

Item ID Number: 00172

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Report/Article Title The Metabolism of High Concentrations of the Organophosphorus Insecticide
Phorate Applied Foliarily to Selected Plant Species

Journal/Book Title

Year 1971

Month/Day February

Color

Number of Images 54

Description Notes Project No. 5066

AFATL-TR-71-22

**THE METABOLISM
OF HIGH CONCENTRATIONS
OF THE ORGANOPHOSPHORUS INSECTICIDE
PHORATE APPLIED FOLIARLY
TO SELECTED PLANT SPECIES**

**PYROTECHNICS BRANCH
FLAME, INCENDIARY, AND EXPLOSIVES DIVISION**

TECHNICAL REPORT AFATL-TR-71-22

FEBRUARY 1971

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AIR FORCE ARMAMENT LABORATORY

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EGLIN AIR FORCE BASE, FLORIDA

**The Metabolism
of High Concentrations
of the Organophosphorus Insecticide
Phorate Applied Foliarly
to Selected Plant Species**

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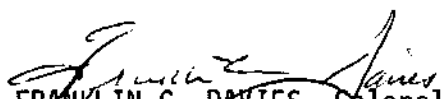
FOREWORD

The active Air Force project directly related to the information discussed in this report is Exploratory Development Project 5066. Requests for further detailed information or any comments on this report may be referred to Air Force Armament Laboratory (DLI), Eglin Air Force Base, Florida 32542.

Statistical analyses were performed by Booz-Allen Applied Research.

The use of trade names is for identification purposes only and does not constitute endorsement by the United States Air Force.

This report has been reviewed and is approved.


FRANKLIN C. DAVIES, Colonel, USAF
Chief, Flame, Incendiary and
Explosives Division

ABSTRACT

Gas chromatographic and enzymatic analyses (cholinesterase-inhibition method) were used to monitor the metabolism of the organophosphorus insecticide 0,0-diethyl S-[(ethylthio)methyl] phosphorodithioate (phorate) applied foliarly to three economically important plants (Homestead tomato, Wiley sorghum, and Honey sorghum). The resulting data provided guidelines in predicting toxicity and persistence of metabolite residues for high concentrations of insecticides employed by the military. An attempt was also made to relate the metabolism of the insecticide to phytotoxic damage among and within plant species. The data indicated that no plant-variety-dependent distinction exists in the formation of toxic phorate metabolites as shown by *in vitro* anticholinesterase activity recorded over a four-week period. Further investigation, with the same high concentrations of phorate placed on glass plates located adjacent to treated plants, indicated the formation of toxic phorate metabolites was without the influence of biological substrates within the plants. There were no statistically significant differences with respect to the rate of increase of cholinesterase-inhibition percentage values between the sorghum and glass plates; the rate of formation of anticholinesterase oxidized metabolites was predominantly through chemical oxidation on the leaf surface and not by plant enzyme catalysis, or at least, the oxidation occurred at such a rate as to mask the enzyme catalysis. The large droplet size in the application of phorate resulted in higher toxic residue values, especially on the surface of the plant, than would normally be expected.

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SECTION I

INTRODUCTION

The research reported in this study constitutes part of a program to elucidate toxicological and ecological hazards associated with repetitive aerial applications and spills of organophosphorus insecticides. Toxicological hazards may exist in plant foliage even after environmental persistence studies determine the absence or safe level of the parent insecticide. These hazards result from the conversion of the parent insecticide to toxic oxidized metabolites. The rate of this conversion determines the nature and magnitude of the toxic residues in the plant tissues.

The use of organophosphorus insecticides is increasing because of their wide spectrum of effectiveness (comparable to the chlorinated hydrocarbons) and their short residual action in water, soils, and plants. Any accident involving ultra-low-volume formulations of insecticides could result in a per-unit-area concentration that would be detrimental to agronomic plants because of unacceptable residue levels. For this reason, data relating the tolerance of agronomic plants to repetitive aerial applications of ultra-low-volume formulations of organophosphorus insecticides are of interest to military pest control programs.

Guidelines were desired for predicting toxicity and persistence of metabolite residues in plants after application of high concentrations of the insecticide. Insecticides in or under considerations for the Air Force inventory include malathion (dithiophosphoric derivative), naled (phosphoric acid derivative), fenthion (thiophosphoric acid derivative with sulfide linkage), and Dursban[®] (thiophosphoric acid derivative).

Phorate, O,O-diethyl S-[(ethylthio) methyl] phosphorodithioate, was the insecticide selected because it is a model compound containing oxidation sites analogous to those found in sulfur-containing organophosphorus insecticides. Its toxicity, expressed as an oral LD₅₀ value, is more than 100 times as great as the most toxic insecticide presently used by the military⁽¹⁾. Thus, persistence data on toxic metabolites of phorate should provide a model system of maximum toxic residues on foliage which should be unapproachable for insecticides presently used by the military when applied at the same concentration. Furthermore, previous studies^(2, 3, 4, 5) have elucidated the plant metabolism pathway (Figure 1) of the insecticide along with solvent-partitioning functions of phorate and its metabolites^(2, 4).

Conclusive research data are not available concerning insecticide phytotoxicity; however, it is known that plant species exhibit a wide range of tolerance to applications of organophosphorus insecticides^(6,7).

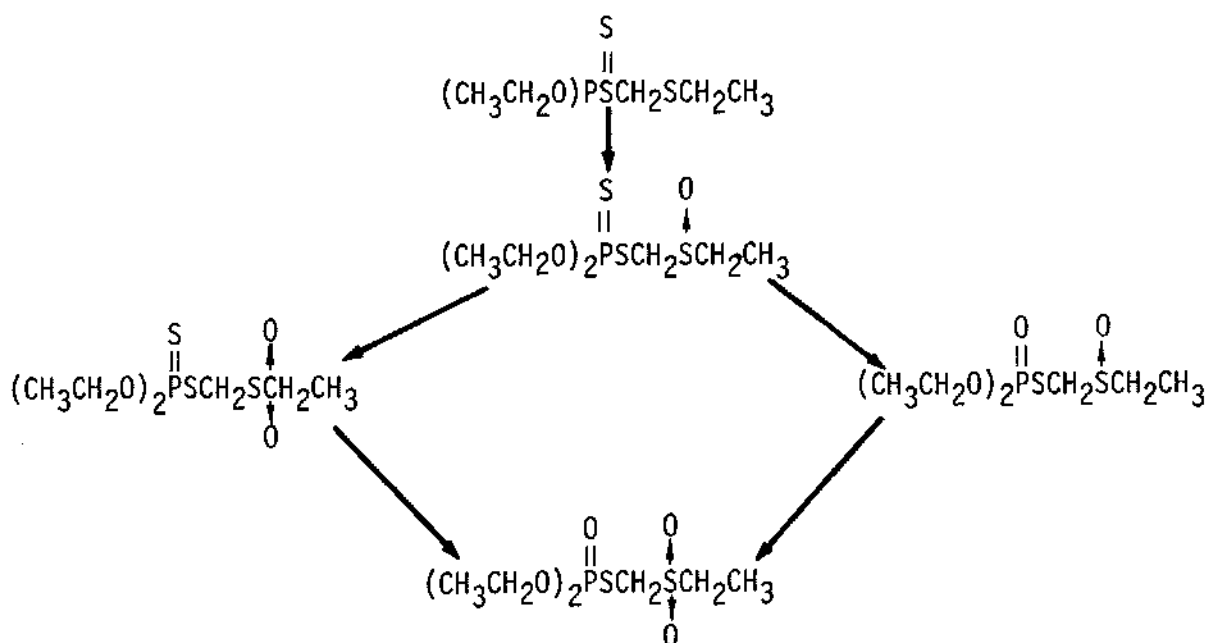


Figure 1. Plant Metabolism Pathway of Phorate
(Based on Reference 5)

The basis for this phytotoxic resistance or susceptibility is not clear. The experimental procedure used in this study allowed an investigation into the role performed by individual plant chemistries in metabolizing organophosphorus insecticides.

Studies of the morphological effects caused by highly concentrated foliar applications of mevinphos and methyl demeton on selected plant species indicated that, in general, broadleaf plants were more susceptible to the insecticides than were grasses⁽⁷⁾. Severe morphological injuries were observed on soybean, cotton, and tomato plants one day after foliar treatment, while seven days were required before comparable injuries were noted on corn and sorghum. Coleman and Dean⁽⁶⁾ found that resistance of sorghum to methyl parathion was genetically controlled in their studies with a resistant and a susceptible variety, Wiley and Honey, respectively. Thus, differential phytotoxicity to organophosphorus insecticides can be expected between plant species as well as within a given species. Differences do occur in the rates of metabolism of insecticides in different plant species. A study⁽⁵⁾ of the plant metabolism of Di-Syston[®] (dithio-Systox[®]) and phorate indicated that the rates of a reaction may vary slightly from one plant species to another and according to the stage of growth, but the data obtained may be used as a guide to the relative proportions of the metabolites present at intervals after application. In

another study (8), the effects of temperature and plant species upon the rates of metabolism of systemically applied Di-Syston[®] were considered, and similar results were obtained. Phorate differs from Di-Syston[®] due to the presence of an ethylene rather than a methylene group in its side chain. Thus, this study attempted to relate the metabolism of the insecticide to phytotoxic damage among plant species and within a plant species.

Oxidation can increase both the water solubility and the anticholinesterase activity of organophosphorus insecticides (9). The logical approach for monitoring anticholinesterase compounds (toxic phosphorus esters) formed during the metabolism of phorate was a cholinesterase-inhibition method. Phorate alone is too weak an inhibitor to be detected in micro amounts, but when applied to plants, it is very rapidly converted to potent anticholinesterase agents. The final unhydrolyzed metabolite (phorate oxygen analog sulfone) in the oxidation series (Figure 1) is the most active inhibitor (2). The I₅₀ value (molarity of inhibitor which results in 50 percent of the activity of the control) of phorate is approximately 250 times that of the phorate oxygen analog sulfone (5). One day after the application of phorate to corn, the residue of phorate sulfoxide, which has an I₅₀ value approximately 1/100 that of phorate, was more than three times that of phorate (4).

SECTION II

EXPERIMENTAL PROCEDURES

1. MATERIALS AND METHODS

a. Apparatus. A Sorvall Omni-mixer[®] was used for macerating plants. A gas chromatograph equipped with a flame photometric phosphorus detector and a digital integrator was employed in the phorate analyses. A recording pH stat was used to determine cholinesterase activity.

b. Reagents and Solvents. Standards, supplied by the American Cyanamid Company, were technical grade phorate (Thimet[®]) of 90-percent purity, analytical grade phorate of 97.8-percent purity, and 94-percent phorate oxygen analog sulfoxide containing 6-percent phorated oxygen analog sulfone.

Reagents were anhydrous sodium sulfate, certified A.C.S.; acetylcholine perchlorate (a "rare and fine" chemical from K and K Laboratories, Inc.); sterile filtered horse serum (Colorado Serum Co.); sodium chloride (crystals), analytical reagent grade; sodium chloride (granular), U.S.P. grade; sodium hydroxide pellets, analytical reagent grade; and potassium hydrogen phthalate, certified A.C.S. acidimetric standard.

Solvents were certified A.C.S. acetone, certified A.C.S. hexanes, and vegetable oil.

c. Plant Parameters. The representative broadleaf plant selected was Lycopersicon esculentum mill. var. Homestead 24 (tomato), and the grasses were Sorghum vulgare Pers. var. Wiley (sorghum), and Sorghum vulgare Pers. var. Honey (sorghum). The two varieties of sorghum were selected to represent a variety (Wiley) that was resistant to an organo-phosphorus insecticide and one (Honey) that was susceptible. The plants were grown in a clear glass greenhouse with a minimum night temperature of 60° to 65°F and a maximum day temperature of 95° to 100°F. Seeds were planted in a soil consisting of a 7:3:1 mixture of sandy loam, peatmoss, and perlite with four pounds of dolomitic limestone and one pound of superphosphate added per cubic yard of soil. The pH of the soil was 6.5. Each tomato plant was transplanted to an individual four-inch plastic pot at the age of four weeks. The sorghum experimental unit consisted of 10 plants per four-inch plastic pot. A 15-15-15 liquid fertilizer was applied bi-weekly.

A 2 percent or 1 percent solution of phorate (0.2 milliliter or 0.1 milliliter of technical grade phorate dissolved in 10 milliliters of vegetable oil medium) was applied foliarly as 0.01 milliliter droplets with microsyringe to the tomato and sorghum at the concentrations shown in Table I. This procedure allowed a uniform and exact application to all

TABLE I. PLANT PARAMETERS EMPLOYED WITH HIGH CONCENTRATIONS OF PHORATE

Species	Age, Weeks	Date of Application	Phorate, Concentration,	
			ppm ^a	lb/A ^b
Homestead Tomato	6	8 August 1969	19,839	2.19
		19 November 1969	16,630	1.84
Wiley Sorghum	4	2 March 1970	8,753	1.26
	3	20 May 1970	(c)	(c)
Honey Sorghum	4	8 April 1970	8,461	1.22
	3	20 May 1970	(d)	(d)

^aConcentration of phorate solution based on gas chromatographic analysis with analytical grade phorate.

^bConcentration applied to plant based on leaf area (pounds of active ingredient per acre).

^cSame concentration applied as 2 March 1970, but no gas chromatographic analysis.

^dSame concentration applied as 8 April 1970, but no gas chromatographic analysis.

the plants. The levels of phorate applied were adjusted to the maximum amount that would result in minimal visible damage. To insure similarity in plant size at each application, the phorate was applied after three to six weeks of initial plant growth. The varying lengths of time between planting and insecticide application were to compensate for seasonal variation in plant growth. Table I shows the age of the plants with the date of application of phorate. During the March (Wiley sorghum) and April (Honey sorghum) portions of the experiment, the phorate in the vegetable oil medium was applied to glass plates located adjacent to the treated and control plants.

d. Extraction Technique. The plants to be sampled were severed at soil level, sectioned, and rinsed in a 400-milliliter beaker containing 25 milliliters of hexanes and 25 milliliters of acetone in distilled water (60 percent by volume) to remove any residues remaining on the plant surface. The solvent mixture, followed with the plant material, was poured into a cup for the Sorvall Omni-mixer[®]. The beaker was rinsed with 5 milliliters of hexanes and then by 5 milliliters of acetone in distilled water (60 percent by volume). After the plant material was thoroughly macerated, the macerate was filtered through three layers of

cheesecloth into a separatory funnel. The cup which had contained the macerate was rinsed with 10 milliliters of hexanes and then by 10 milliliters of acetone in distilled water (60 percent by volume). The rinsings were added to the separatory funnel followed by 15 milliliters of a saturated sodium chloride solution which aided in separating the organic and aqueous phases.

Acetone was removed from the aqueous layer by use of a rotary evaporator attached to a water aspirator. Complete removal of the acetone was essential because of its ability to inhibit cholinesterase. The aqueous layer was filtered (to remove minute pieces of plant material) through Whatman No. 2 paper into a 50-milliliter volumetric flask and brought to volume with distilled water. The organic phase was placed over 10 grams of anhydrous sodium sulfate, filtered into a 50-milliliter volumetric flask, and brought to volume with hexanes. If the samples could not be analyzed immediately, they were stored at 5°C.

The aqueous layer was analyzed for cholinesterase-inhibiting metabolites and breakdown products. Gas chromatographic analysis of the organic phase for phorate allowed monitoring of the rapidity of breakdown and oxidation. The efficiency of the extraction technique, based on the recovery of phorate in the organic phase, is shown in Table II.

TABLE II. EFFICIENCY OF EXTRACTION TECHNIQUE FOR PHORATE WITH TOMATO AND SORGHUM			
Species	Date of Experiment	Efficiency of Extraction, Percent	
		Controls ^a	Insecticide-Treated Plants ^b
Homestead Tomato	August	^a 66.2	32.4
	November	^a 87.7	63.2
Wiley Sorghum	March	63.3	46.7
Honey Sorghum	April	75.9	51.7

^aConcentration of phorate applied to plant placed through extraction scheme.
^bPhorate-treated plants from day 0 placed through extraction scheme.
^cGlass surface residues from day 0 placed through extraction scheme.

e. Cholinesterase-Inhibition Method. The advantages of using cholinesterase-inhibition methods for determining organophosphorus residues are that (a) the sensitivity is far greater than for chemical methods and (b) the method is particularly suitable when the insecticide undergoes

changes in the plant to produce metabolites with a high inhibitory activity(10,11). One of the disadvantages is the lack of specificity or inability to distinguish among different types of cholinesterase inhibitors found within the plant.

The persistency of toxic metabolites and breakdown products in the respective plant species was monitored using an automated pH stat method(12) to determine cholinesterase activity. The cholinesterase inhibition for each species and glass plate sample was measured immediately after the application of the insecticide and on various succeeding days for one month. Control plants were treated with only the corresponding amounts of vegetable oil and were similarly measured. The experiment was repeated for each species.

The cholinesterase activity of the prepared water sample was recorded with a recording pH stat. A sample (0.2 milliliter tomato or 0.4 milliliter sorghum sample) was placed in a microbeaker containing 5 milliliters of a 0.154-molar saline solution (9.000 grams of analytical reagent grade sodium chloride in 1000 milliliters distilled water) and 0.5 milliliter horse serum. The microbeaker solution was heated to 37.5°C and adjusted to pH 8.0 prior to addition of 0.3 milliliter of the cholinesterase enzyme substrate, 0.110-molar acetylcholine perchlorate (0.675 grams crystalline substrate in 10 milliliters distilled water). The titrant, 0.0100N sodium hydroxide, was standardized with 0.0100N potassium acid phthalate (0.20423 grams acid in 1000 milliliters distilled water).

Normal-activity curves (no samples added) and control-activity curves (aqueous samples from control plants) were obtained prior to measuring cholinesterase activity for the aqueous samples from phorate-treated plants. Cholinesterase inhibition of the phorate metabolites and breakdown products was expressed as a percentage value obtained from a ratio of cholinesterase activity (expressed in units of micromoles of acetylcholine hydrolyzed per minute per milliliter of horse serum) of the samples from the phorate-treated plants to samples from the control plants. A second percentage of cholinesterase inhibition was calculated using the normal activity value as the base.

f. Calibration Curve. To equate the percentage of cholinesterase inhibition to the concentration of the metabolite extract, a log-linear plot was made of the phorate oxygen analog sulfoxide equivalent, in parts per million (ppm) of 0,0-diethyl S-[(ethylsulfinyl) methyl] phosphorothiolate, versus the percentage of cholinesterase inhibition. Figure 2 contains the calibration curve based on 0.4 milliliter samples of the standard parts-per-million solutions of the phorate oxygen analog sulfoxide. Sample sizes of 0.2 milliliter gave cholinesterase inhibition percentages that were approximately half the values shown in Figure 2.

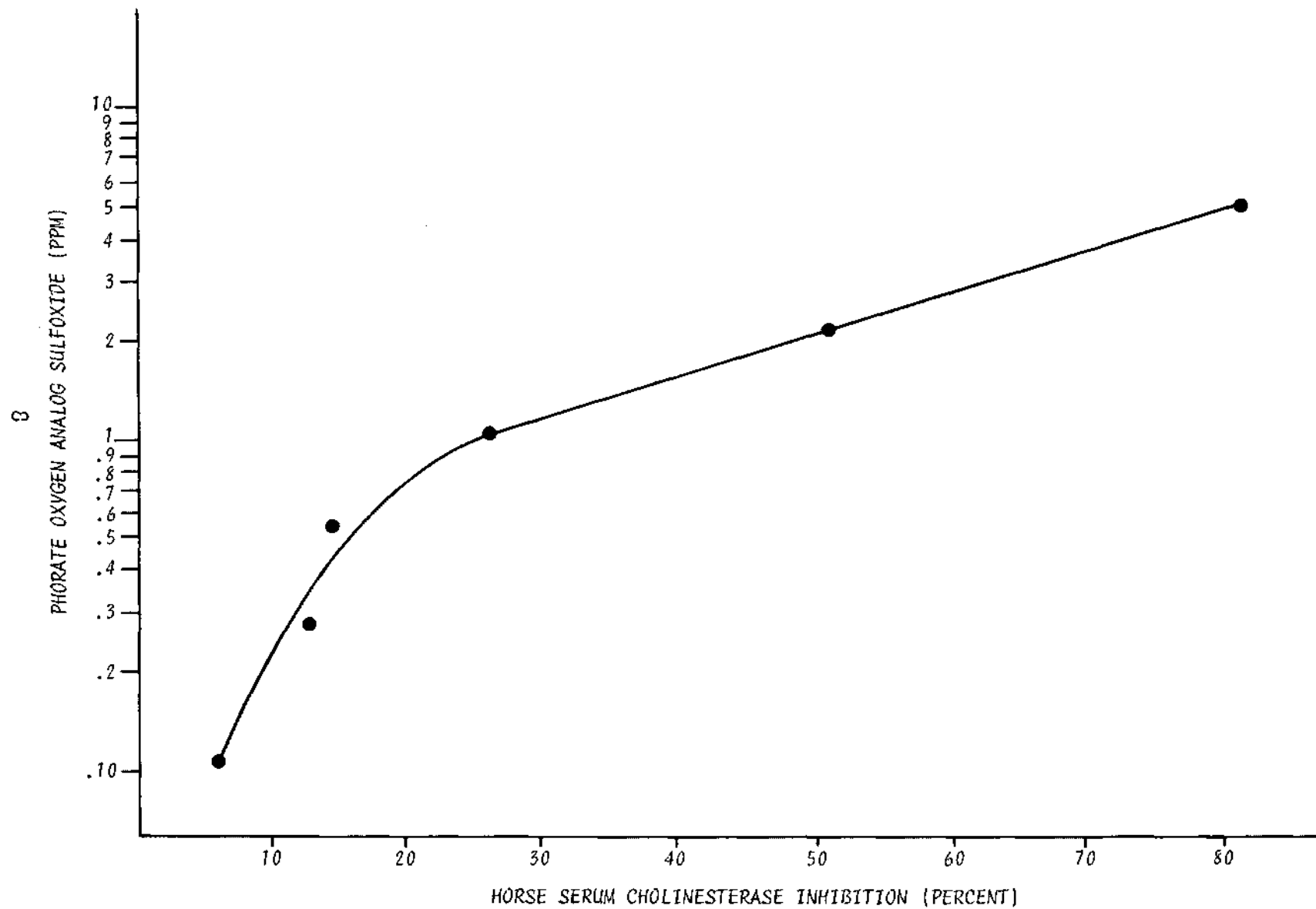


Figure 2. Horse Serum Cholinesterase Inhibition Versus Concentration of 0,0-Diethyl S-[(Ethylsulfinyl)methyl] Phosphorothiolate

g. Gas Chromatographic Analysis. Gas chromatography was used to detect unaltered phorate in the organic phase from the plant extracts. A gas chromatograph equipped with a flame photometric phosphorus detector was employed. A six foot by one-fourth inch stainless steel column containing Chromport XXX, 80/90 mesh, coated with 3 percent SE-30, and conditioned for 24 hours at 220°C, was used for the phorate analysis of the August tomato extracts. A retention time of 105 seconds was recorded with the inlet temperature set at 250°C, the column-oven temperature at 200°C, and the detector temperature at 235°C. Gas flow rates in cubic centimeters per minute were nitrogen 80, hydrogen 150, air 20, and oxygen 20.

A six foot by one-fourth inch glass column containing Chromosorb W, 80/90 mesh, coated with 3 percent OV-1 and conditioned for 24 hours at 210°C was used for the phorate analysis of the November tomato extracts, March Wiley sorghum extracts, April Honey sorghum extracts, and glass plates. A retention time of 130 seconds was recorded with the inlet temperature set at 245°C, the column-oven temperature at 190°C, and the detector temperature at 185°C.

Injection sample sizes for August tomato extracts were 5 microliters, and all others were 2.7 microliters. Concentrations of phorate present in the August analysis were determined by integration of peak area by a digital integrator. Concentrations of all other analyses were determined by peak height. All determinations were expressed in parts per million based on standard curves.

2. STATISTICAL ANALYSES.

Before any of the data were analyzed, all of the cholinesterase inhibition percentages were transformed using the arc sine formula:

$$\theta = 2 \arcsin P$$

where θ is the new variable to be analyzed

and P is the percent of cholinesterase inhibition divided by 100.

This transformation minimized and stabilized the variation of the data and created the homogeneity of variance that was essential to the use of the analysis of variance technique, and the other statistical procedures used for the analysis of these experiments. The results were stated in percentage notations for presentation throughout the report.

It was not possible to analyze all of the cholinesterase inhibition percentages on an experiment-wide basis because the measurements of the three experimental species were taken on various and differing days

during the month following application of the insecticide. To make comparisons that were valid and meaningful, it was necessary to select those days on which all species under consideration were measured. Table III shows the days of measurement for each species. During the first week

TABLE III. MEASUREMENT DAYS BY SPECIES AND EXPERIMENT

Days After Application	Homestead Tomato		Wiley Sorghum		Honey Sorghum	
	August	November	March	May	April	May
0	X	X	X	X	X	X
1	X	X	X		X	
2		X	X	X	X	X
3	X					
4	X	X	X	X	X	X
5		X	X	X		X
6	X	X			X	
7	X		X	X	X	X
8				X		X
9		X	X		X	
10	X					
11			X		X	
12		X		X		X
13	X					
14			X	X	X	X
15		X				
16				X	X	X
17	X					
18			X			
19		X		X		X
20					X	
21	X		X	X		X
22		X				X
23					X	
25			X	X		
26		X				
27					X	

following application of the insecticide, only those measurements that were made on the same day were compared; during the next three weeks, measurements that were made on contiguous days were also included in the comparison.

Only one measurement of cholinesterase inhibition was made on each of the days for the Homestead tomato experiments and for the March and April Wiley and Honey sorghum experiments. It was not possible, therefore, to test for significance of the interaction between seasonal effects and the number of days following application. Since this interaction would have been used to test the seasonal and time-passage effects individually, an unduly large interaction would have masked significance of the main effects. Paired t-tests with the same or contiguous days' results were used to circumvent this possibility. When nonsignificance of the paired values was determined, an analysis-of-variance technique was used to test for significance of the passage of time upon the cholinesterase-inhibition percentage. In those cases in which these results were significant, Duncan's new multiple range test was used to identify where the differences did, in fact, exist. In the absence of significance, means were computed, and the effects of the passage of time upon the cholinesterase inhibition were studied. Two replicates of measurements were made for the May Wiley and Honey sorghum plants, and it was, therefore, possible to use the analysis of variance technique when only these data were involved in comparisons.

Initially, all testing was conducted at the 95-percent probability level (significant). When this proved significant, subsequent testing was conducted at the 99-percent probability level (highly significant).

SECTION III

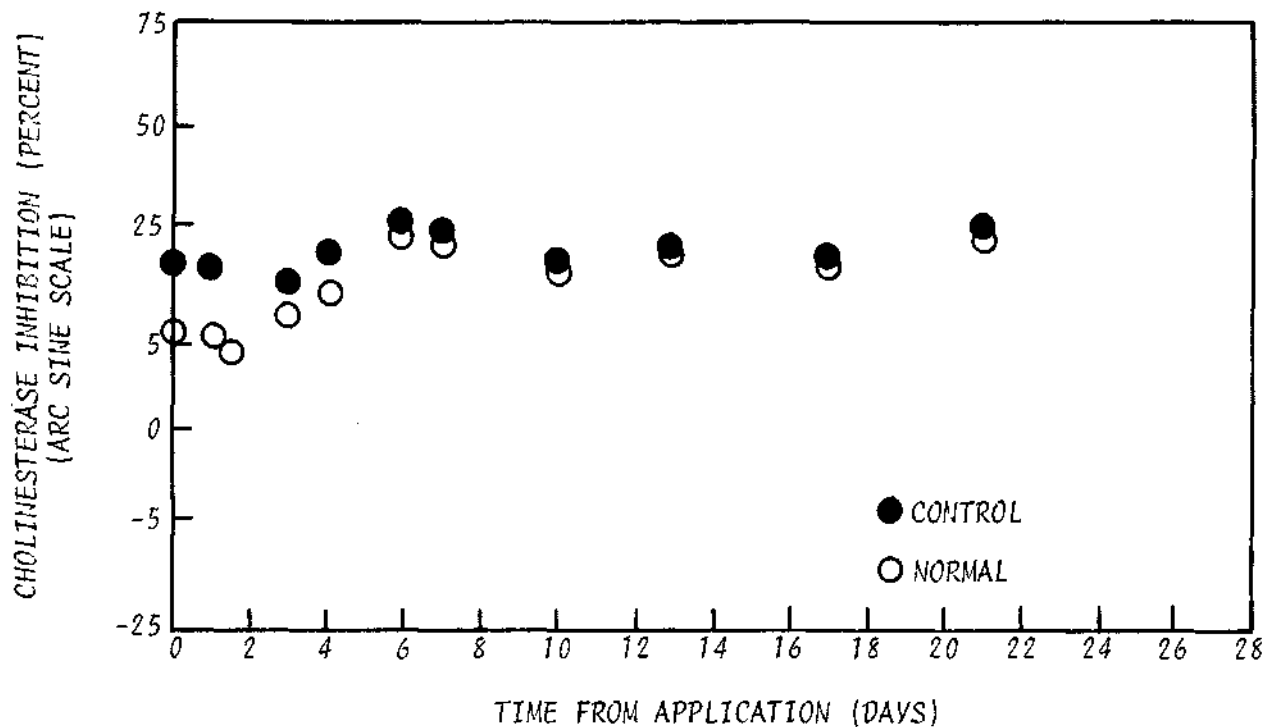
RESULTS AND DISCUSSION

Ultra-low-volume formulations of insecticides used by the military involve the aerial application of a low-volume concentrate (0.75 to 10 ounces per acre, undiluted). Phorate is applied with normal formulations at rates of 0.5 to 3 pounds of active ingredient per acre. Table I presents the application rates within this range. However, the defined, directed application of phorate to the leaf surface without spraying resulted in a maximum interface between insecticide droplet (10 microliters) and leaf area. This large droplet size represented the application of high concentrations of phorate; the droplet contained more phorate (0.25 milligram) and is larger than that ordinarily found following routine application of insecticides. The optimum diameter for insecticide spray droplets is in the range of 20 microns⁽¹³⁾.

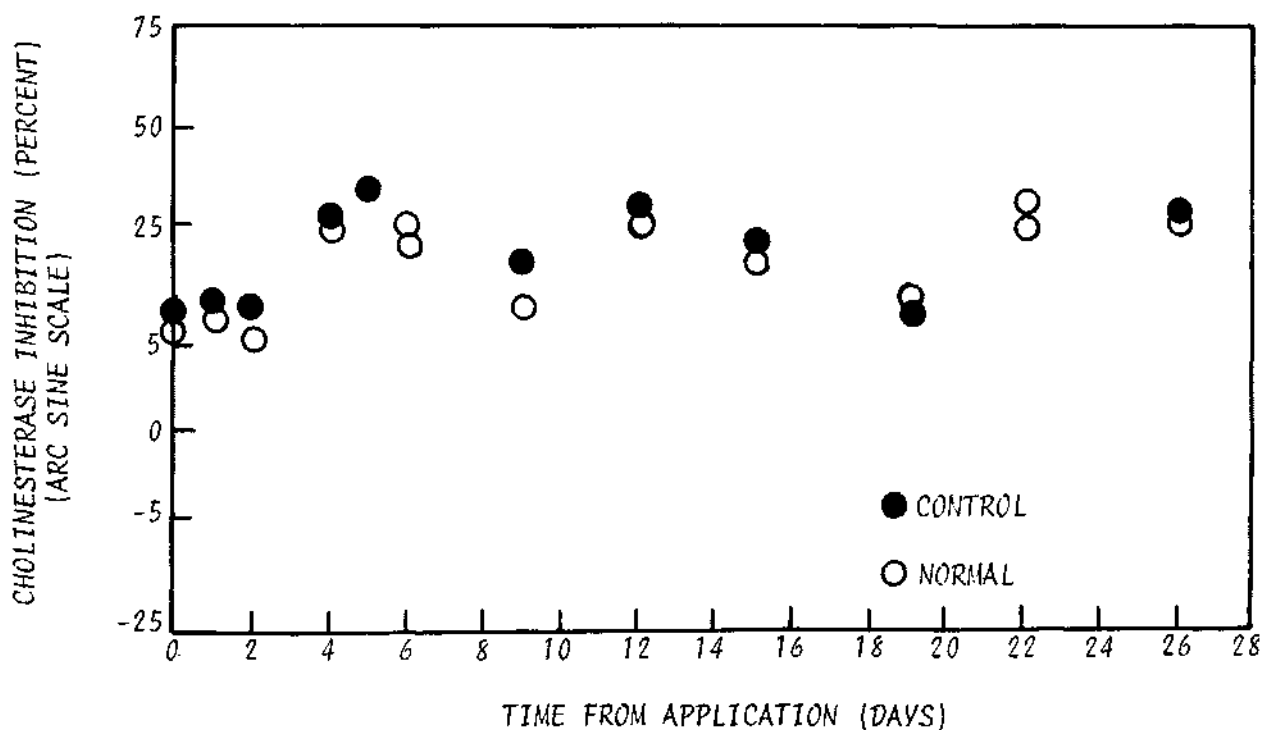
The percentages representing the efficiency of the extraction technique employed (Table II) are minimum values because of the volatility of phorate. Phorate is lost from the soil by volatilization and about 25 percent of the loss occurs in the first hour after treatment⁽¹⁴⁾. Similar results would be expected on the leaf surface, therefore, the length of time between treatment and initial extraction of the plant would result in a value of phorate present that is slightly less than that which was applied. The time before initial extraction was approximately the same in all cases; however, the extraction of the August tomato after application of phorate during the late morning heat probably accounts to some degree for the lower value in extraction efficiency. Phorate was applied to the other plants in the early morning.

A comparison of the percentage of cholinesterase inhibition obtained using the normal-activity value and the percentage obtained using the control-activity value as a base is shown in Figures 3 and 4 for Homestead tomatoes and for Wiley sorghum for each of the two applications of insecticide. A comparison for Honey sorghum is shown in Figure 5 for the May application; however, the April control plants were not usable due to contamination. The results of paired t-tests in analyzing the differences between percentages for each species allowed the use of all the cholinesterase-inhibition percentages based on the normal activity value. Cholinesterase-inhibition values obtained showed no correlation with plant weight.

A comparison of the percentages of cholinesterase inhibition during the month following application of the insecticide for each of the three plants, using the analysis of variance technique, indicated that the average for the Homestead tomato was significantly lower than those for

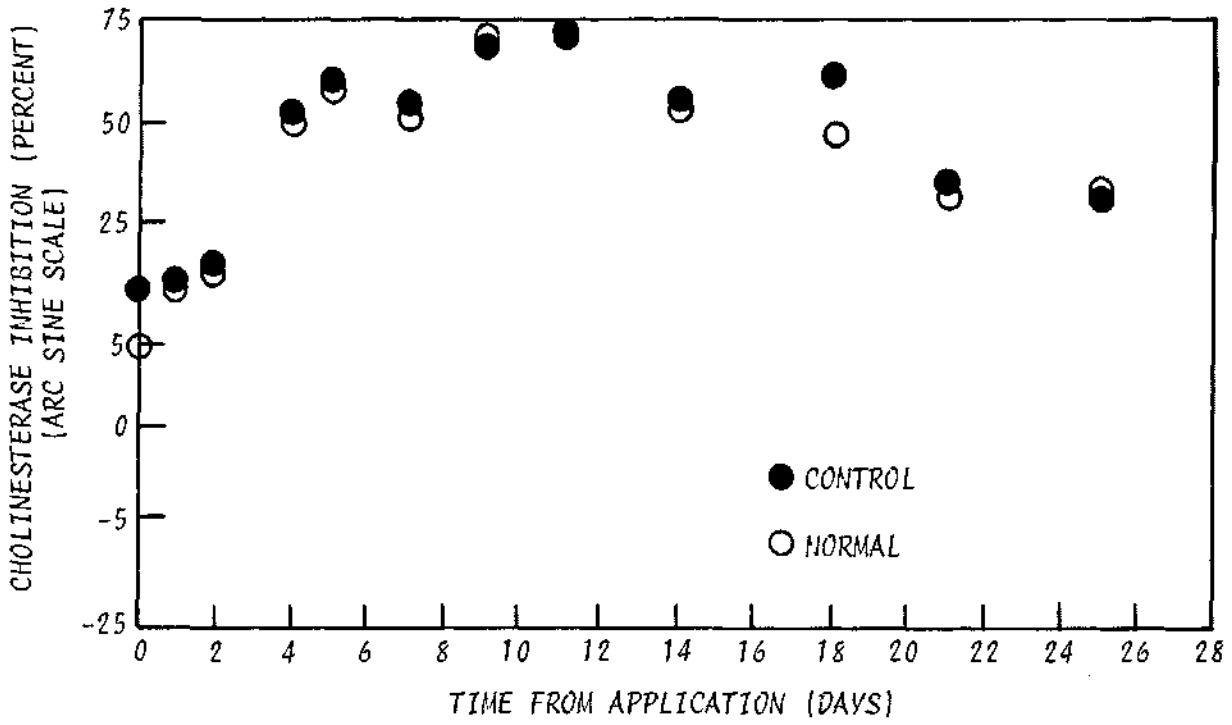


a. Phorate Applied in August

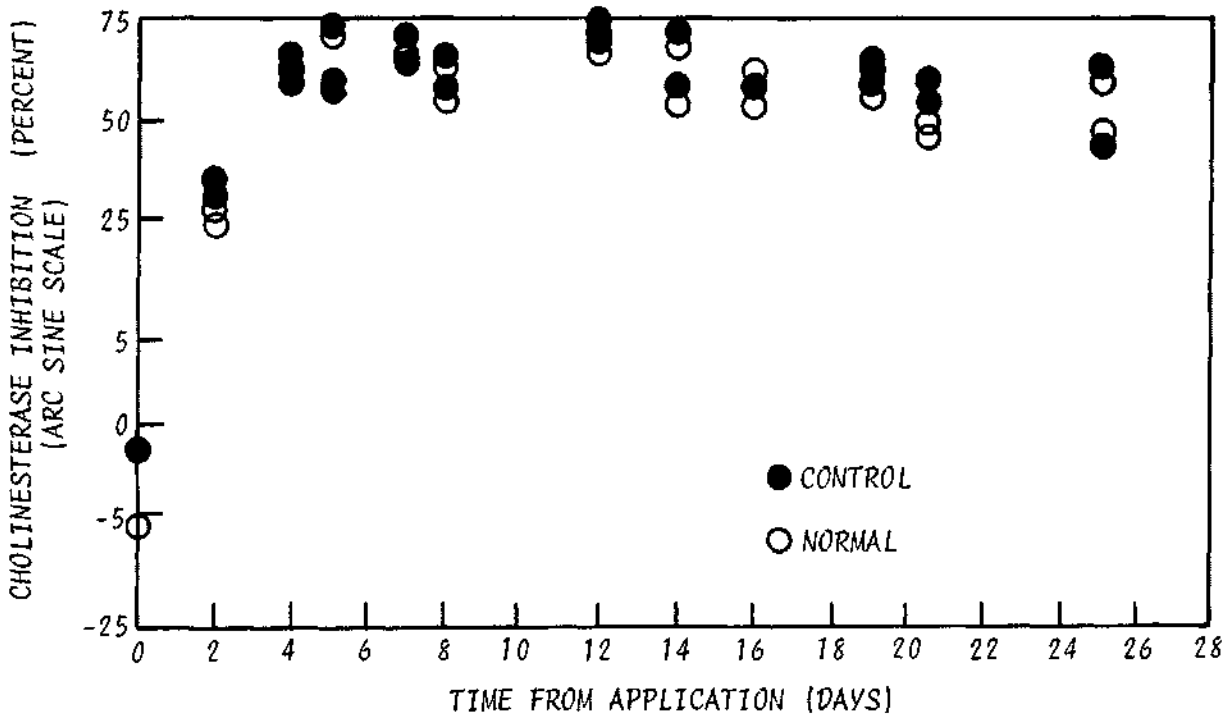


b. Phorate Applied in November

Figure 3. Cholinesterase Inhibition of Homestead Tomato



a. Phorate Applied in March



b. Phorate Applied in May

Figure 4. Cholinesterase Inhibition of Wiley Sorghum

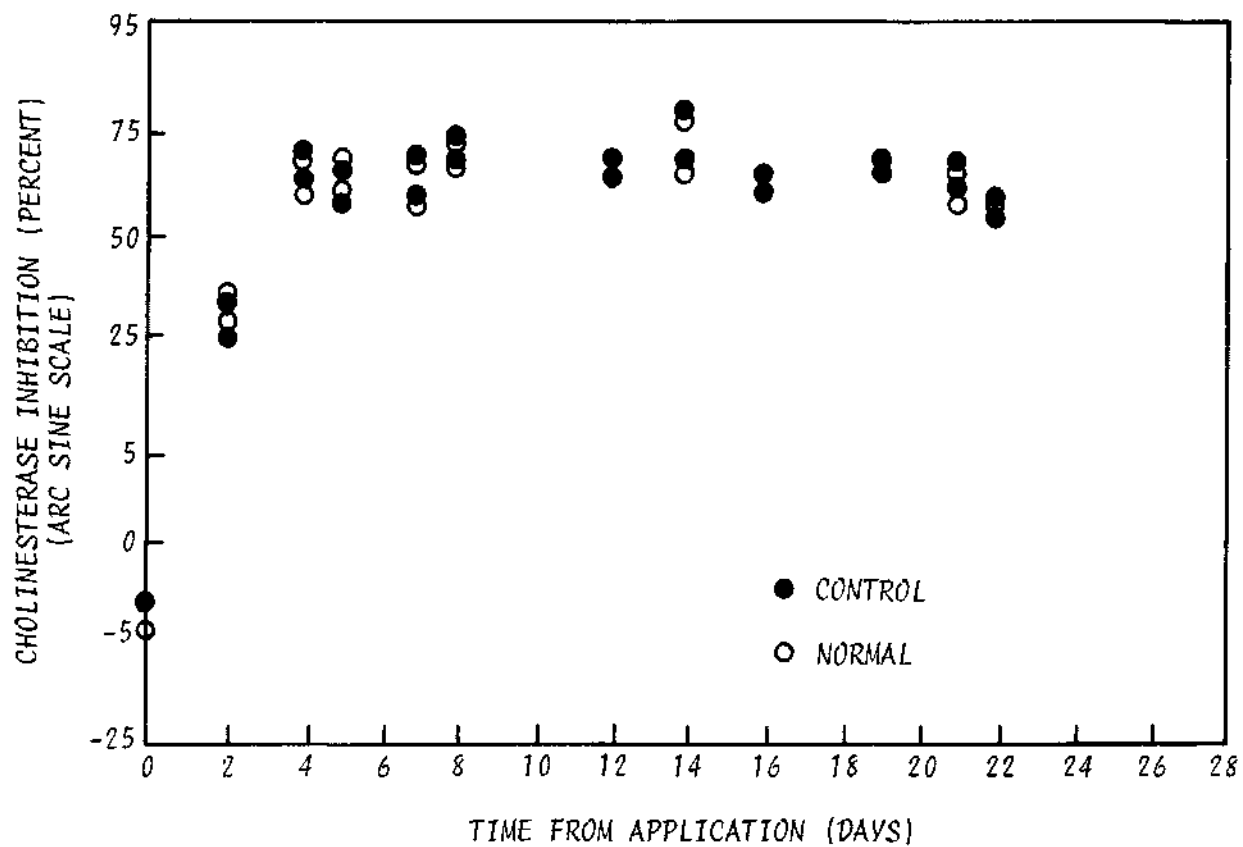


Figure 5. Cholinesterase Inhibition of Honey Sorghum (Phorate Applied in May)

the two varieties of sorghum. Figure 6 shows the inhibition percentage values for each of the three plants; the lower level for the tomato plants is evident. Therefore, for statistical purposes, data from the tomato plant experiments were analyzed independent of the sorghum data.

Gas chromatographic analysis of the hexanes phase after extraction from tomato and sorghum indicated less than one ppm phorate present by the sixth day (Table IV). A determination of residues of phorate and five of its metab-

TABLE IV. GAS CHROMATOGRAPHIC ANALYSIS FOR PHORATE FROM TOMATO AND SORGHUM

Day	Concentration of Phorate, ppm			
	Tomato		Sorghum	
	August	November	March (Wiley)	April (Honey)
0	128	42	16	18
1	140	34	9-12	9
2		28	6-7	5-6
3	23			
4	<1	9	<3	1
5		4		
6		1		<1

olites from various parts of corn plants (treated with one pound of the insecticide per acre) indicated that phorate was essentially gone in 14 days⁽⁴⁾. During this determination, phorate was recovered from fortified samples with 96 percent efficiency using a Soxhlet apparatus in an eight-hour extraction technique. The higher values of phorate in the August tomato samples, as compared to the November samples, were due to the concentration of the sample to 10 milliliters instead of the 50-milliliter final volume of all other samples. Small differences in the data are probably due to the slight variations in extraction efficiencies for each plant. The disappearance of phorate proceeds at approximately the same rate in each plant variety.

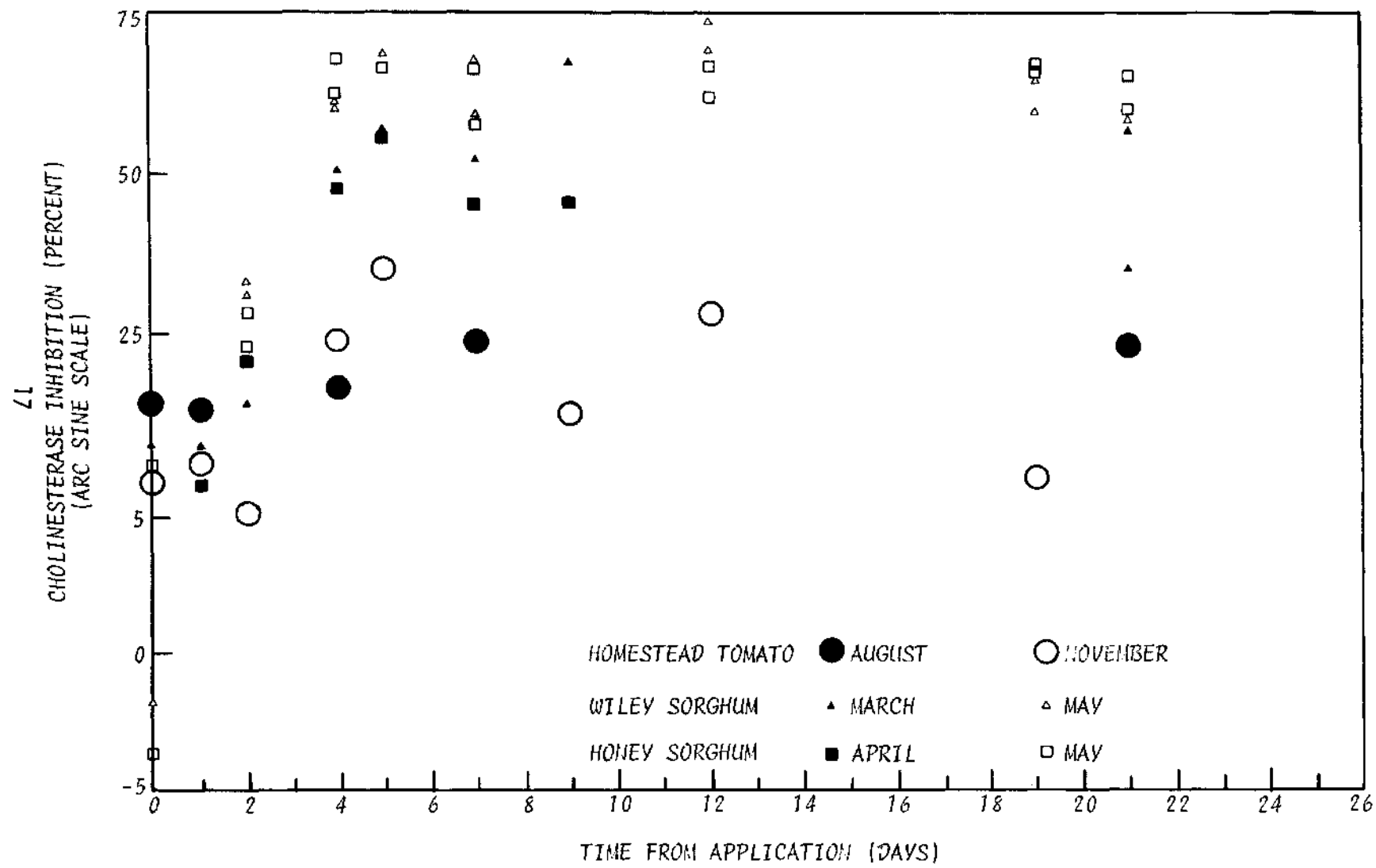


Figure 6. Comparison of Percent Cholinesterase Inhibition for Three Varieties of Plants

The results of a cholinesterase-inhibition analysis for the Homestead tomato plants for all of the days measured for the August and November applications of insecticide are shown in Figure 7. An analysis of comparable daily measurements in August and November indicated no significant differences between the two application dates or by the number of days after application. The average percentages of cholinesterase inhibition for the days used in the comparison are given in Table V and Figure 8 and are not significantly different at the 95 percent probability level.

TABLE V. AVERAGE PERCENT CHOLINESTERASE INHIBITION, HOMESTEAD TOMATO

Days After Application	Cholinesterase Inhibition, Percent
21,22	27.1
6	24.4
12,13	23.9
4	21.4
9,10	15.9
1	12.4
0	11.3

Figures 9 and 10 show the percentages of cholinesterase inhibition for Wiley and Honey sorghum, respectively. Analyses of the possible daily comparisons for each variety indicated significant differences between the March and May data for the Wiley sorghum and highly significant differences between the April and May data for the Honey sorghum.

Similar analyses of those cholinesterase-inhibition percentages which were obtained on the same or contiguous days for Wiley sorghum in March and Honey sorghum in April indicated no significant differences between the two varieties for those two months (Figure 11). An analysis of the differences in cholinesterase-inhibition percentages with respect to the number of days after application of phorate to sorghum during March and April indicated highly significant differences. Table VI shows average daily percentages. In Figure 12, the average values with associated letters are shown graphically. The cholinesterase-inhibition percentage peak was reached by the ninth day following application of phorate, and an

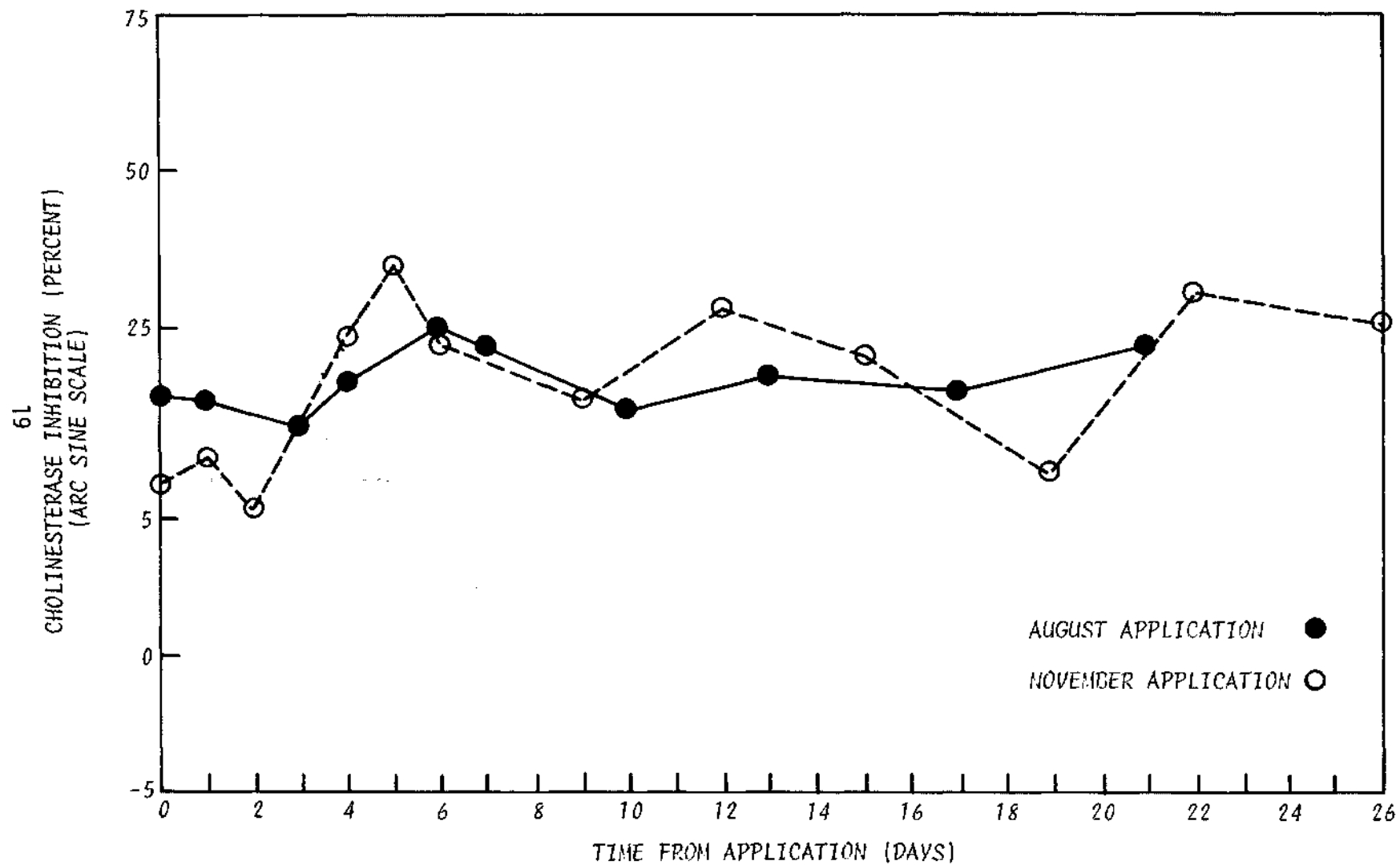


Figure 7. Percent Cholinesterase Inhibition, Homestead Tomato

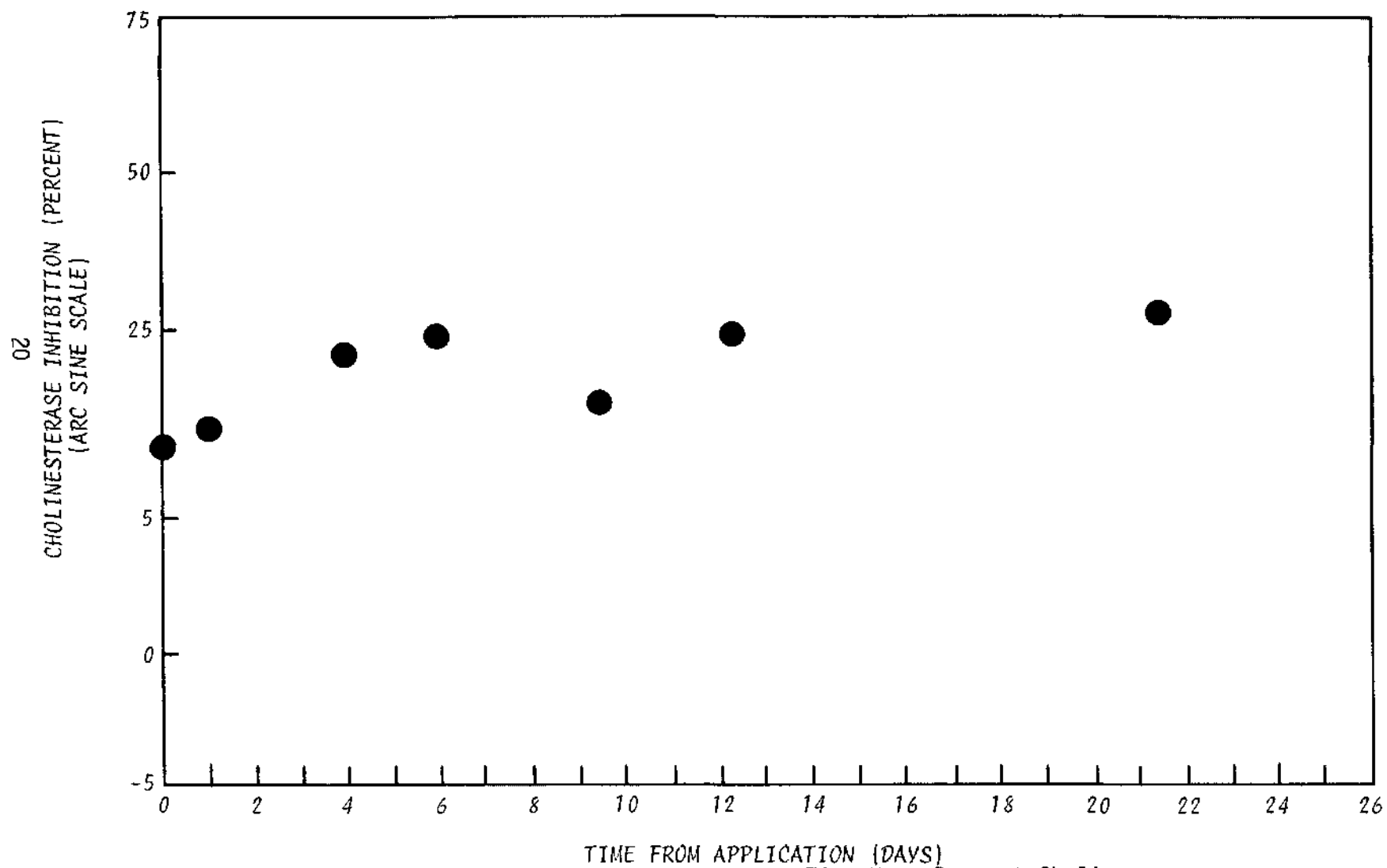


Figure 8. Effects of Passage of Time Upon Percent Cholinesterase Inhibition, Homestead Tomato

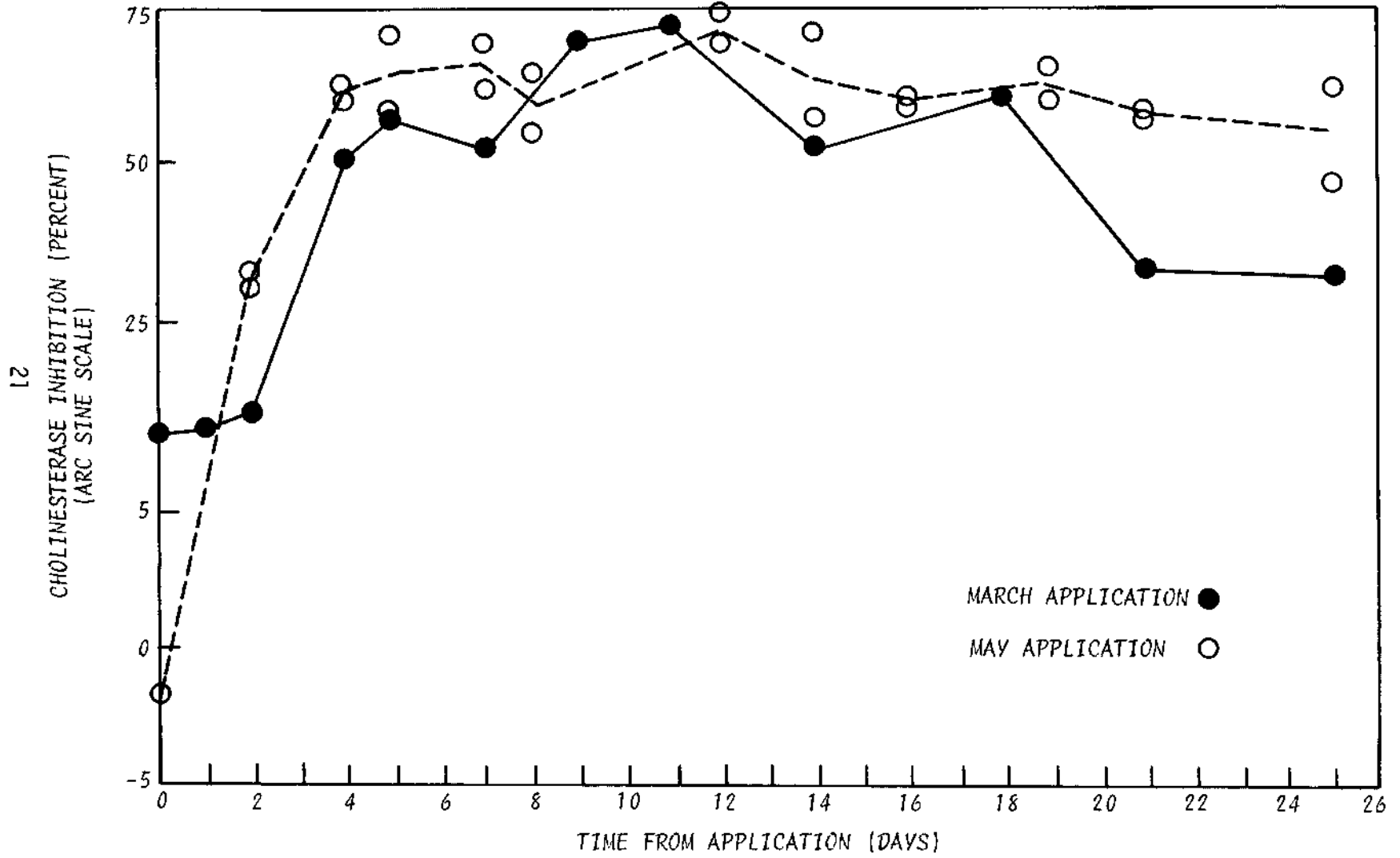


Figure 9. Percent Cholinesterase Inhibition, Wiley Sorghum

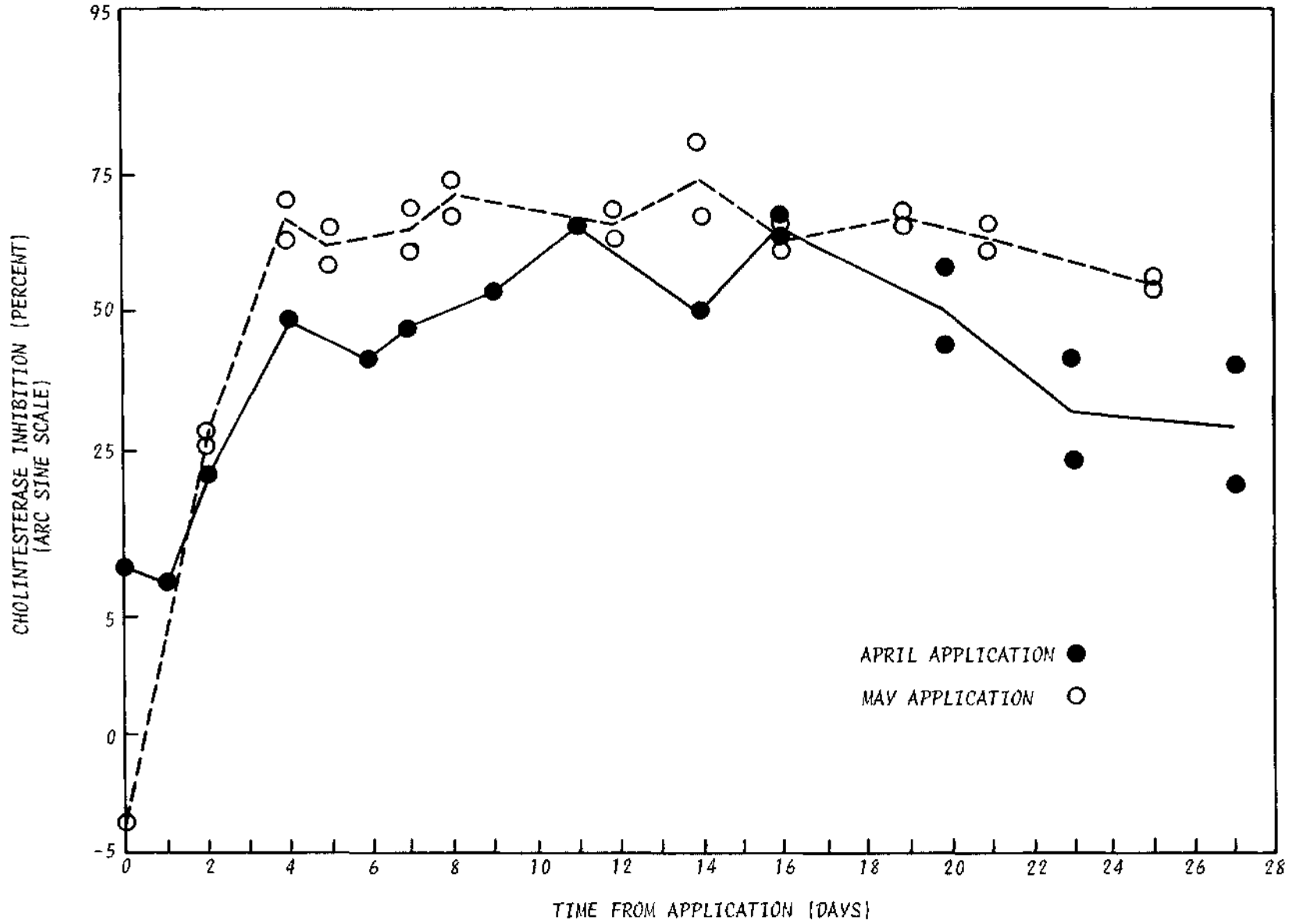


Figure 10. Percent Cholinesterase Inhibition, Honey Sorghum

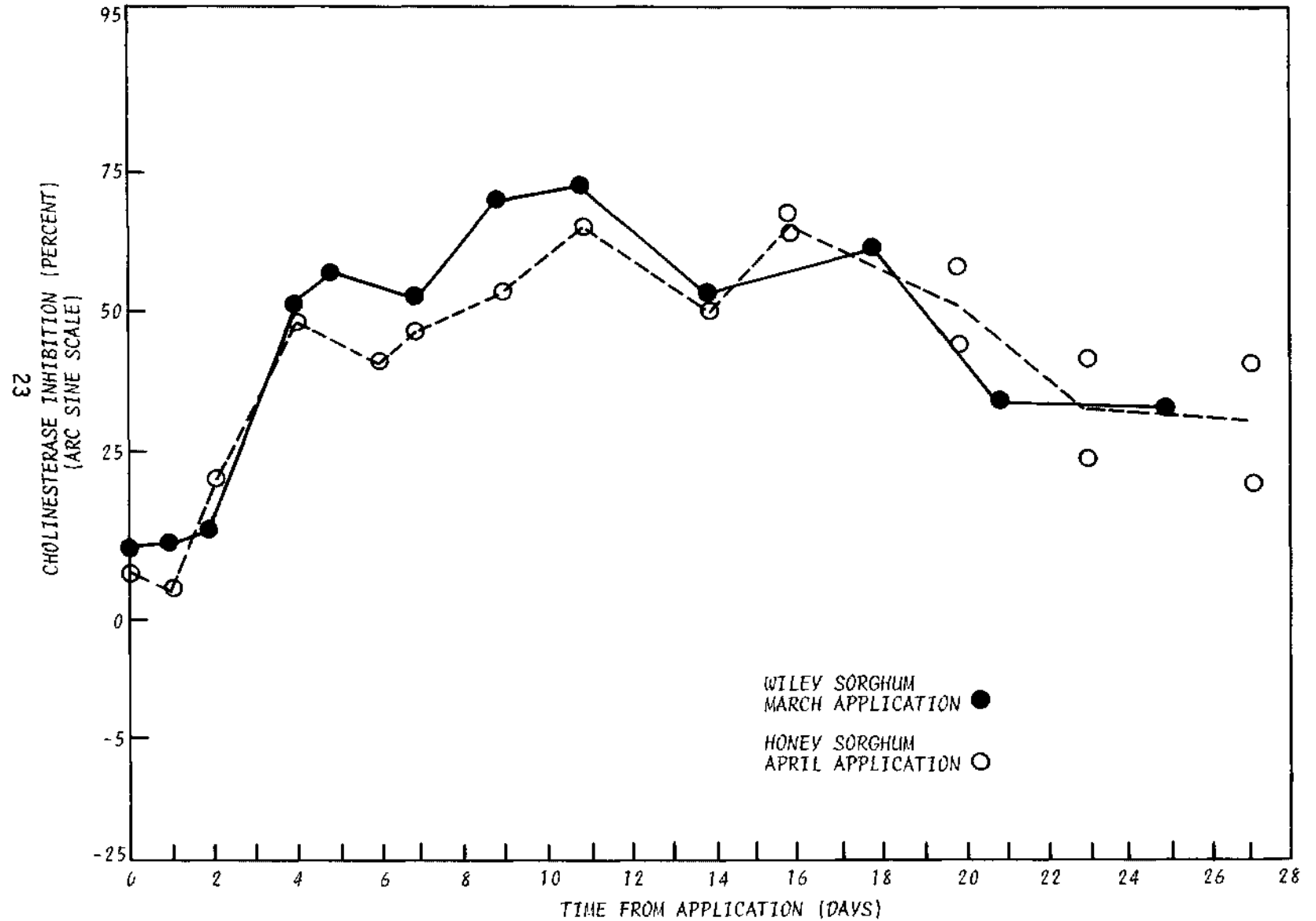


Figure 11. Percent Cholinesterase Inhibition, Wiley and Honey Sorghum

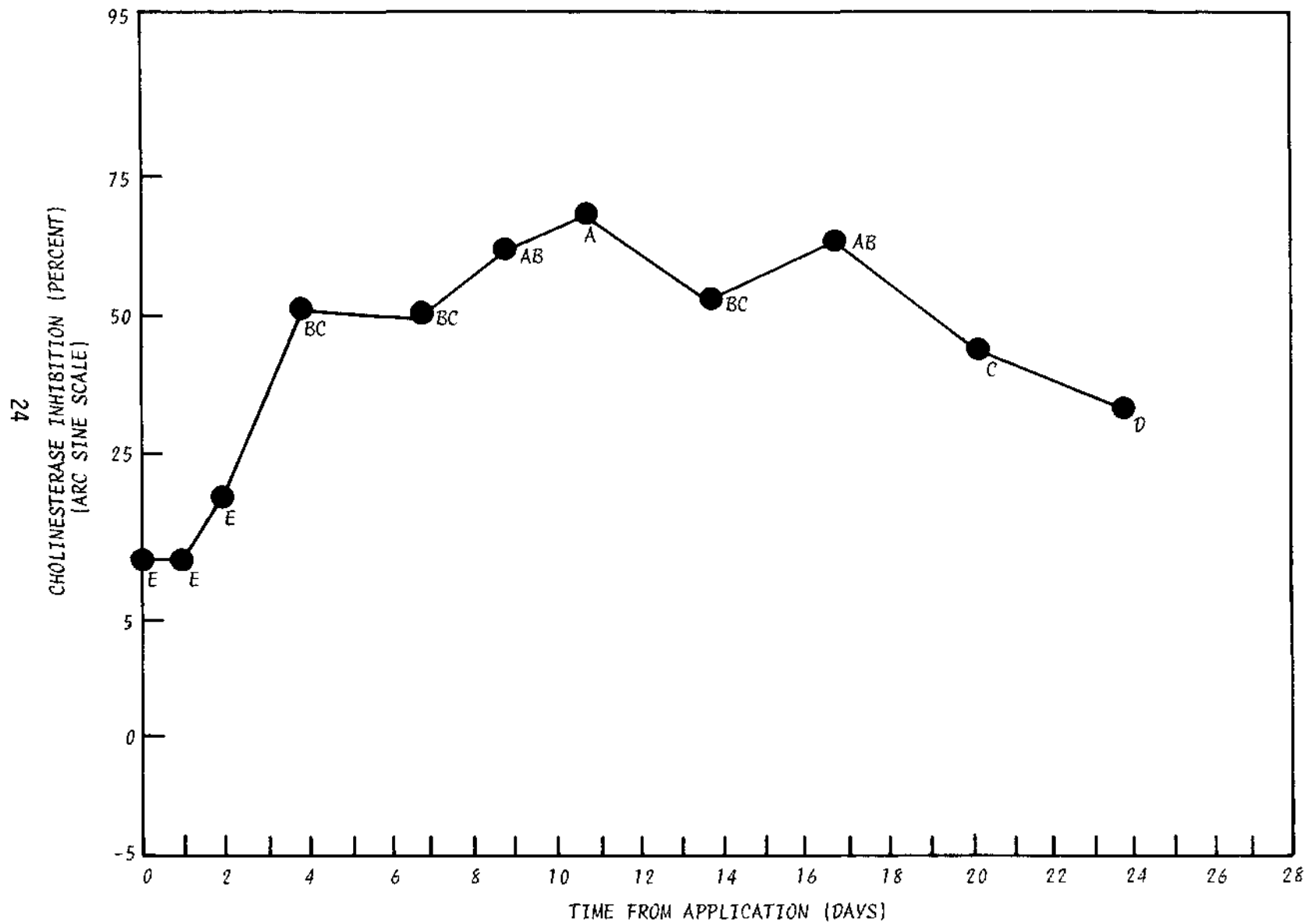


Figure 12. Effects of Passage of Time Upon Percent Cholinesterase Inhibition, Wiley Sorghum (March) and Honey Sorghum (April)

average peak level of 63.9 percent inhibition was maintained until the eighteenth to twenty-first day, when it began decreasing. By the twenty-third to twenty-fifth day it had decreased to 31.5 percent.

TABLE VI. AVERAGE PERCENT CHOLINESTERASE INHIBITION, WILEY SORGHUM (MARCH) AND HONEY SORGHUM (APRIL)

Days After Application	Cholinesterase Inhibition, Percent	Remarks (Common letter indicates no significant difference at 99-percent Probability Level)
11	67.9	a
16,18	62.5	ab
9	61.1	ab
14	51.0	bc
4	49.1	bc
7	49.0	bc
21,20	42.1	c
25,23	31.5	d
2	18.5	e
0	10.2	e
1	9.8	e

Figure 13 shows the percentages of cholinesterase inhibition for Wiley and Honey sorghum on various days during May. The analysis indicated highly significant differences only with respect to the number of days after application; neither the species of sorghum nor the species/day interaction yielded results which indicated any significant effect. The average percentages for the various days after application are shown in Table VII. All averages that are not significantly different at the 99 percent probability level have a common letter. In Figure 14, the average values with associated letters are shown graphically. May Wiley and Honey sorghum plants reached a peak percentage

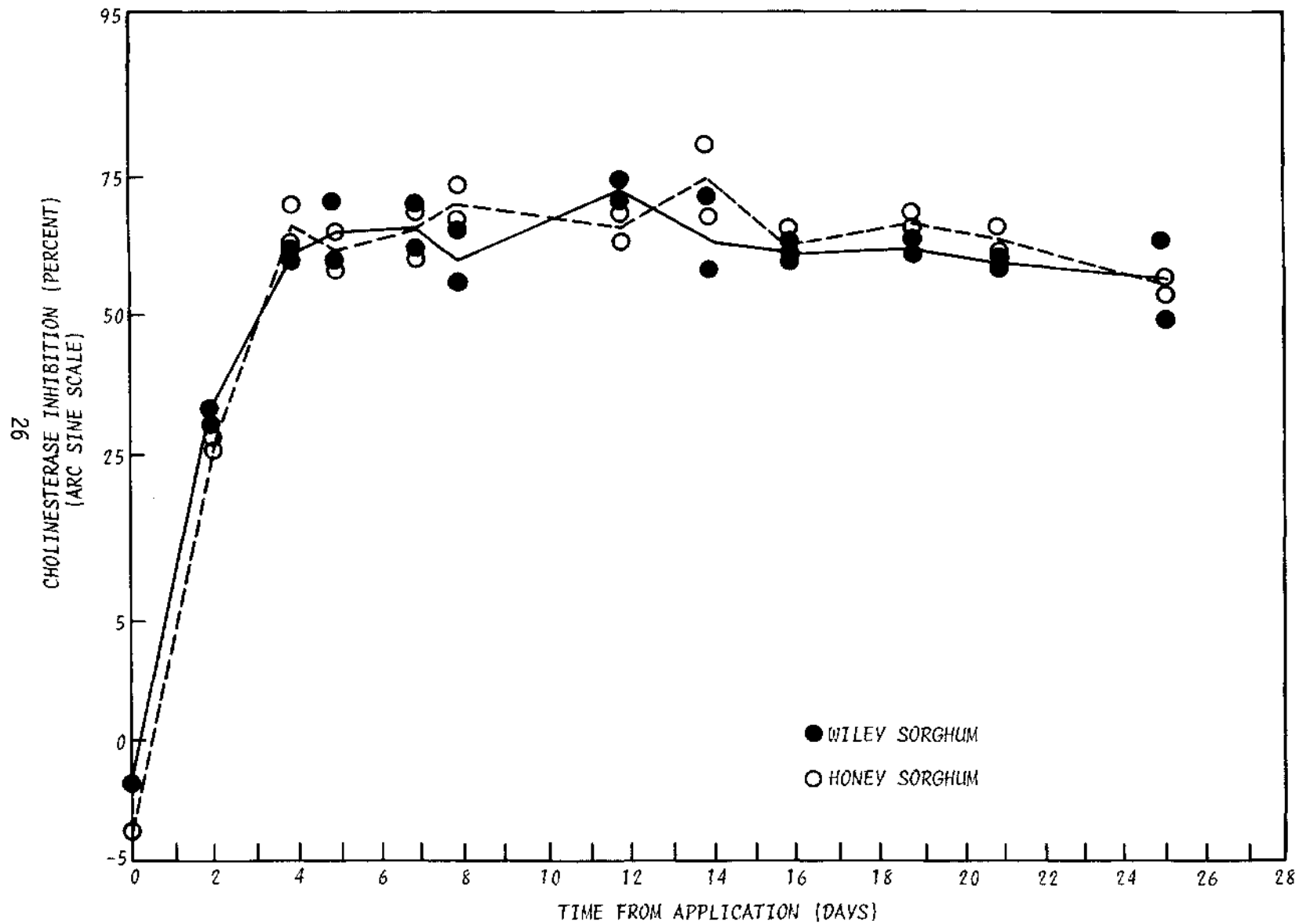


Figure 13. Percent Cholinesterase Inhibition, Wiley and Honey Sorghum (May)

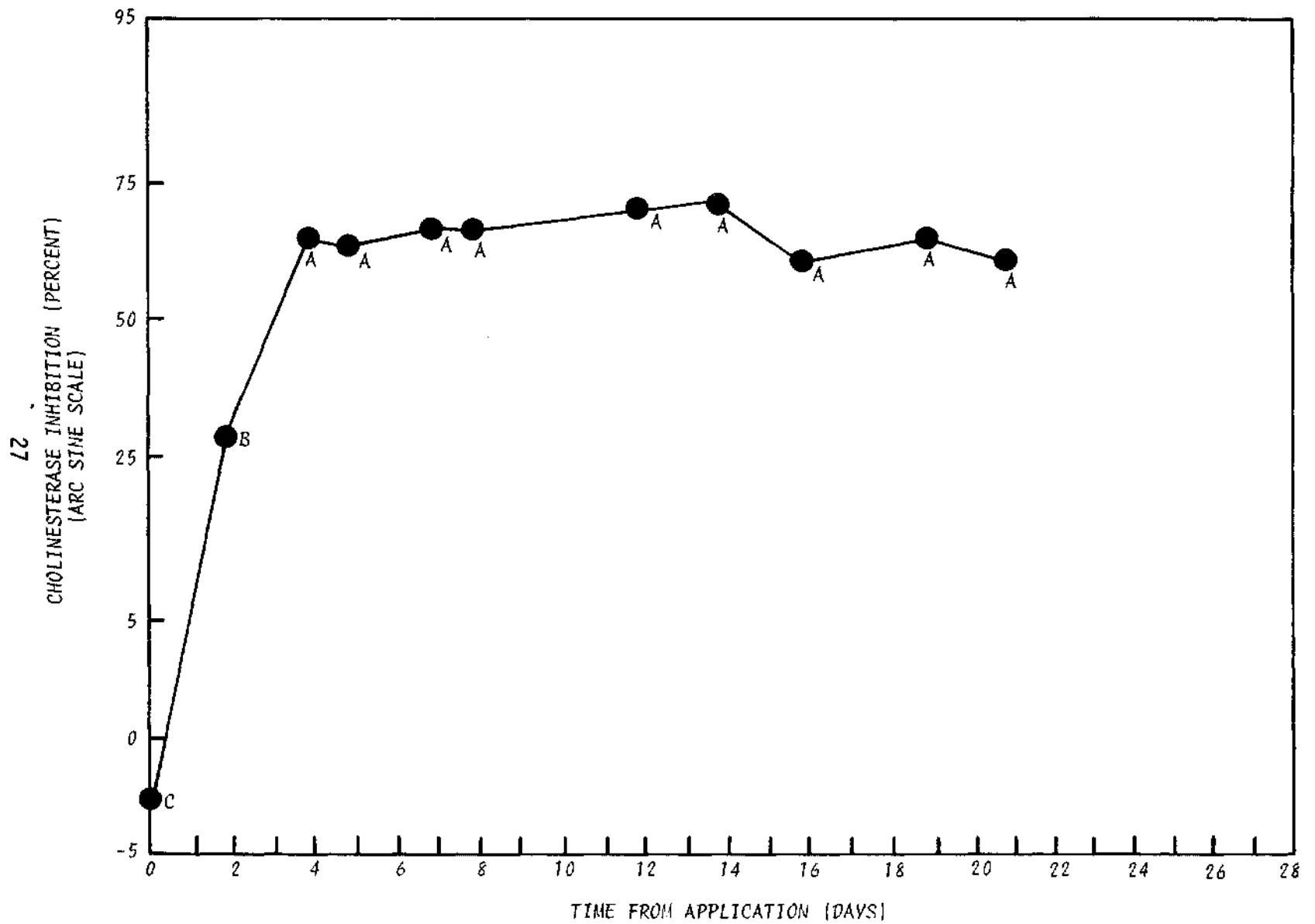


Figure 14. Effects of Passage of Time Upon Percent Cholinesterase Inhibition, Wiley and Honey Sorghum (May)

of cholinesterase inhibition by the fourth day and maintained an average peak level of 64.5 percent for the remainder of the month.

TABLE VII. AVERAGE PERCENT CHOLINESTERASE INHIBITION, WILEY AND HONEY SORGHUM (MAY)

Days After Application	Cholinesterase Inhibition, Percent	Remarks (Common letter indicates no significant difference at 99-percent Probability Level)
14	68.8	a
12	68.7	a
8	65.2	a
7	65.1	a
19	64.5	a
4	64.0	a
5	63.1	a
16	60.6	a
21	60.4	a
2	28.9	b
0	-1.5	c

With no significant differences existing between the two varieties of sorghum in May or during continuous experiments in March and April, it appeared that the metabolism of high concentrations of phorate proceeded at the same rate in each variety; however, distinct visible differences occurred between the two plant varieties.

Despite slight initial visible damage, both sorghum and tomato recovered from insecticide damage within 30 days of treatment. The preliminary data did show, however, that neither variety of sorghum recovered from insecticide damage when exposed to the same concentration of phorate as was used on the tomato plants. This is indicative of the

effects of a high concentration of phorate due to the large droplet size, since any visible damage at the rates used in this experiment would not be expected. With the tomato, minor curling of the leaves was observed and many plants had a loss of apical dominance indicating that possibly, at the concentrations of phorate used, there was a change in the auxin content or hormonal distribution within the plant. The first injury symptoms for both sorghum varieties were characterized by a localized bleaching of the blade pigments (i.e., chlorophyll) to a yellow-green coloration with a slightly flaccid condition. These necrotic blotches were more distinct with Honey sorghum as they acquired a red-brown coloration. This characteristic color difference was apparent in the aqueous samples, as Honey samples were much darker than those of Wiley. The flaccid condition was more evident with Honey sorghum. The more visible damage to sorghum compared to tomato in preliminary experiments, with both exposed to the same concentration of phorate, may appear to contradict the research reported in Reference 7. However, that report notes that (a) conclusions regarding the possible effects of organophosphorus insecticides, except mevinphos and methyl demeton, could not be made since the experiment was a fixed-effects model and not a random selection of possible organophosphorus insecticides; and (b) differences in plant response (susceptibility or resistance) could be accounted for by a postulation that differences may be related to leaf area interception of the insecticide.

The reason for existing differences between March/April and May sorghum can only be postulated. Peak percentages of cholinesterase inhibition by the fourth day (64.5 percent) in May versus the ninth day (63.9 percent) in March/April indicate a more rapid oxidation of phorate to anticholinesterase metabolites. A significant factor may be the relatively higher mean temperatures in May. In an evaluation of the effects of environmental temperature on Di-Syston[®] systemically applied to cotton leaves, the oxidation of the sulfide, Di-Syston[®], to the sulfoxide occurred so rapidly at temperatures above 70°F that only traces could be detected, even at intervals as short as one hour after treatment⁽⁸⁾. The major component in the leaves during the one-week experiment was the Di-Syston[®] sulfoxide. The rate of disappearance of sulfoxide was increased approximately 1.86 times for each 10°C rise in temperature (energy of activation of 10 kcal/mole). The initial oxidation of phorate in cotton leaves was less rapid than the oxidation of Di-Syston[®] with traces of phorate found up to three days, although these never exceeded 5 percent of the total radioactivity in the labeled experiment⁽⁵⁾. It was also noted⁽⁸⁾ that the rate of oxidation of the sulfoxides in the oxidation series (Figure 1) was measurably slower for phorate than for Di-Syston[®]. The sum of the rate constants for the disappearance of phorate sulfoxide due to oxidation is half that of Di-Syston[®] sulfoxide. This indicates that phorate sulfoxide was probably the major metabolite during the first two weeks of this investigation. Also, when alfalfa seed was treated with

treated with phorate or Di-Syston[®], the effectiveness for aphid control varied by as much as several weeks depending on the rate of plant growth⁽¹⁵⁾. The rate of metabolism is slower in cooler weather, and the slower the plant growth, the longer the persistence of toxic residues.

Tomato behaved similarly to the May sorghum. Tomato maintained a mean percentage value of 19.5 throughout the experiment; sorghum maintained a constant mean value throughout the month after the third day.

Thus, the results indicated that the metabolism of high concentrations of phorate proceeded at approximately the same rate in each species and between plant varieties. These results were not in complete harmony with those from earlier experiments. In previous metabolism studies of phorate and Di-Syston[®] on various plants such as cotton, alfalfa, lemon, and bean, it was found that the rates of reaction may be expected to vary slightly among plant species and according to the stage of growth⁽⁵⁾. Later studies⁽⁸⁾ specifically oriented toward the metabolism of Di-Syston[®] in a variety of plant species, indicated that at 70°F, the metabolism of Di-Syston[®] sulfoxide and hydrolytic decomposition of the toxic products occurred two to three times faster in tomato leaves than in cotton leaves.

Differences in results and insecticide application parameters indicated the experimental data resulted from the chemical nature of phorate on the plant surface without the influence of biological substrates. Application methods⁽⁵⁾ included topical application of 5 to 25 microliters of insecticide to the base of a young plant or placement of isolated leaves in a water dispersion (0.1 percent solution) of the insecticide to permit the study of the rates of metabolism uncomplicated by the continual accumulation of translocated material. The method of application used in this work was foliar with a definite quantity of phorate applied as large droplets to each intact plant.

An earlier study⁽¹⁶⁾ with Systox[®] (similar to phorate in structure) showed that the chemical nature of the surface washes from fruit treated with thiono- and thio- isomers changed rapidly upon exposure to light and air. Exposure of the isomers to light and air under controlled conditions on glass plates, uncomplicated by biological substrates, resulted in a surprisingly rapid conversion of the Systox[®] isomers into compounds which appeared to be chromatographically similar to those found within the plant tissues. Another study⁽¹⁷⁾ showed that the action of air and sunlight on surface residues of Systox[®] isomers has a rapid effect and appears to promote their oxidation in the same sequence as found *in vitro* with hydrogen peroxide and in plant tissues. Thin films of phorate exposed to ultraviolet light or sunlight and air gave similar results^(2, 18, 19, 20). Exposure to sunlight on paper, glass, and leaf surfaces indicated that the initial stable residues of phorate may not be the original compound or its simple oxidation products; prolonged exposure resulted in the formation of more polar compounds⁽¹⁸⁾. Results of ultra-

violet irradiation of phorate on the surface of a liquid suggested that the oxidation products are the sulfoxide and sulfone of the parent compound, with the sulfone showing greater persistency and the sulfoxide being in greater quantity during the early stages of irradiation^(19, 20).

To substantiate the concept that the experimental results in these investigations were without the influence of biological substrates within the plants, the same concentrations of phorate were applied to glass plates as were applied to the treated plants. The glass plates were located on the greenhouse bench adjacent to the control and treated plants. This was done with Wiley and Honey sorghum in March and April, respectively.

Table VIII shows a comparison of the gas chromatographic data for the plants and glass plates. Within 48 hours, phorate could no longer be detected on the glass plates; the same rapid disappearance was noted with the plants--approximately one ppm detected after 96 hours.

TABLE VIII. GAS CHROMATOGRAPHIC ANALYSIS FOR PHORATE FROM GLASS PLATES AND SORGHUM				
Day	Phorate Concentration, ppm			
	March		April	
	Wiley Sorghum	Glass Plates	Honey Sorghum	Glass Plates
0	16	22	18	26
1	9-12	5-6	9	5
2	6-7		5-6	
4	<3		1	
6			<1	

The cholinesterase-inhibition percentages obtained from the glass plates for the March and April experiments (Figure 15) were compared with the values obtained for the treated plants (Figure 11). The plots are very similar, with the glass plate cholinesterase-inhibition values being significantly higher on all days considered. However, most noteworthy is that the data through the twentieth day following application exhibited logarithmically linear trends (99 percent probability level) with no quadratic tendencies for both the sorghum and glass plates in March and April. The best-fitting straight lines have been plotted (Figures 16 and 17), and the equations for each of the lines are:

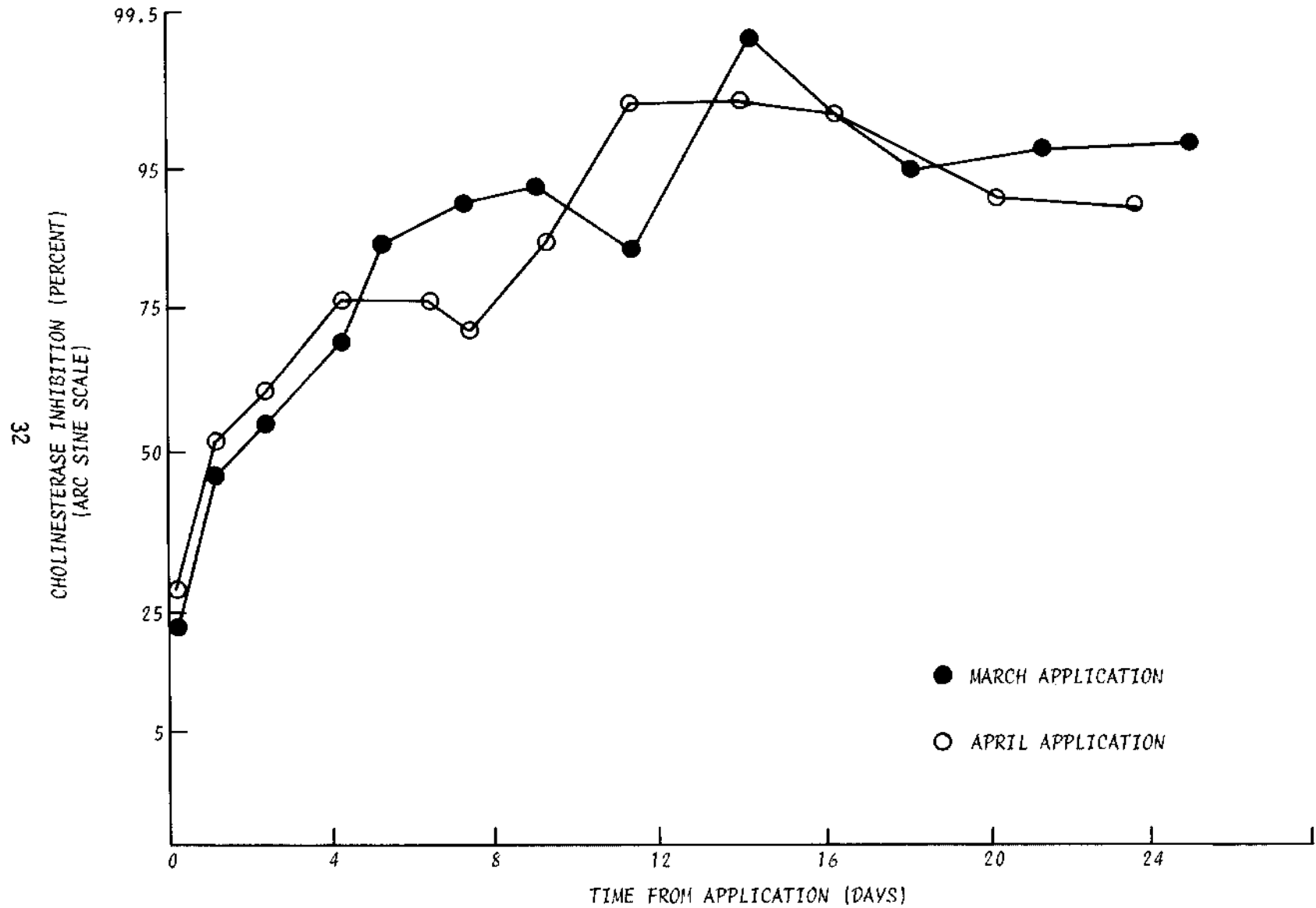


Figure 15. Percent Cholinesterase Inhibition, Glass Plates

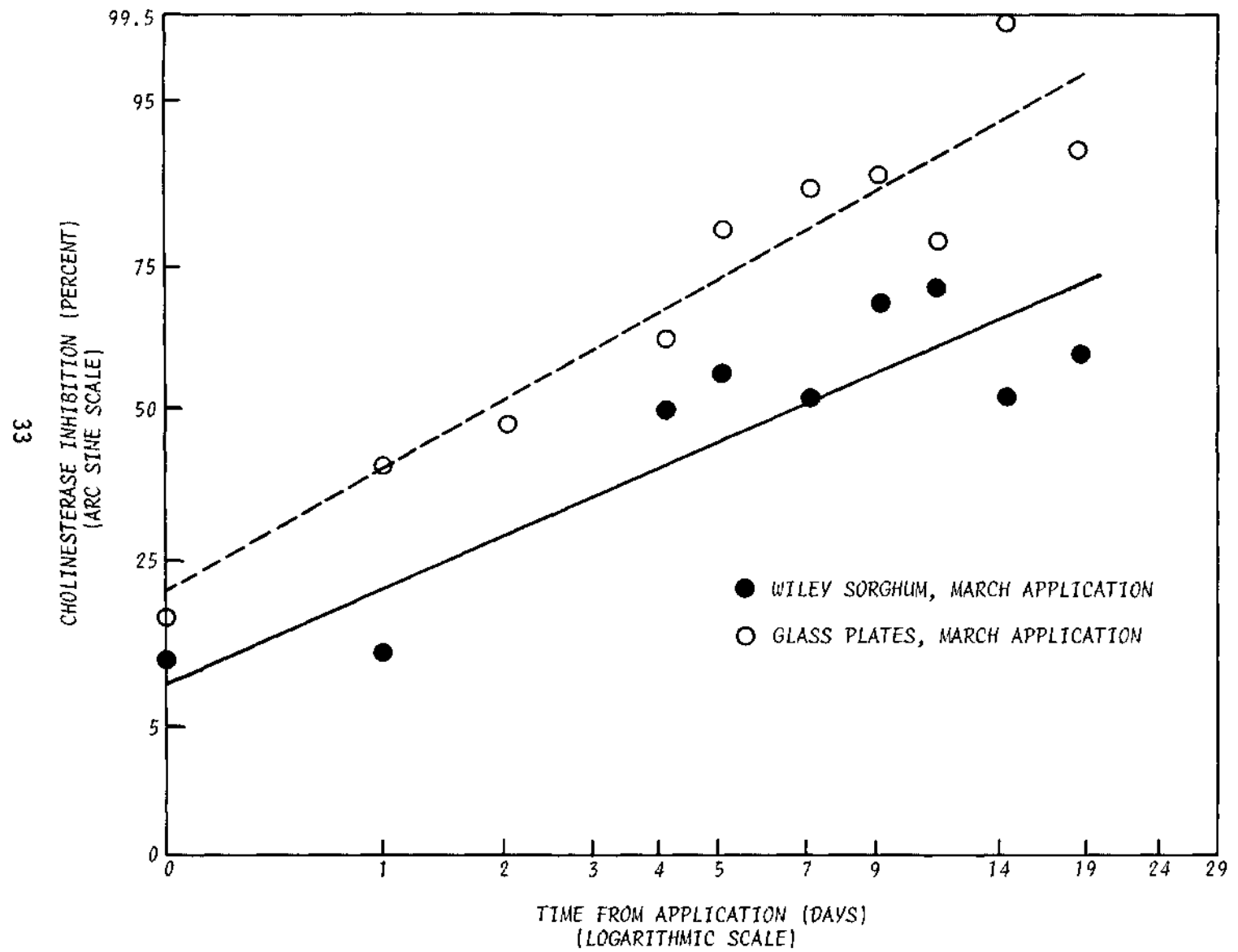


Figure 16. Comparison of Percentage Cholinesterase Inhibition for Glass Plates and Wiley Sorghum

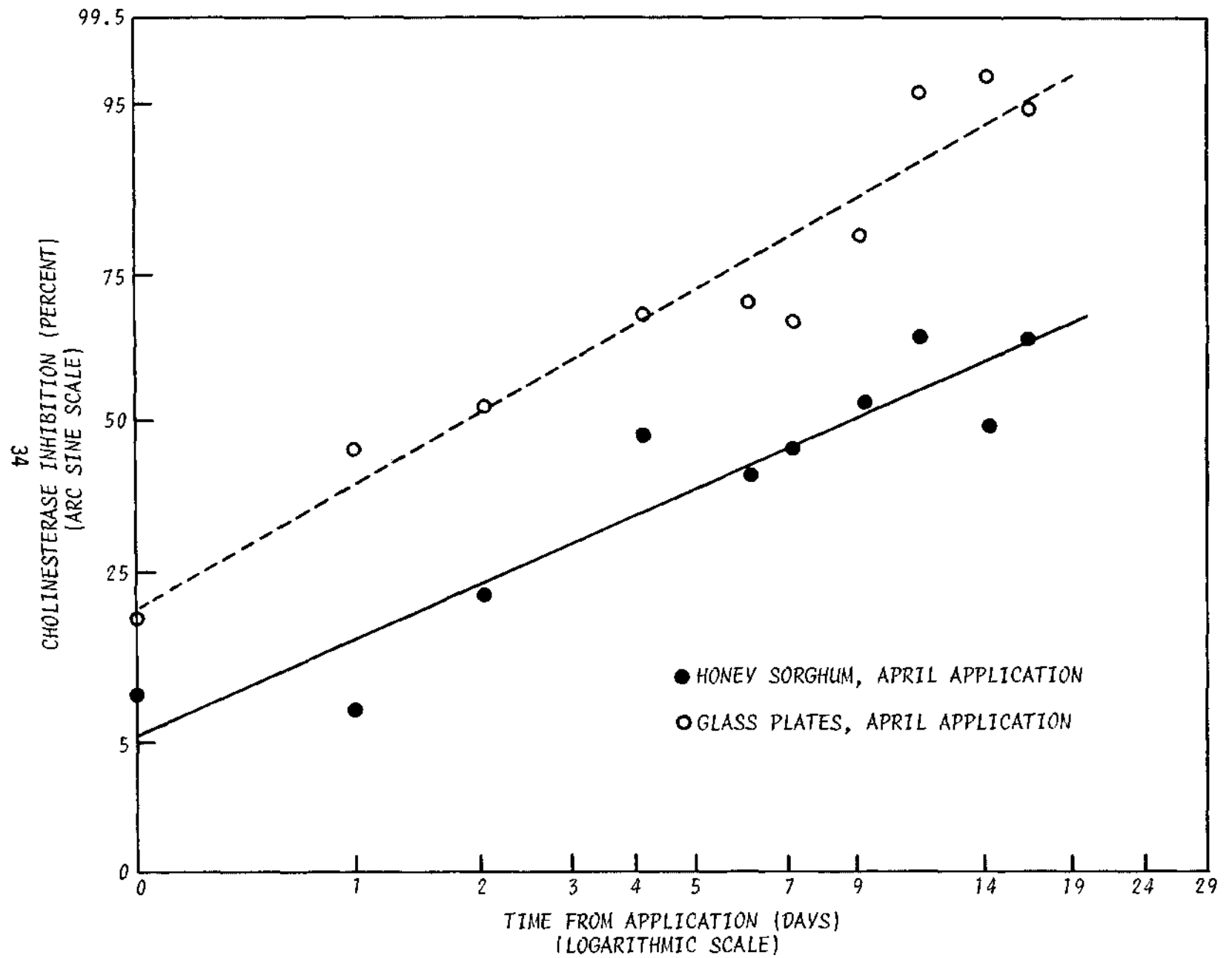


Figure 17. Comparison of Percentage Cholinesterase Inhibition for Glass Plates and Honey Sorghum

Wiley sorghum, March	$Y = 0.597 + 0.484 \log (X + 1)$
Glass plate, March	$Y = 0.922 + 0.626 \log (X + 1)$
Honey sorghum, April	$Y = 0.489 + 0.480 \log (X + 1)$
Glass plate, April	$Y = 0.917 + 0.621 \log (X + 1)$

where Y is the arc sine transformation of the percent cholinesterase inhibition

and X is the number of days after the application of phorate in vegetable oil.

An analysis of comparison of the linear plots gave significant results. The percentage of cholinesterase inhibition increased at the same rate for both varieties of sorghum. The percentages of cholinesterase inhibition for Wiley and Honey sorghum increased at the same rate as did those for the glass plates. The percent of variation explained by the linear trend is as follows:

Wiley Sorghum	78.6
Glass Plate, March	89.3
Honey Sorghum	88.2
Glass Plate, April	92.4

The rate of formation of cholinesterase-inhibiting compounds appeared the same between the glass plates and sorghum plants. It appeared that, at least at high concentrations of phorate, formation of anticholinesterase oxidized metabolites was predominantly through a chemical oxidation on the leaf surface, and not plant enzyme catalysis. This took place at least at such a rate as to mask enzyme catalysis. With low concentrations of phorate within sorghum blades, methods similar to those described in References 5 and 8 could distinguish any difference in the rates of metabolism between the two varieties of sorghum.

The higher cholinesterase inhibition values for the glass plates, (Figure 15) in comparison with the sorghum (Figure 11), can be attributed to the higher extraction efficiency with the glass plates. However, the phorate plant residue analysis by gas chromatography indicated the presence of phorate 48 hours after it could no longer be detected in samples from

the glass plates. Although the cholinesterase-inhibition analysis indicates the same rapid oxidation of phorate on leaf and glass plate, phorate could be present within certain portions of the leaf, e.g., within stomatal pores, and hence, within a potentially low-oxygen environment. This could explain the gas chromatographic data. An examination of roots resulted in no detectable cholinesterase inhibitors. Metabolism studies with lemon leaves showed the presence of large amounts of intact Di-Syston[®] accompanied by very slow conversion to other oxidative products⁽⁸⁾. This suggested that the oil-soluble esters were being protected from aqueous hydrolysis by the oil content of the leaves. This was confirmed by a radioautograph which showed nearly all of the radioactivity from P³² Di-Syston[®] translocated into a lemon leaf is confined to the oil glands. A similar radioautograph of Systox[®]-thiol-isomer translocated into lemon leaves showed that most of the radioactivity was located in the aqueous tissues of the plants⁽²¹⁾. These differences were correlated with the relative water solubilities of the compounds, i.e., Di-Syston[®] 66 ppm and Systox[®]-thiol-isomer 3900 ppm. The water solubility of phorate was recorded as 85 ppm⁽⁵⁾.

With the rate of formation of cholinesterase inhibitors the same on glass plates and sorghum leaf surfaces, the lower cholinesterase-inhibition percentage values for tomato (Figure 6) are difficult to explain. The values are lower than expected with tomato having received a concentration of phorate double that received by sorghum. Since the gas chromatographic data for tomato and sorghum agree, the distinct differences in cholinesterase-inhibition percentage values could have resulted from poorer efficiency in recovering oxidized metabolites from tomato compared to sorghum. The similarities in graphic plots for May sorghum (Figure 14) and tomato (Figure 8) would support this rationale.

Evaluation of the cholinesterase-inhibition percentage values with the calibration curve resulted in values relating the concentration of toxic residues present in and on the plant foliage. Fourteen days after application of phorate to Honey sorghum in April, sample preparation of a foliar rinse of the surface of the plant accounted for approximately 50 percent of the total cholinesterase inhibition of the plant. A lack of detection of phorate within seven days indicated that residues of the oxidized metabolites in sorghum occurred to a very large degree via oxidation of phorate on the leaf surface, absorption within the leaf, and possible translocation within the plant. This is not in agreement with the previous studies presented in References 5 and 9. Other researchers have postulated⁽⁵⁾ that the relative rates of absorption and translocation of phorate and Di-Syston[®] increased as the experiment proceeded because of the formation of more water-soluble oxidative metabolites in the subcuticular layers of plant tissue around the region of application. In Reference 9, the postulation is that the parent compounds were fairly persistent on the surface of the leaves but were metabolized rapidly once they had penetrated.

These variations can be explained by the differences in application method and in insecticide concentration: i.e., a 5 microliter topical application to the base of the stem of a cotton plant versus a 0.2 milliliter application of a 2 percent solution of phorate in vegetable oil to the blades of sorghum.

It must be remembered that as the toxic metabolites are forming, they are concurrently being hydrolyzed to nontoxic phosphoric or thiophosphoric acids. Though the oxygen-analogs of phorate can inhibit cholinesterase activity more than their thionophosphate precursors, they appear to have a higher degree of instability^(2, 4). The phosphorus is considerably more electrophilic in the P=O compounds, thus weakening the P-S ester bond and facilitating hydrolysis and accelerating phosphorylation of the enzyme⁽⁵⁾. Consequently, the presence of relatively large amounts of a highly oxidized metabolite in a plant would result in higher cholinesterase inhibition and higher apparent residue values than would an equivalent amount of a metabolite with less cholinesterase activity in another plant. The higher cholinesterase-inhibition values mean the presence of metabolites which are easily hydrolyzed, resulting in an overall faster rate of metabolic detoxification.

The concentrations of phorate metabolites in tomato and sorghum were expressed in parts per million (ppm) as phorate oxygen analog sulfoxide equivalent via a cholinesterase-inhibition method of analysis. The calibration curve for the residue method is given in Figure 2. It is independent of plant material analyzed and of the sample preparation technique. It reflects none of the losses that may occur in the various steps of sample preparation. A sample calculation follows the formula:

$$\frac{wxV/v}{W} = \text{parts per million of phorate oxygen analog sulfoxide equivalent in sample analyzed.}$$

Where w is the phorate oxygen analog sulfoxide equivalent obtained in the analyses, micrograms.

v is the aqueous extract in the determination, milliliters.

V is the total solvent in sample extraction, milliliters.

W is the sample extracted, grams (fresh weight).

The toxic residues present in tomato foliage were based on the average weight of tomato plants initially after application of phorate (six weeks) and at the conclusion of the experiment (nine weeks), 6 grams and 28 grams, respectively. Thus, residues in the tomato foliage ranged from 1.1 to 5.3 ppm phorate oxygen analog sulfoxide equivalent.

Residue persistence in sorghum (Table X) was higher. The May sorghum had concentrations with a range of 0 to 20.2 ppm. The average residue value after the second day was 17.9 ppm. The March/April sorghum had concentrations with a range of 2.4 to 18.5 ppm. The fourfold increase in residues by the ninth day is comparable to that found by Bowman and Casida⁽²⁾ in considering the persistence of phorate-P³² and its metabolites in vegetable crops. The total anticholinesterase activity of greenhouse pea plants, sprayed with phorate at one pound per acre, increased for about the first four days and then declined, but inhibitors persisted for 20 to 30 days⁽²⁾. Foliage application of O,O-diethyl S-[(isopropylthio) methyl] phosphorodithioate to pea plants resulted in the appearance of anticholinesterase metabolites within one day and persistence of such metabolites in high concentration for at least nine days with detectable amounts present for 21 days⁽²⁾. The results of this investigation were comparable: the main difference was higher residue levels.

In crops treated with phorate, the ultimate toxic residues are present in a fractional part per million⁽⁵⁾. When applied to corn at a rate of one pound per acre, phorate was essentially gone in 14 days, while very low levels of its sulfoxide and sulfone (0.1 ppm or less) persisted through the 28-day experimental interval. At harvest time, the plant was essentially free of insecticide, less than 0.01 ppm⁽⁴⁾.

The concentration of phorate metabolite residues present, though high, would probably be at a safe level by harvest time. The ultimate toxic metabolites present in harvest time residues are dependent upon both the interval between application and harvest and the method of application. Older plants having phorate applied at high rates would definitely have to be monitored for toxic residues.

The lower residue values for tomato foliage are due in part to a larger daily plant weight--approximately threefold that of sorghum. The result could be a more rapid metabolism and hydrolysis and provides another possibility for the cholinesterase-inhibition percentage values being lower for tomato than for sorghum.

Generally, oxidation in plants never increases the toxicity of an application significantly⁽⁹⁾. However, the large residue values obtained in this study indicate that the toxicity is increased considerably on the surface of the plant when high concentrations are involved. A number of researchers, in speculating upon potential residue problems after various methods of treatment with systemic insecticides, have concluded that persistence curves should be established on different crops grown under different environmental conditions⁽⁸⁾. Military application of insecticides at normal rates results in residues which can be monitored with guidance from available literature. Residue breakdown of these organophosphorus insecticides is usually rapid with no persistency problems. However, the result of repetitive

aerial application or spillage of insecticides in cropland areas may result in concentrations higher than usual. This study indicates exposure studies of the respective insecticides on glass plates alone under different environmental conditions would serve as a guide in predicting residues from high concentrations of insecticides.

TABLE IX. PERSISTENCE OF PHORATE OXYGEN ANALOG SULFOXIDE EQUIVALENT IN SORGHUM		
Time From Application, Day	Concentration, ppm	
	March/April ^a	May ^b
0	2.4-5.3	0
1	2.4-5.3	8.3
2	2.4-5.3	
4	10.3-14.5	17.1
5		19.7
7	10.3-14.5	18.7
8		18.5
9	12.6-18.5	
11	16.0-18.5	
12		16.6
14	10.3-14.5	20.2
16	12.6-18.5	18.0
18	12.6-18.5	
19		15.3
20	10.3-11.9	
21	10.3-11.9	17.0
23	6.9-7.9	
25	6.9-7.9	

^aConcentration range is the result of considering the standard deviation for the average plant weight for the entire experimental period in May; this is due to the lack of plant weight values for March/April. Results are averages of two samples, three replications each.

^bResults are averages of four samples, three replications each.

SECTION IV

SUMMARY AND CONCLUSIONS

Data on the metabolism of foliar applications of high concentrations of the organophosphorus insecticide phorate on Homestead tomato and Wiley and Honey sorghum are reported. The investigation of phorate metabolism, monitored by gas chromatographic and enzymatic analysis, produced the following results:

1. The cholinesterase activity values obtained showed no correlation with plant weight.
2. The disappearance of phorate appeared to proceed at the same rate in each plant species and variety; phorate disappeared more quickly from glass plates than from March/April sorghum under the same experimental parameters.
3. No significant differences were apparent between the two varieties of sorghum in May or during continuous experiments in March and April. It appeared that the formation of anticholinesterase metabolites, after high foliar applications of phorate, proceeded at the same rate in each variety although distinct visible differences occurred between the Wiley and Honey sorghum.
4. The peak percentages of cholinesterase inhibition from sorghum samples by the fourth day in May versus the ninth day in March/April indicated more rapid oxidation of phorate to anticholinesterase metabolites at higher temperatures.
5. The phorate metabolism in tomato was similar to metabolism in the May sorghum; however, actual comparison of percentage values of cholinesterase inhibition during the month for each of the three plants indicated that the average for the Homestead tomato was significantly lower than those for the two varieties of sorghum.
6. The percentage values of cholinesterase inhibition for the Wiley and Honey sorghum increased at the same rate as for the glass plates, indicating that the rate of formation of anticholinesterase-oxidized metabolites was predominantly through chemical oxidation on the leaf surface and not by plant enzyme catalysis; this surface oxidation took place at least at such a rate as to mask enzyme catalysis.
7. The larger droplet size in application technique resulted in higher toxic-residue values for phorate metabolites, especially on the surface of the plant, than would normally be expected.

This study was initiated to find a basis for predicting toxicity and persistence of metabolite residues in plants after application of high concentrations of sulfur-containing organophosphorus insecticides during military spray operations. The results indicate that exposure studies of high concentrations of insecticides on glass plates alone, under different environmental conditions, would serve as a guide in predicting residues from repetitive aerial application or spillage of insecticides used by the military in cropland areas. Such studies with a controlled environment would yield toxic-residue-persistence data under various conditions for high concentrations of insecticides.

REFERENCES

1. Thomson, W.T. Agricultural Chemicals, Book I. Insecticides, Acaricides, and Ovicides. Thomson Publications, Davis, California, 1967.
2. Bowman, J.S. and J.E. Casida. Metabolism of the Systemic Insecticide O,O-Diethyl S-Ethylthiomethyl Phosphorodithioate (Thimet) in Plants. *J. Agr. Food Chem.* 5: 192-197, 1957.
3. Bowman, J.S. and J.E. Casida. Further Studies on the Metabolism of Thimet by Plants, Insects, and Mammals. *J. Econ. Entomol.* 51:838-843, 1958.
4. Bowman, M.C., M. Beroza, and J.A. Harding. Determination of Phorate and Five of Its Metabolites in Corn. *J. Agr. Food Chem.* 17:138-142, 1969.
5. Metcalf, R.L., T.R. Fukuto, and R.B. March. Plant Metabolism of Dithio-Systox and Thimet. *J. Econ. Entomol.* 50:338-345, 1957.
6. Coleman, O.H. and J.L. Dean. Inheritance of Resistance to Methyl Parathion in Sorgo. *Crop Sci.* 4:371-372, 1964.
7. Wolverton, B.C., W.J. Wallace, A.L. Young, and D.D. Harrison. Studies on the Systemic Uptake of Toxic Phosphorus Esters by Plants. Morphological Effects of Foliar Applications of the Organophosphate Insecticides Mevinphos and Methyl Demeton on Selected Plant Species. Air Force Armament Laboratory Technical Report AFATL-TR-69-116, Eglin Air Force Base, Florida, September, 1969.
8. Metcalf, R.L., H.T. Reynolds, M. Winton, and T.R. Fukuto. Effects of Temperature and Plant Species upon the Rates of Metabolism of Systemically Applied Di-Syston. *J. Econ. Entomol.* 52:435-439, 1959.
9. Heath, D.F. Metabolism in Plants and Soils. In Organophosphorus Poisons, Anticholinesterases and Related Compounds edited by D.F. Heath. Pergamon Press, New York, 1961.
10. Archer, T.E. Enzymatic Methods. In Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives, Volume I edited by G. Zweig. Academic Press, New York, 1963.
11. Sutherland, G.L., P.A. Giang, and T.E. Archer. Thimet. In Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives, Volume II edited by G. Zweig. Academic Press, New York, 1964.

12. Nabb, D.P. and Florence Whitfield. Determination of Cholinesterase by an Automated pH Stat Method. Arch. Environ. Health. 15:147-154, 1967.
13. Himel, C.M. The Optimum Size for Insecticide Spray Droplets. J. Econ. Entomol. 62:919-925, 1969.
14. Young, A.L. and B.C. Wolverton. Military Herbicides and Insecticides. Air Force Armament Laboratory Technical Note AFATL-TN-70-1, Eglin Air Force Base, Florida, January, 1970.
15. Reynolds, H.T., T.R. Fukuto, R.L. Metcalf, and R.B. March. Seed Treatment of Field Crops with Systemic Insecticides. J. Econ. Entomol. 50:527-539, 1957.
16. Metcalf, R.L., R.B. March, T.R. Fukuto, and M.G. Maxon. The Nature and Significance of Systox Residues in Plant Materials. J. Econ. Entomol. 48:364-369, 1955.
17. Fukuto, T.R., R.L. Metcalf, R.B. March, and M.G. Maxon. Chemical Behavior of Systox in Biological Systems. J. Econ. Entomol. 48:347-354, 1955.
18. Cook, J.W. and R. Ottens. Note on the Conversion of Some Organophosphate Pesticides to Less Polar Compounds by Ultraviolet Light. J. Assoc. Offic. Agr. Chemists. 42:211-212, 1959.
19. Mitchell, T.H., J.H. Ruzicka, J. Thomson, and B.B. Wheals. The Chromatographic Determination of Organophosphorus Pesticides. Part III. The Effect of Irradiation on the Parent Compounds. J. Chromatog. 32:17-23, 1968.
20. Ruzicka, J.H., J. Thomson, and B.B. Wheals. The Gas Chromatographic Examination of Organophosphorus Pesticides and Their Oxidation Products. J. Chromatog. 30:92-99, 1967.
21. Metcalf, R.L., R.B. March, T.R. Fukuto, and M.G. Maxon. The Behavior of Systox-isomers in Bean and Citrus Plants. J. Econ. Entomol. 47:1045-1055, 1954.

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1. ORIGINATING ACTIVITY (Corporate author) Flame, Incendiary, and Explosives Division Air Force Armament Laboratory Eglin Air Force Base, Florida		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	
		2b. GROUP	
3. REPORT TITLE THE METABOLISM OF HIGH CONCENTRATIONS OF THE ORGANOPHOSPHORUS INSECTICIDE PHORATE APPLIED FOLIARLY TO SELECTED PLANT SPECIES			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Final Report (May - December 1970)			
5. AUTHOR(S) (First name, middle initial, last name) George S. Kotchmar, Jr., Capt, USAF, Billy C. Wolverton, Elizabeth E. Boothe, Sandra M. Lefstad			
6. REPORT DATE February 1971	7a. TOTAL NO. OF PAGES 53	7b. NO. OF REFS 21	
8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S) AFATL-TR-71-22		
b. PROJECT NO. 5066			
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)		
d.			
10. DISTRIBUTION STATEMENT Approved for public release; distribution unlimited.			
11. SUPPLEMENTARY NOTES Available in DDC		12. SPONSORING MILITARY ACTIVITY Air Force Armament Laboratory Air Force Systems Command Eglin Air Force Base, Florida 32542	
13. ABSTRACT Gas chromatographic and enzymatic analyses (cholinesterase-inhibition method) were used to monitor the metabolism of the organophosphorus insecticide 0,0-diethyl S-[(ethylthio)methyl] phosphorodithioate (phorate) applied foliarly to three economically important plants (Homestead tomato, Wiley sorghum, and Honey sorghum). The resulting data provided guidelines in predicting toxicity and persistence of metabolite residues for high concentrations of insecticides employed by the military. An attempt was also made to relate the metabolism of the insecticide to phytotoxic damage among and within plant species. The data indicated that no plant-variety-dependent distinction exists in the formation of toxic phorate metabolites as shown by <i>in vitro</i> anticholinesterase activity recorded over a four-week period. Further investigation, with the same high concentrations of phorate placed on glass plates located adjacent to treated plants, indicated the formation of toxic phorate metabolites was without the influence of biological substrates within the plants. There were no statistically significant differences with respect to the rate of increase of cholinesterase-inhibition percentage values between the sorghum and glass plates; the rate of formation of anticholinesterase oxidized metabolites was predominantly through chemical oxidation on the leaf surface and not by plant enzyme catalysis, or at least, the oxidation occurred at such a rate as to mask the enzyme catalysis. The large droplet size in the application of phorate resulted in higher toxic residue values, especially on the surface of the plant, than would normally be expected.			

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Phorate O,O-diethyl S-[(ethylthio)methyl] phosphorodithioate Organophosphorus Insecticides Plants Insecticide Residues Insecticide Metabolism						