Persistence of Residues of Velsicol VCS-506 and Two of Its Metabolites in Tomatoes and Grapes

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The persistence of Velsicol VCS-506 [*O*-(4-bromo-2,5-dichlorophenyl)-*O*-methyl phenylphosphonothioate] insecticide, its O-analog, and phenol derivative (2,5-dichloro-4-bromophenol) was studied on tomatoes and grapes grown in the coastal plain of Israel during the summer and fall. Chemical analysis of tomato plants sprayed with VCS-506, 3500 g of emulsifiable concentrate/ha, showed that the residue level in the fruit was the same 1 and 21 days posttreatment. During the same time interval, there was only a 20% reduction in the residue level of Velsicol VCS-506 in the leaves, whereas between the third and fifth weeks after treatment, the rate of degradation increased. In grapes that were dipped in a 2.5% VCS-506 emulsifiable concentrate emulsion the residue level was 12 ppm 1 day after treatment and 7.5 ppm 44 days later. The concentration of the phenol derivative in all the samples was 1–10% of that of the parent compound. In both tomatoes and grapes, the highest concentration of this derivative was found 3 weeks after treatment. Accumulation of the O-analog was not found. Translocation of the parent compound from leaves to fruit was not observed, but some translocation of the phenol derivative was detected. Results from the chemical analysis were confirmed by the tlc enzymic inhibition technique. Additional cholinesterase-inhibiting metabolites were not detected. Field application was according to the recommended amounts and procedures.

An organophosphorus insecticide Velsicol VCS-506 [*O*-(4-bromo-2,5-dichlorophenyl)-*O*-methyl phenylphosphonothioate; also known as Phosvel, Abar, and Leptophos] was chosen as a potential candidate for insect control in cotton and vegetable crops in Israel. Its relatively low mammalian toxicity (acute oral LD_{50} of technical VCS-506, 90.5 mg/kg (albino rats) and its promising performance in the field gave a valid reason for further investigation. Leuck *et al.* (1969, 1970) reported that this compound degrades rather slowly in coastal Bermuda grass and in forage corn. A decrease of 50% in the residue level in corn plants was recorded 2–3 weeks after treatment. Johnson *et al.* (1971) showed that disappearance of VCS-506 was less rapid than that of many other organophosphorus insecticides.

In this work the persistence of Velsicol VCS-506 and two of its major metabolites was studied in two edible crops, tomato and grapes. Chemical analysis of the parent compound, its O-analog, and the phenol derivative (2,5-dichloro-4-bromophenol) was based on the glc method described by Bowman and Beroza (1969).

EXPERIMENTAL SECTION

Insecticides. Analytical samples of Velsicol VCS-506, its O-analog, the phenol derivative, and a commercial formulation of the insecticide (emulsifiable concentration 36%) were supplied by the Velsicol Chemical Corp. (Chicago, Ill.). Analysis of the emulsifiable concentrate formulation showed: Velsicol VCS-506 content, 32%; O-analog, less then 0.0013%; and the phenol derivative, 0.13%.

Experiments with Tomato Plants. Tomato plants (variety EILON) were grown in the coastal area and sprayed on Nov 14 with a 36% emulsifiable concentrate of Velsicol VCS-506. The insecticide was applied at a rate of 1250 g of active ingredient/ha to fruit-bearing plants. Samples of leaves and fruits were taken on selected days after treatment. On each date three samples of 1 kg of fruit and 50 g of leaves were taken at random and kept at −17° until analyzed. On each sample two analyses were performed.

Owing to the relatively low temperatures prevailing (daily average temperature, 14–20°), there was no marked change in the size of the fruits during the course of the experiment. The first rain fell 4 weeks after treatment.

Experiments with Grapes. The experiments were carried out on two varieties of grapes, Dabouki (white) and Muskat Hamburgii (black), during midsummer (daily average temperature, 22–30°). Bunches of grapes of similar size (2 weeks before ripening) were marked and then dipped in 10 l. of 2.5% of the emulsifiable concentrate emulsion. At different times after application, samples of four bunches each were taken and kept frozen until analyzed.

Analytical Methods. Residue determination of Velsicol VCS-506, its O-analog, and phenol derivative was carried out according to the method reported by Bowman and Beroza (1969), with some modifications in the extraction and column chromatography steps. Per cent recovery and limit of detection are presented in Table I.

Extraction Procedure. *Tomato Leaves.* Samples of 50 g of tomato leaves were extracted in a Waring Blendor with 200 ml of acetone. The mixture was filtered through Pyrex No. 3 fritted glass funnel and the leaf debris was reextracted twice more with 200 and 150 ml of acetone. The extracts were combined and reduced under vacuum to about 250 ml. A quantity of 150 ml of 10% NaCl solution was added (to bring the ratio between acetone and water to 1:1), and the solution was extracted three times with benzene (100, 100, 50 ml).

The combined benzene extracts (containing the VCS-506, its O-analog, and the phenol derivative) were dried over anhydrous Na_2SO_4, and the volume was reduced on a rotary evaporator to 1–2 ml. Twenty milliliters of benzene was added, and the content was again evaporated to 1–2 ml (to remove all the acetone) and the residue dissolved in 10 ml of benzene.

Fruits. Samples of 400 g of tomato fruits or grapes were homogenized in a Waring Blendor with 400 ml of acetone. A subsample of 100 g (about 200 ml) was taken and filtered through a fritted glass funnel. The sample was reextracted two more times, with 150 and 100 ml of acetone, and prepared for column chromatography following the same procedure described for tomato leaves.

Column Chromatography. The parent compound, the O-analog, and the phenol derivative were separated by two columns (20 mm i.d.) working in tandem: upper column, deactivated silica gel (mixing 200 g with 40 ml of 0.2

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Table I. Per Cent Recovery[a] of Velsicol VCS-506, Its O-Analog, and Phenol Derivative

Plant organ	Level of fortification, ppm	Velsicol VCS-506	O-Analog	Phenol derivative
Tomato, leaves	0.2	90	83	100
	1.0	100	85	105
	4.0	95	90	
Tomato, fruit	0.2	103	87	96
	1.0	97	91	92
	2.0	95		
Grapes, fruit	0.5	96	87	98
	2.0	94		

[a] Limit of detection: Velsicol VCS-506; 0.02–0.05 ppm; O-analog, 0.05–0.08 ppm; phenol derivative, 0.005–0.008 ppm.

M phosphate buffer (pH 7) diluted 1:1 with distilled water and then dried at 105° for 90 min); lower column, alumina (10 g, grade III). In each of these columns 2 g of anhydrous Na_2SO_4 was placed above and below the adsorbent layer. Both columns were prewet with benzene. The benzene extract (in 10 ml) was applied to the upper column and eluted with 50 ml of benzene. The benzene eluate collected from the lower column contained the Velsicol VCS-506. The columns were then separated. The silica gel column was first washed with 50 ml of 10% ether in petroleum ether (this fraction was discarded) and then with 150 ml of 50% ether in petroleum ether to elute the O-analog.

The alumina column was washed with 100 ml of acetone (this fraction was discarded) and the phenol derivative was eluted with 75 ml of 2% glacial acetic acid in benzene. The acidic benzene eluate was washed with 50 ml of 5% sodium bicarbonate followed by 25 ml of water and dried over Na_2SO_4.

The VCS-506 eluate and those of the phenol derivative and O-analog were evaporated almost to dryness and the residue dissolved in the appropriate volume of acetone for glc determination.

Gas Chromatography. The column and the conditions used were those described by Bowman and Beroza (1969). The instrument used was a Tracor MT-220 gas chromatograph.

RESULTS AND DISCUSSION

Residues in Tomato Plants. Results from this experiment have shown that in the plant the insecticide Velsicol VCS-506 degraded relatively slowly. During the first 3 weeks after treatment, losses of Velsicol VCS-506 from the leaves amounted to 20% of the total applied (Figure 1). Later on, the rate of degradation almost doubled, but, even so, after 5 weeks the concentration of the insecticide was still 35% of that applied to the leaves.

Similar results were obtained from the analysis of tomato fruits (Figure 2). During the first 3 weeks after treatment there was no reduction in the amount of Velsicol VCS-506 in the fruit. Degradation of the insecticide was observed between the third and fifth weeks only.

This experiment was conducted in the fall (November–December), on plants that were at their final stage of growth, and therefore the concentration of the insecticide in the tissue was not affected by the growth of the plant.

The rate and shape of the degradation curve of Velsicol VCS-506 in tomato plants were different from those usually obtained for organophosphorus insecticides. The degradation of this insecticide was very slow at the beginning and was accelerated only 3 weeks after treatment, whereas the more common type of curve shows rapid degradation on the first days after treatment and a slower rate later on.

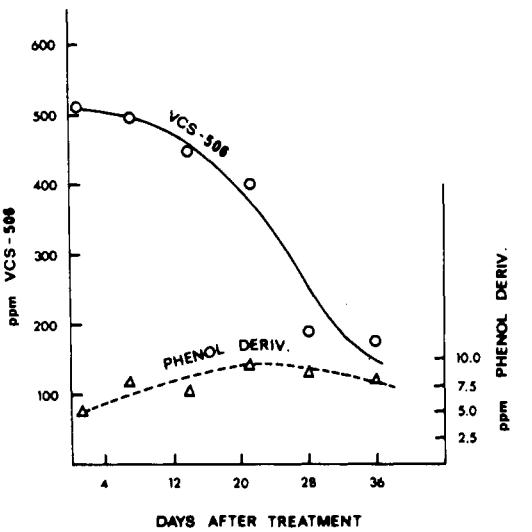

Figure 1. Residues of Velsicol VCS-506 and its phenol derivative in tomato leaves.

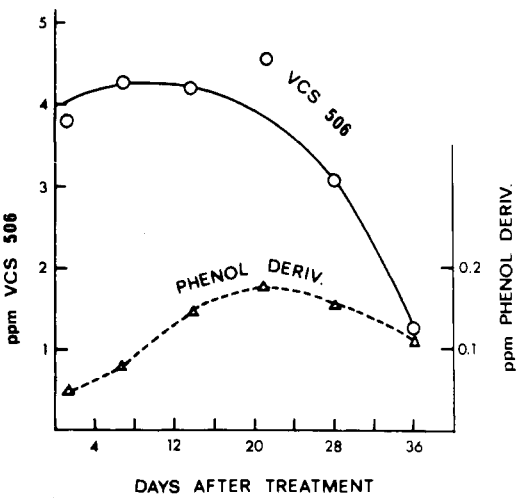

Figure 2. Residues of Velsicol VCS-506 and its phenol derivative in tomato fruits.

Chemical analysis of tomato plants showed that during the first 3 weeks after treatment the amount of the phenol derivative increased from 0.05 to 0.18 ppm in the fruit and from 5 to 9 ppm in the leaves. Later on, the amount of this derivative decreased in both leaves and fruit.

The ratio between the concentrations of the phenol derivative and Velsicol VCS-506 changed during the first 36 days posttreatment from 1:100 to 1:25 in the leaves, and from 1:100 to 1:10 in the fruit.

Although the concentration of the phenol derivative was relatively low, a clear pattern of slow accumulation during the first 3 weeks was observed in tomato fruits and leaves and also in grapes.

The O-analog was not detected in the fruit, but its concentration in the leaves was 0.6 and 0.2 ppm 2 and 3 weeks after treatment, respectively. The concentration of this derivative in the plant was much lower then that of the phenol derivative and was approximately 0.1% of the concentration of the parent compound.

Small fruits (2–3 cm wide) were covered with plastic bags at the time of treatment and removed 3 hr after. Other fruits, of the same size, were marked before spraying and kept uncovered. In the covered fruits no residues of the parent compound were detected 2 and 4 weeks posttreatment, whereas in the uncovered fruit the residue level of Velsicol VCS-506 was 4–5 ppm. Thus, translocation from leaves to fruit was not observed.

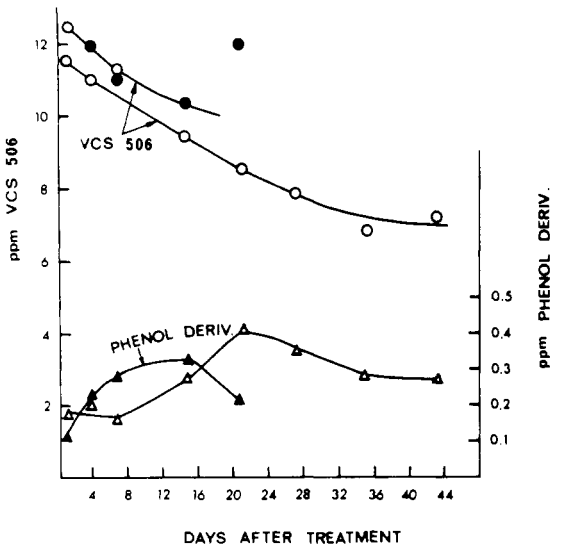

Figure 3. Residues of Velsicol VCS-506 and its phenol derivative in grapes: (●,▲) variety Muscat (black); (○,△) variety Dabuki (white).

During the same period of time the amount of the phenol derivative in the covered fruit was 0.08–0.09 ppm whereas in the uncovered fruit it was 0.35–0.37 ppm. This suggests the possible translocation of this derivative from leaves to fruit.

Tomato fruits and grapes were dipped in tap water for 24 hr employing mild agitation; it was found that the amount of the insecticide on the fruit was the same before and after the treatment.

Residues in Grapes. Bunches of grapes (two varieties) 2 weeks before ripening were dipped in the insecticide emulsion, and at selected time intervals samples were taken for analysis. At the time of treatment the fruits had reached their final size and therefore the effect of growth on the residue level during the experiment was eliminated. The results of this experiment are presented in Figure 3. The degradation rate in both varieties was very slow. Only 20% of the insecticide disappeared during the first 2 weeks after treatment. On the 44th day after application, 60% of the amount applied was still present in the white (Dabouki) variety.

In grapes there was a gradual increase in the amount of the phenol derivative in both varieties during the first 2–3 weeks, with a moderate decrease later on. These results were similar to those obtained for tomato fruits and leaves.

Analysis by the Tlc Enzyme Inhibition Technique. Tomato extracts from this and other experiments and various fractions obtained from column chromatography were subjected to the tlc enzyme inhibition test, in a search for additional cholinesterase inhibitor breakdown products (A. Ben-Aziz and N. Aharonson, unpublished results).

The parent compound and two of its metabolites were separated on silica gel G and analyzed by the enzymic inhibition test (Mendoza *et al.*, 1968), using either 5-bromoindoxyl acetate or 1-naphthyl acetate as the substrate. In this experiment, the presence of only the parent compound and its O-analog in the tomato plants was confirmed, and the residue level was similar to that obtained by chemical analysis.

In the plant the organophosphorus insecticide Velsicol VCS-506 was found very stable, a phenomenon which would suggest a relatively long biological lasting effect correlated with improved insecticidal performance. Field trials, however (P. M. Vermès, personal communication), gave disappointing results against larvae of *Spodoptera littoralis* when compared to the efficacy of other organophosphorus insecticides with comparable stomach and contact toxicity and which were found less persistent in the plant.

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COMMUNICATIONS

Effect of Flue Curing on the Amylose/Amylopectin Ratio of Tobacco Starch

Methods are evaluated for isolating starch and fractionating amylose and amylopectin in tobacco. Flue curing did not alter the amylose/amylopectin ratio, which was found to be approximately 30%/70% before and after curing, but did decrease the solubility of amylose from 23% before curing to 5% after curing. Amylose was retrograded during the flue curing process either because of high temperature and dehydration, or enzymatic activity. The data suggest that the amylases responsible for conversion of starch to reducing sugars during the flue-curing process do not alter the amylose/amylopectin ratio but only the total starch content.

The amylose/amylopectin ratio of tobacco starch has not been well documented although this ratio has been established for many other plant species including potato, corn, wheat, rice, banana, tapioca, sago and lily bulbs (Bates *et al.*, 1943), peas (McCready *et al.*, 1950), apples (Carter and Neubert, 1954), and sugar cane (Cashen and Friloux, 1966). This lack of information for tobacco starch is regrettable because tobacco leaf starch is subjected to drastic conditions during the flue-curing process, when it is reduced by enzymatic activity from amounts as high as 30% (Bacon *et al.*, 1952) to as low as 1% in 4–5 days.

There are conflicting reports on diurnal changes in the