compound is used for the control of a wide range of annual broadleaf and grass weeds in soybeans. With further development, registration for corn and rice (*Oryza sativa* L.) can be expected. Its use in food crops makes it essential that we know the extent and type of degradation and/or dissipation in soil and plants.

Information on the biochemistry of bifenox degradation is limited to the manufacturer's technical bulletin[1] and three abstracts (1, 2, 3). Biochemical reactions known to occur in other herbicide degradation (in plants and/or micro-organisms) include: 1) beta oxidation; 2) cleavage of an ether linkage; 3) ring hydroxylation; 4) ring cleavage; 5) ester hydrolysis; 6) dehalogenation; and 7) N dealkylation. The objective of this investigation was to elucidate, in greenhouse and laboratory studies of soil and plant reactions, the degradative pathway of bifenox.

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[1] Mobil Chemical, Technical Bull. MODOWN™ Herbicide, 4 Feb. 1974.

MATERIALS AND METHODS

Radiochemicals. Bifenox, randomly labeled in either the benzene ring (specific activity 9.4 μCi/mg) or phenoxy ring (specific activity 2.42 μCi/mg) was used throughout these studies with the exception of the soil retention experiment which utilized benzene-ring-labeled bifenox (specific activity 17.25 μCi/mg). The ^{14}C-bifenox (>98% purity) was supplied by Mobil Chemical Company, Edison, New Jersey.

Greenhouse studies. Two test regimes were utilized to study the metabolism of ^{14}C-bifenox applied preemergence to seeded corn (Dekalb XL66), soybean (York), oat (*Avena sativa* L. Clinton), and velvetleaf. In the first test, galvanized metal flats (30 by 20 by 8 cm) were filled with a soil mixture of loam, peat moss, and sand in a 2:1:1 (v/v/v) ratio. Each flat was seeded by row with corn, soybean, and velvetleaf. The soil surface was then sprayed with ^{14}C-bifenox labeled in either the benzene or phenoxy ring (parallel experiments) at the rate of 1.7 kg/ha. The flats were maintained under greenhouse conditions utilizing subirrigation for a period of 313 days. Corn and soybeans were harvested on days 7, 14, and 28 after seeding. Velvetleaf was harvested on days 7, 14, and 60. Soil samples were taken to 8 cm at intervals throughout the test period.

The second test utilized a Frederick clay loam field soil contained in plastic pots with a capacity of 11.7 L. Corn and soybean were seeded in separate pots and the soil sprayed with ^{14}C-bifenox at the rates of 2.2 and 4.5 kg/ha. Again, parallel experiments were performed

with benzene-ring- and phenoxy-ring-labeled bifenox. Plants were harvested from each pot on days 21, 42, and at maturity (day 101 - soybeans; day 117 - corn).

After the final harvest, the soil was allowed to remain fallow with regular surface irrigation until day 250, at that time the soil was tilled and seeded with oat and velvetleaf in combination. The plants were harvested after 60 days. Soil samples to a 12 cm depth were taken on days 21, 117, and 250.

All soil samples were dried and a 300-500 mg sample removed for oxidation. The remaining soil was extracted with 80% methanol by soxhlet, concentrated and used for thin layer chromatography (TLC) and liquid scintillation counting (LSC).

Plant samples removed from the two experiments were dried at 60 C in an oven. After drying, the plants were ground in a Wiley mill fitted with a 20 mesh screen, thoroughly mixed and after removal of a 300-500 mg sample for combustion, placed in a 43 by 123 mm cellulose extraction thimble. When the samples were large, only enough material was used to fill the thimble to within 30 mm of the top. The samples were then extracted by soxhlet for 24 hr with 80% methanol. The resulting extract was concentrated to 10 ml and used for TLC and an aliquot for LSC.

Soil retention. Field soil from the control pots used in the second greenhouse study was sifted through a 5 mm mesh screen to remove stones and roots. The sifted soil was then moistened and placed in a glass column having a 2 cm inside diameter and a 50 cm length. The column

was vibrated to allow some packing of the soil while filling. The soil column was 2 by 40 cm at the start of the experiment.

Three mg of benzene-ring-labeled ^{14}C-bifenox (51.75 µCi) was dissolved in 50 ml benzene and placed drop-wise on the column at a slow rate to allow for evaporation of the benzene. After a 3-hr equilibration period, elution of the column with distilled water contained in a separatory funnel was started. The flow rate was adjusted to the rate of movement through the column to avoid pooling on top of the soil.

The eluate was collected in 50 ml fractions which were then evaporated to dryness and re-suspended in methanol. Each fraction was assayed for total ^{14}C and chromatographed on silica gel thin-layer plates for metabolites and/or breakdown products.

A total volume of 2,650 ml was collected over a period of 100 days. To prevent establishment of complete anaerobic conditions, the experiment was terminated when the flow rate slowed to 8 ml per day. The final soil column length was 32 cm.

In vitro metabolism. Seedlings of corn (culm and leaves) were macerated by blender (slow speed for 10 min) in 0.01 M potassium phosphate buffer, *p*H 6.2, in a ratio of 5 ml buffer to 1 gm fresh tissue. After maceration, 19 ml of the slurry were placed in 50 ml flasks and ^{14}C-bifenox added. The final volume of 20 ml had a concentration of 20 ppm (w/v) active ingredient. Duplicate experiments were performed utilizing both benzene and phenoxy-ring-labeled bifenox.

The incubation mixture was slowly shaken at room temperature (25° C ± 3) under constant fluorescent light. At the appropriate times of 0.5, 1, 2, 4, 8, 16, 24, and 48 hr, 20 ml of methanol was added to the mixture of three flasks each time to stop any reaction. Further extraction was by soxhlet with 90% methanol for a 24 hr period. The resulting extract was concentrated for TLC. The experiment was repeated with soybean and velvetleaf seedlings.

Chromatography. Extracted soil and plant samples were assayed by comparative and co-chromatography with 18 synthesized possible metabolites and ^{14}C-bifenox on silica gel plates (Eastman Chromagram with fluorescent indicator). Areas of ^{14}C were detected by strip scanning and/or autoradiography. One-dimensional solvent systems included: chloroform-methanol-acetic acid (75:20:5), benzene-acetic acid (50:4), and benzene-chloroform (8:2). Table IV-1, lists the synthesized possible metabolites and degradation products of bifenox with the Rf values obtained in the three solvent systems.

Table IV-1. Synthesized possible metabolites and degradation products of bifenox with the Rf values obtained in three solvent systems.

Chemical[a]	Solvent system[b]		
	I	II	III
Bifenox	0.84	0.54	0.51
2,4-Dichlorophenyl 4'-nitrophenyl ether	0.88	0.44	0.63
Methyl 5-(2',4'-dichlorophenoxy) anthranilate	0.84	0.48	0.49
5-(2',4'-Dichlorophenoxy) anthranilic acid	0.76	0.33	0.03
5-(2',4'-Dichlorophenoxy)-2-nitrobenzoic acid	0.65	0.19	0.03
Methyl 5-hydroxy-2-nitrobenzoate	0.80	0.17	0.02
Methyl 5-hydroxyanthranilate	0.77	0.08	0.04
5-Hydroxy-2-nitrobenzoic acid	0.33	0.00	0.00
5-Hydroxyanthranilic acid	0.24	0.00	0.00
4-(2',4'-Dichlorophenoxy) aniline	0.83	0.24	0.36
p-Nitrophenol (Aldrich)	0.72	0.20	0.07
p-Aminophenol (MCB)	0.47	0.00	0.00
2,4-Dichlorophenol (Aldrich)	0.77	0.43	0.35
MC 1440, azoxy-nitrofen	0.90	0.54	0.58
MC 1437, azo-nitrofen	0.89	0.61	0.56
MC 1308, azoxy-bifenox	0.90	0.57	0.49
MC 1431, azo-bifenox	0.90	0.58	0.45
MC 1289, azoxy-bifenox acid	0.78	0.11	0.00
MC 1369, azo-bifenox acid	0.77	0.11	0.00

[a] Chemicals synthesized by Mobil Chemical Co., Edison, N. J.

[b] Solvent systems (I) chloroform-methanol-acetic acid (75:20:5); (II) benzene-acetic acid (50:4); and (III) benzene-chloroform (8:2).

RESULTS AND DISCUSSION

Fate of ^{14}C-bifenox in soil. Residue analysis of the soil samples taken from the greenhouse mixture study showed a rapid loss of ^{14}C over a 7 day period (Table IV-2) with an apparent half-life of 3 to 7 days. The residue was characterized by an increase in the amount of bound material (that not extractable by methanol) which resulted in little additional loss of ^{14}C after day 7. Similar results were obtained with field soil in that there was little decrease in the total ^{14}C during the sampling period beginning 21 days after treatment (Table IV-3).

The nature of bifenox dissipation during the 7 day period after application was not determined. Leaching was not a factor in the soil mixture study since subirrigation was the mode of watering and the soil samples were taken to include a core of the full depth contained in the flats. Movement to remote areas of the flat was not considered responsible for dissipation since bifenox has a low solubility (0.35 ppm @ 25 C) in water. The rapid loss coincided with the period of germination and emergence of the crop plants. However, the uptake by the plants[2] was not of the magnitude to account for the 50% loss in bifenox. Further, balance sheet type studies are needed to account for the loss in total ^{14}C-bifenox, particularly during the initial 7 day period following application.

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[2] See Section II - Uptake and Distribution of ^{14}C-Bifenox in Crop and Weed Species.

Table IV-2. Residue analysis of a greenhouse soil mixture after preemergence application of 1.7 kg/ha ^{14}C-bifenox.[a]

Day	Benzene-ring		Phenoxy-ring	
	Total	Bound[b]	Total	Bound[b]
	(ppmw)	(%)	(ppmw)	(%)
0	9.47	4	6.66	3
1	9.31	9	8.92	14
3	4.09	22	5.74	19
7	1.14	26	2.90	20
14	2.22	46	3.43	40
28	1.60	66	1.76	74
180	1.48	64	2.40	78
313	2.28	78	1.49	67

[a] Results are the average of two samples and expressed as ppmw of bifenox or its equivalent.

[b] The percent of bound material was determined by comparison of the total ^{14}C by combustion and the amount extractable by methanol (80%).

Table IV-3. Residue analysis of a Frederick clay loam soil after preemergence application of ^{14}C-bifenox.[a]

Day	Benzene-ring[b]		Phenoxy-ring[b]	
	2.2 kg/ha	4.5 kg/ha	2.2 kg/ha	4.5 kg/ha
	(ppmw)	(ppmw)	(ppmw)	(ppmw)
21	0.97 (49)	1.39 (61)	2.43 (51)	4.21 (53)
117	0.99 (86)	1.27 (88)	3.24 (81)	6.19 (80)
251	1.24 (83)	2.93 (80)	2.53 (81)	5.52 (75)

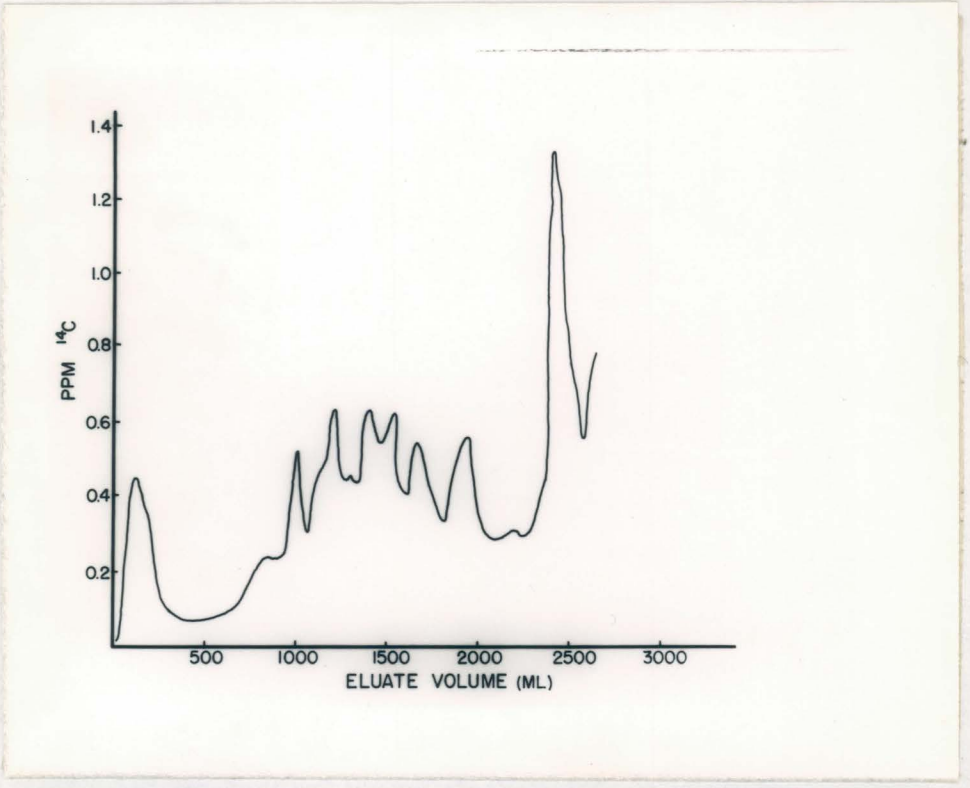
[a] Results are the average of two samples and expressed as ppmw of bifenox or its equivalent.

[b] Numbers enclosed by parentheses are the percent of soil bound residue after methanol (80%) extraction.

Thin layer chromatography of the methanol extracts of soil gave areas identifiable as: (1) bifenox, (2) the acid of bifenox [5-2,4-dichlorophenoxy)-2-nitrobenzoic acid], (3) nitrofen (2,4-dichlorophenyl 4-nitrophenyl ether, (4) 5-(2,4-dichlorophenoxy) anthranilic acid, and (5) unidentified compounds, possible conjugation products. Over the time of the experiment, there was a decrease in the intensity of the areas (as determined by autoradiography) corresponding to bifenox and its acid and an increase in the anthranilic acid. Quantification of the TLC showed that 40% of the total extract applied, remained at the origin and was not identified. These results and those shown in Tables IV-2 and IV-3, indicated that the identifiable compounds, by the procedure used, accounted for less than 15% of the total ^{14}C remaining at the end of the sampling period. Further extraction and more elaborate identification methods are needed to characterize the nature of the remaining compounds.

Results obtained from the eluate of the soil retention study indicated that the initial rapid loss found in the greenhouse study may be characterized by metabolism of the parent compound. The pattern of elution from the soil column was irregular (Figure IV-1) but an overall increase in ^{14}C per fraction was evident over the time period and total eluate collected. TLC of the fractions using the three solvent systems gave two areas of equal intensity through 200 ml of eluate. The first was identified as the hydrolysis product of bifenox, while the second area did not correspond to the synthesized compounds

Figure IV-1. Elution pattern of ^{14}C (ppm w/v) from a soil column following application of ^{14}C-bifenox to the top surface and leached with distilled water. At the end of the elution period, a total of 16.7% of the applied ^{14}C was collected in 2,650 ml eluate.


1.4
1.2
1.0
0.8
0.6
0.4
0.2
PPM ^{14}C
500
1000
1500
2000
2500
3000
ELUATE VOLUME (ML)

and was not identified. The unknown was only slightly detectable after 200 ml of eluate.

The acid of bifenox remained the major metabolite (95%) throughout the remainder of the eluate. After 200 ml, nitrofen was identified in the remainder of the fractions but was present at very low concentrations. In the fractions collected beyond 1000 ml, unaltered bifenox was identified but, like nitrofen, at low concentrations in the remainder of the fractions. No attempt was made to further characterize the ^{14}C-compound(s) remaining on the soil column.

Metabolism of bifenox by plants. Metabolites identified by TLC of the 80% methanol extracts from crop and weed species grown in the greenhouse soil mixture and the field soil were identical to those extracted from the soil. The percent of methanol extractable ^{14}C from the dried plant material averaged 50% of the total and less than 20% of that extract remained at the origin of the TLC plates. It could not be determined if the degradation products in these plants were a result of uptake of the bifenox with subsequent metabolism within the plants or uptake of the altered compounds from the soil.

The *in vitro* studies with shoot tissue macerates of corn and soybean showed no metabolism of bifenox. Velvetleaf metabolized bifenox to its acid but to less than 1% over the 48 hr incubation period. These results are contrary to those of a previous study

utilizing the same species,[3] where the root absorption of ^{14}C-bifenox from nutrient solution resulted in metabolism of the herbicide to its acid and nitrofen. The possibility exists that the metabolism was a result of microbial action on the root surface. Further studies are needed to elucidate the nature of metabolism which apparently takes place in (or on) the roots and not in the shoots of the plants used in these studies.

Metabolic pathway of bifenox. From the studies presented, a proposed metabolism scheme of bifenox was drawn (Figure IV-2). The major pathway of degradation appeared to be through the acid of bifenox to nitrofen and the anthranilic acid. Although not unequivocally identified, methyl 5-(2,4-dichlorophenoxy) anthranilate was included in this scheme as a logical step to its acid. Theissen[4] suggested that the anthranilate was unstable in metabolic systems and was quickly converted to the anthranilic acid which may explain its absence in extracts of soil and plants.

Although the scheme shows ring hydroxylation and conjugation as end products, it is understood that these reactions can and probably do occur with all the compounds shown. As was stated previously, further studies are needed to characterize such products.

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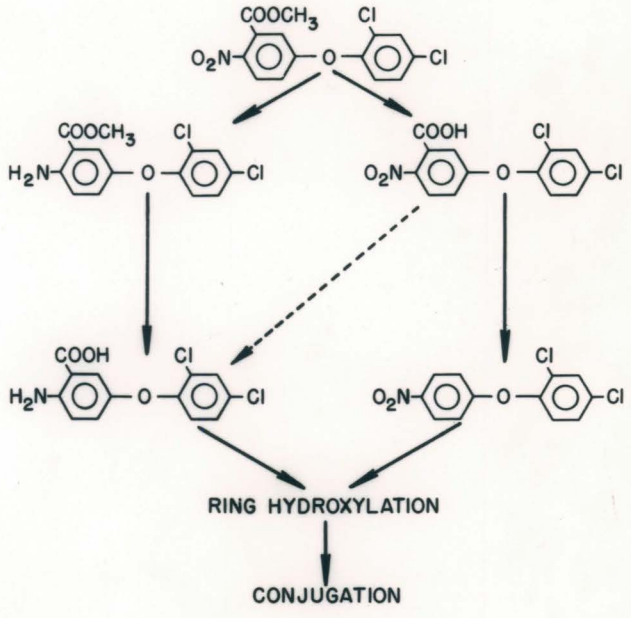
[3] Section III. Differential Absorption and Distribution as a Basis for the Selectivity of Bifenox.

[4] Theissen, R. J. Mobil Chemical Co. (personal communication).

Figure IV-2. Proposed metabolism scheme of bifenox. Compounds in clockwise order are: bifenox, 5-(2,4-dichlorophenoxy)-2-nitrobenzoic acid, nitrofen, 5-(2,4-dichlorophenoxy) anthranilic acid, methyl 5-(2,4-dichlorophenoxy) anthranilate.

PROPOSED METABOLISM SCHEME OF BIFENOX



It was not established in these studies whether cleavage of the ether linkage occurred as part of the degradation of bifenox. Frear and Swanson (4) reported little ether-link-cleavage of bifenox by glutathione S-transferase from pea. An indication that cleavage does occur is found in the greater amount of residue in plants growing with phenoxy-ring-labeled bifenox treatment over that with benzene-ring label.[5] In addition, Hawton and Stobbe (5) reported that cleavage of the ether link in nitrofen occurred in plants and was rapidly conjugated to plant substances. Since nitrofen is a degradation product of bifenox, the possibility of ether-link-cleavage exists.

The results presented here show that bifenox is rapidly degraded to the acid which may be removed from the soil surface by leaching. This loss amounted to about 50% of the total and occurred over a period of 3 to 7 days. The remaining was apparently bound to the soil in an increasing quantity over the time periods measured. Further metabolism probably occurred while the substances were bound to soil colloids.

Plants grown in bifenox treated soil contained the same degradation products as those found in the soil. This indicated that the plants absorbed these products without further breakdown and/or the pathway of degradation in the roots was similar to that in the soil.

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[5] Section II. Uptake and Distribution of ^{14}C-Bifenox in Crop and Weed Species.

In both the plant and soil studies, the greater amounts of radioactivity was not extractable with 80% methanol and was apparently bound to plant constituents or soil colloids. Of that portion extracted, 20 to 40% was of a polar nature and remained at the origin during TLC. Therefore, the pathway of degradation as presented here, was based on a small fraction of the total radioactivity and further elucidation of the nature of the unknown compounds would be in order.

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ABSORPTION, DISTRIBUTION AND METABOLISM OF BIFENOX

by

Gerald Roger Leather

(ABSTRACT)

The fate of bifenox [methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate] in soil and plants following preemergence application of this herbicide, was investigated in greenhouse and laboratory studies.

Radiolabeled ^{14}C-bifenox had a half-life of 3 to 7 days after application to the soil and was characterized by hydrolysis to 5-(2,4-dichlorophenoxy)-2-nitrobenzoic acid. The acid was found to be highly mobile in a soil elution column, accounting for the short half-life in soil as a result of leaching of the acid. Additional metabolites identified in the soil included: 2,4-dichlorophenyl-4-nitrophenyl ether and 5-(2,4-dichlorophenoxy) anthranilic acid. Comparison of the total ^{14}C in the sampling zone of 8 cm to that extractable by methanol showed increased binding of the compounds to the soil over a 313-day period.

The uptake by plants was rate dependent in the range of 1.7 to 4.5 kg/ha, preemergence application. Velvetleaf (*Abutilon theophrasti* Medic.), a weed susceptible to the herbicide, absorbed and translocated more ^{14}C-bifenox during early seedling growth than corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.).

The patterns of absorption and distribution of ^{14}C-bifenox, were different among corn, soybean, and velvetleaf. Autoradiographs of seedlings growing in treated soil showed the ^{14}C to be confined to

those areas of the crop plants in contact with the soil during emergence. Velvetleaf absorbed and translocated the labeled compound(s) to all areas, including the immature leaves. Root absorbed ^{14}C-bifenox from nutrient culture was transported in equal quantity to the shoots of the three species. However, in corn and soybean the ^{14}C-compound(s) was confined to the primary and secondary leaf veins while velvetleaf showed a general distribution of ^{14}C throughout the leaf tissue.

The test species were capable of metabolizing ^{14}C-bifenox after root absorption from nutrient culture. Tissue macerates of the shoots of corn and soybean *in vitro* did not metabolize the bifenox, velvetleaf hydrolized it to the acid but to less than 1% of the total incorporated.