

Determination and Persistence of Phoxim and Its Oxygen Analog in Forage Corn and Grass

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Residues of the insecticide phoxim (phenylglyoxylonitrile oxime *O,O*-diethyl phosphorothioate; Bay 77488) and its oxygen analog were determined in Coastal bermudagrass, *Cynodon dactylon* (L.) Pers., and corn after the insecticide was applied in the field as aqueous sprays of an emulsifiable concentrate at rates of 6, 12, and 24 oz (active ingredient) per acre. Also, glass jar (qt) silage was made from 1-day posttreatment corn. Recoveries

of phoxim and its O analog from spiked samples by the method used were about 96 and 65%, respectively; sensitivity was about 0.004 ppm. Phoxim diminished rapidly and was not detectable at 21 days posttreatment in either forage, no matter what the rate of application. Residues of the O analog were detected at 0-day posttreatment only. In corn silage, the residues of phoxim (the O analog was not detected) were not stable.

Phoxim (phenylglyoxylonitrile oxime *O,O*-diethyl phosphorothioate; also known as Bay 77488, Valexon, and Baythion) has been reported to show promise for control of such economically important forage pests as grasshoppers, *Melanoplus* spp., and the alfalfa weevil, *Hypera postica* (Gyllenhal) (Chemagro Corp., 1969). It is a broad spectrum contact and stomach poison with a short residual life (Harris, 1970) that also has potential as a control for soil insects (Harris and Svec, 1970) and for some cole crop and cotton insects (Judge and McEwen, 1970; Beckham, 1970). Its acute oral LD₅₀ is 8500 to 8800 mg per kg in rats (Kenaga and Allison, 1969).

Residues of phoxim and its oxygen analog (O analog) applied as an emulsifiable concentrate (E.C.) to two extensively grown southern forage crops were therefore determined by gas chromatography. The chemical formulas of these compounds are shown in Figure 1. The present report describes details of the analytical procedure, the persistence of phoxim and its O analog in corn and Coastal bermudagrass, *Cynodon dactylon* (L.) Pers., and the stability of the compounds in corn silage.

EXPERIMENTAL

Apparatus. A Hewlett-Packard Co. Model 5750 gas chromatograph was equipped with the Melpar flame photometric detector (Tracor, Inc., Austin, Texas) operated in the phosphorus mode (526-nm filter).

The liquid chromatographic columns (Kontes No. 42000) were 12 mm i.d. glass. The Soxhlet extractors were Fisher Scientific Co., No. 9-556 B.

Reagents and Solvents. Analytical-grade samples of phoxim and its O analog and phoxim E.C. (4 lb per gal) were supplied by the Chemagro Corp.

The silica gel for the liquid chromatography of the extract was used as received from the J. T. Baker Chemical Co. (no. 3405). It lost 3.6% of its weight after overnight heating at 110° C.

Benzene, acetonitrile, hexane, acetone, and absolute methanol were used as C.P. grade solvents; all except methanol were distilled before use. Sodium sulfate was the anhydrous granular reagent grade chemical.

Sample Preparation and Extraction. Field samples of Coastal bermudagrass or forage corn were finely chopped in a Hobart cutter and mixed well. Then, 20 g of each sample were transferred to a Soxhlet apparatus containing a plug of glass wool to prevent insoluble plant material from siphoning over during the solvent exchanges. Each sample was extracted under nitrogen for 6 hr by using 150 ml of 10% methanol in benzene at the rate of about six solvent exchanges per hour. The extract was allowed to cool and then percolated through a plug of sodium sulfate (about 25-mm diam. × 30-mm thick). Next the flask and plug were washed with 10 ml of fresh benzene. Four drops of diethylene glycol were added to the extract to serve as a keeper. The extract was evaporated just to dryness on a 40° C water bath under water pump vacuum (any residues of methanol or water must be removed to avoid difficulty in the subsequent liquid chromatography). The residue was taken up in 10 ml of benzene for liquid chromatography.

Liquid Chromatography. A silica gel column was prepared by adding successively to the glass chromatographic column a plug of glass wool, 2 g of sodium sulfate, 4 g of silica gel, and 2 g of sodium sulfate. The column was washed with 25 ml of benzene, and the filtrate was discarded. Collection of the eluate was begun when the extract was added to the column. After the extract had percolated into the absorbent, the vessel and the column were rinsed three times with 5-ml portions of benzene, and the column was eluted with 25 ml more of benzene (total = 50 ml). The eluate (fraction A) contained phoxim (I).

The receiver was changed and the column was eluted with 50 ml of 5% acetone in benzene. This eluate (fraction B) contained the O analog.

Two drops of diethylene glycol were added to each eluate; then the fractions were evaporated just to dryness under water pump vacuum by using a 40° C water bath. The residue of fraction A was transferred to a calibrated tube with benzene, and its volume was adjusted to 2 ml for gas chromatographic analysis. Equal volumes of equilibrated hexane-acetonitrile (2 ml of each for corn and silage; 5 ml of each for grass) were used to transfer the residues of fraction B to a glass-stoppered tube. After the contents were vigorously shaken for 1 min, the hexane layer was discarded and the acetonitrile layer was reserved for subsequent gas chromatography.

Gas Chromatographic Analysis. The following conditions were used for gas chromatography. Column: glass, 50-cm ×

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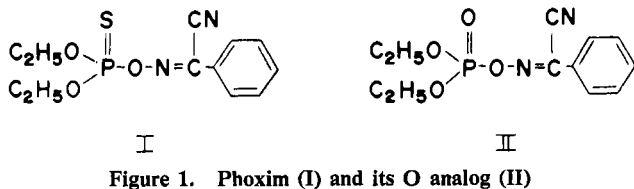


Figure 1. Phoxim (I) and its O analog (II)

4-mm i.d. (6-mm o.d.); Packing: OV-101, 5% (w/w) on 80 to 100 mesh Gas-Chrom Q (Applied Science Laboratories, State College, Pa.); Gases: nitrogen (carrier) at 160 ml per min; oxygen at 40 ml per min; hydrogen at 200 ml per min; and Temperature: column 150° C; injection port 160° C; detector (external) 170° C.

The column was conditioned overnight at 250° C and then conditioned further with the instrument operated as described by injecting 250-ng amounts of the appropriate insecticide until several 5-ng amounts produced a constant response. The accumulation of plant extractive in the gas chromatographic column tended to reduce sensitivity; however, this was overcome by periodically replacing the first 5 cm of the column. A portion of each fraction (5 μ l or a dilution thereof) was injected alternately with standard solutions, and residues in the unknowns were calculated on the basis of peak height.

Field Treatment of Coastal Bermudagrass and Forage Corn and Preparation of Corn Silage. Aqueous sprays were prepared of E.C. phoxim (4 lb active ingredient per gal) and applied at rates of 6, 12, and 24 oz per acre to forage corn (DeKalb-805) and Coastal bermudagrass. (Analysis of the concentrate showed the phoxim and the O analog to be 53.7 and <0.001% by weight, respectively.)

The corn was planted in 38-in. rows and separated into four blocks, each of which received a different treatment (0, 6, 12, or 24 oz insecticide per acre). The experimental design, spraying of plots, sampling, and sample preparation were as reported previously (Leuck and Bowman, 1968).

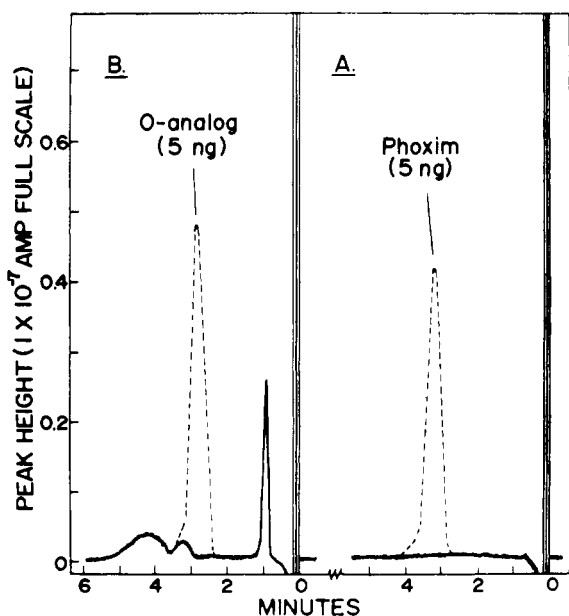


Figure 2. Gas chromatograms of phoxim and its O analog in fractions A and B obtained by liquid chromatography on silica gel. Solid lines are unfortified controls of corn equivalent to 50 mg; broken lines are the same fractions spiked with 5 ng (0.10 ppm) of the insecticides

Also, a pasture of Coastal bermudagrass handled in the same way was sprayed at rates of 0, 6, 12, and 24 oz per acre. Samples of both forages were taken immediately after the sprays had dried and at 1, 7, 14, 21, and 28 days posttreatment.

Corn silage was prepared in 1-qt glass jars (six jars per treatment) from finely chopped corn plants taken concurrently with the 1-day field samples, packed tightly in the jars, and sealed. Three per treatment were frozen immediately, and three per treatment were stored for 30 days at 85° F and then frozen at 15° F until analyzed for residues.

RESULTS AND DISCUSSION

The extraction procedure was chosen, based on the maximum recoveries obtained by exhaustive extraction of field-weathered samples. Benzene plus 10% methanol gave slightly better recovery than chloroform plus 10% methanol which is normally used at this laboratory (Bowman *et al.*, 1968). An extraction period of 6 hr was necessary to remove all residues recoverable by the method. The extractions were performed under an atmosphere of nitrogen to prevent possible oxidation of phoxim to the O analog.

Gas chromatograms of fractions A and B obtained from the liquid chromatography of a corn sample on silica gel are shown in Figure 2. Retention times for phoxim and its O analog were 3.2 and 2.8 min, respectively. In the analysis of fraction B, the peak that occurred at about 1 min was caused by an impurity in the acetone; those that occurred at about 3 and 4 min were also present in untreated corn and grass. The use of OV-210 or a mixture of OV-210 and OV-101 as the liquid phase gave complete separation of the two compounds. However, OV-210 was more difficult to condition to the insecticides, so for this reason and because small amounts of the O analog were sought in the presence of larger residues of phoxim, we chose to separate the insecticides before gas chromatography and to use an OV-101 column. The chromatograms of extracts of grass and corn silage were similar to those for corn (Figure 2), so they are not shown. Recoveries of phoxim and its O analog from corn, grass, and corn silage spiked with 0.05 to 5.0 ppm were 96 ± 2 and $65 \pm 2\%$, respectively. The sensitivity of the method was about 0.004

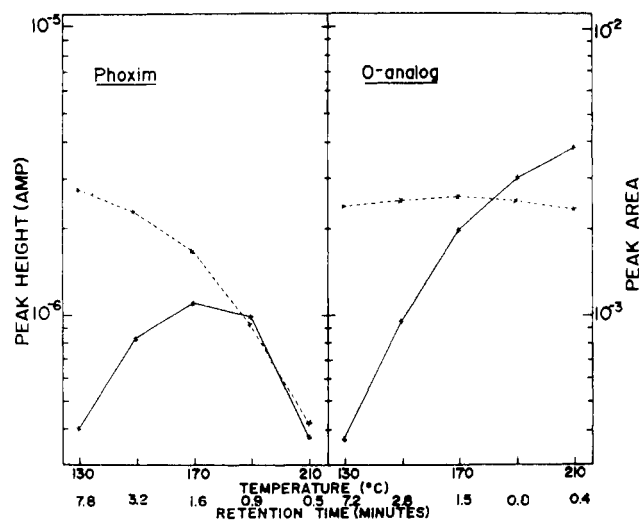


Figure 3. Effect of column temperature on the gas chromatographic response of phoxim and its O analog. Peak height (solid line) was measured in amperes and peak area (broken line) in disc integrator counts (1 traversal = 100) \times the full scale electrometer setting

Table I. Residues of Phoxim and Its O Analog in Forage Corn after Treatments with Phoxim E.C. at Three Levels (Tifton, Ga., 1970)

Days post-treatment	Rain-fall, in. ^a	Treatment level, oz per acre	% Dry matter, $\bar{x} \pm$ S.E.M.	ppm, $\bar{x} \pm$ S.E.M., wet basis ^b		
				Phoxim	O Analog ^c	Total
0	0.00	6	17.7 \pm 0.2	3.01 \pm 0.08	0.012 \pm 0.000	3.02 \pm 0.08
		12	17.8 \pm 0.1	8.01 \pm 0.28	0.052 \pm 0.001	8.06 \pm 0.28
		24	17.2 \pm 0.1	14.4 \pm 2.4	0.214 \pm 0.040	14.6 \pm 2.4
1	0.00	6	16.1 \pm 0.4	0.092 \pm 0.010	<0.004 \pm 0.000	0.092 \pm 0.010
		12	14.6 \pm 0.1	0.270 \pm 0.021	<0.004 \pm 0.000	0.270 \pm 0.021
		24	15.2 \pm 0.3	1.09 \pm 0.04	<0.004 \pm 0.000	1.09 \pm 0.04
7	0.00	6	17.4 \pm 0.1	<0.004 \pm 0.000	<0.004 \pm 0.000	<0.008
		12	17.4 \pm 0.3	<0.004 \pm 0.000	<0.004 \pm 0.000	<0.008
		24	17.8 \pm 0.2	0.022 \pm 0.003	<0.004 \pm 0.000	0.002 \pm 0.003
14	0.65	6	22.0 \pm 0.1	<0.004 \pm 0.000	<0.004 \pm 0.000	<0.008
		12	22.3 \pm 0.5	<0.004 \pm 0.000	<0.004 \pm 0.000	<0.008
		24	23.3 \pm 0.2	0.009 \pm 0.004	<0.004 \pm 0.000	0.009 \pm 0.004
21	1.90	6	27.6 \pm 0.4	<0.004 \pm 0.000	<0.004 \pm 0.000	<0.008 \pm 0.000
		12	25.9 \pm 0.3	<0.004 \pm 0.000	<0.004 \pm 0.000	<0.008 \pm 0.000
		24	25.5 \pm 0.7	<0.004 \pm 0.000	<0.004 \pm 0.000	<0.008 \pm 0.000

^a Between samplings; total rainfall = 2.80 in. ^b No residues were detected in samples taken from untreated plots at the various intervals or from treated plots at 28 days posttreatment. ^c Corrected for 65% recovery.

ppm (based on twice noise) except for the O analog analysis in grass (0.010 ppm).

During the preliminary investigations concerned with the gas chromatographic characteristics of phoxim and the O analog, the parent insecticide was found to be thermally unstable in the gas chromatograph; however, the O analog was essentially stable. These results were surprising because O analogs are generally more unstable than the parent phosphorothioates. The effect of column temperature on the response of the compounds is shown in Figure 3. The response (peak area) of phoxim at 210° C was only about 15% of the response at 130° C; the response of the O analog was relatively unaffected over the same temperature range. The

operating temperature of the column (150° C) was therefore selected to provide a relatively short retention time and an acceptable level of thermal stability.

The *p* values (Beroza and Bowman, 1965; Beroza *et al.*, 1969) may be useful in developing methods of analyzing other substrates or for identification at the nanogram level; for phoxim and its O analog in hexane-acetonitrile, they were 0.056 and 0.012, respectively.

Residues of phoxim and its O analog in field corn are reported in Table I. At rates of 6 and 12 oz per acre, residues of phoxim declined to below detectable levels (<0.004 ppm) during 7 days of weathering; however, traces (0.009 ppm) persisted through 14 days after treatment at a rate of 24 oz per acre. Low residues (0.21 ppm or less) of the O analog were found in corn taken immediately after the sprays had dried, no matter what the treatment. However, after 1 day of weathering, none (<0.004 ppm) could be detected in samples from any treatment.

Table II lists the residues found in the bermudagrass.

Table II. Residues of Phoxim and Its O Analog in Coastal Bermudagrass after Treatment with Phoxim E.C. at Three Levels (Tifton, Ga., 1970)

Days post-treatment	Rain-fall, in. ^a	Treatment level, oz per acre	% dry matter, $\bar{x} \pm$ S.E.M.	Phoxim
				ppm, $\bar{x} \pm$ S.E.M., wet basis ^b
0	0.00	6	32.6 \pm 0.3	1.75 \pm 0.02
		12	33.8 \pm 0.5	5.46 \pm 0.25
		24	32.7 \pm 0.3	13.0 \pm 0.2
1	0.00	6	33.0 \pm 0.8	0.544 \pm 0.017
		12	34.3 \pm 0.7	1.12 \pm 0.08
		24	33.4 \pm 0.9	2.71 \pm 0.21
7	0.00	6	37.8 \pm 1.1	0.036 \pm 0.008
		12	37.0 \pm 0.5	0.069 \pm 0.003
		24	35.5 \pm 0.5	0.208 \pm 0.024
14	0.00	6	42.6 \pm 0.7	<0.004 \pm 0.000
		12	41.3 \pm 1.5	<0.004 \pm 0.000
		24	39.1 \pm 0.7	0.048 \pm 0.013
21	1.86	6	41.5 \pm 0.5	<0.004 \pm 0.000
		12	42.9 \pm 0.4	<0.004 \pm 0.000
		24	37.7 \pm 1.0	<0.004 \pm 0.000

^a Between samplings; total rainfall = 5.96 in. ^b No residues were detected in samples taken from untreated plots at the various intervals or from treated plots at 28 days posttreatment. No residues of the O analog (<0.010 ppm) were detected in any samples except those taken from plots treated at a rate of 24 oz per acre sampled immediately after the sprays had been dried; these averaged 0.063 ppm (corrected for 65% recovery).

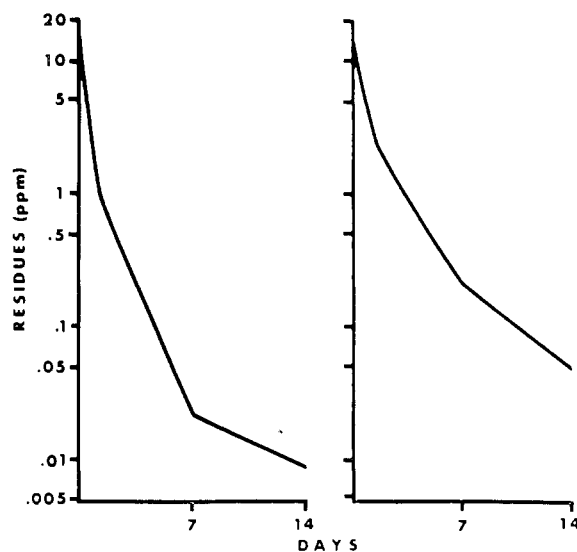


Figure 4. Residue of phoxim in Coastal bermudagrass (right) and field corn (left) after a spray application at a rate of 12 oz per acre

Table III. Residues of Phoxim in Corn Silage (Aged and Unaged) Prepared from Field Samples Taken at 1-Day Post-treatment with Three Levels of Phoxim E.C. (Tifton, Ga., 1970)

Treatment, level, oz per acre	% dry matter, $\bar{x} \pm S.E.M.$	Phoxim
		ppm, $\bar{x} \pm S.E.M.$, wet basis ^a
Unaged silage		
6	16.3 \pm 0.1	0.084 \pm 0.008
12	16.1 \pm 0.5	0.214 \pm 0.017
24	16.4 \pm 0.1	1.28 \pm 0.04
Aged silage ^b		
6	15.0 \pm 0.1	0.008 \pm 0.000
12	15.7 \pm 0.6	0.083 \pm 0.016
24	15.8 \pm 0.2	0.522 \pm 0.062

^a No residues were detected in untreated silage. No residues of the O analog (<0.004 ppm) were detected in any samples. ^b Sealed in glass jars and aged at 85° F for 30 days.

After 14 days of weathering, all detectable residues of phoxim disappeared from plots treated at a rate of 6 and 12 oz per acre, but low levels (0.048 ppm) persisted in plots treated with 24 oz per acre; however, these dissipated during the following week. Residues of the O analog averaging 0.063 ppm were detected only in samples from grass treated at the highest rate and taken immediately after the sprays had dried.

The dissipation of phoxim from corn and grass treated at a rate of 12 oz per acre is illustrated graphically in Figure 4.

The stability of residues of phoxim in corn silage is reported in Table III. No residues of the O analog were detected in any samples, and residues of phoxim in aged samples were significantly lower (*P* 0.05) than in unaged samples (frozen immediately after packing). The residues in silage are contrasted graphically in Figure 5. From these data, residues of phoxim in aged corn silage could be expected to dissipate.

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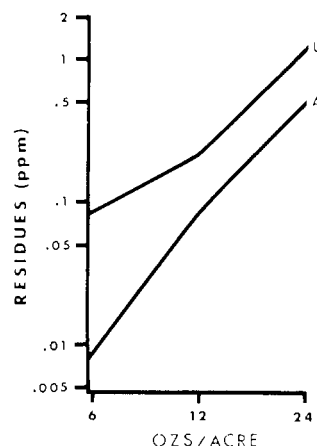


Figure 5. Residues of phoxim in corn silage in jars prepared from corn at 1-day posttreatment with 6, 12, or 24 oz insecticide per acre. (A) Silage aged 30 days at 85° F; (U) silage unaged and frozen immediately

the insecticide sprays and collecting the field samples and F. G. Crumley for assistance in analyzing for residues.

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