ISRAEL JOURNAL OF ENTOMOLOGY Vol. VIII 1973

MONOCROTOPHOS RESIDUES IN CITRUS, 1970/71

N. Aharonson and Ch. Resnick

Pesticides Division

Plant Protection Department

Tel-Aviv-Yafo, Israel

A B S T R A C T

Residues of monocrotophos, 3-hydroxy-N-methyl-cis-crotonamide dimethyl phosphate, were determined by gas chromatography and by an enzymatic bioassay procedure. Samples of orange and lemon fruits and leaves were analyzed by gas chromatography with a stacked thermionic flame detector. limit of detection for the above analysis was 0.2 ppm for the leaves and 0.05 ppm for the fruit. Samples for the analysis were taken at random from one to 120 days after spraying.

I N T R O D U C T I O N

The organophosphorus insecticide monocrotophos, 3- hydroxy-N-methyl-cis-crotonamide dimethyl phosphate, has pro- vided very promising control of some important insects in the citrus grove. Since it is regarded as a potential candidate for pest control in citrus trees a study was conducted as to the persistence of this compound, and the residue level at time of harvest. Monocrotophos is a water soluble systemic insecticide (Voss and Dittrich, 1967), thus it seems important to examine the distribution of monocrotophos within the tree, its translocation, and rate of degradation.

Menzer and Casida (1965) reported only trace amounts of monocrotophos metabolites in plants. Giang and Beckman

(1969) described an analytical procedure for the detection of monocrotophos and its major metabolites. The analysis of monocrotophos by gas chromatography and its dissipation in plants was studied by Bowman and Beroza (1967), and Westlake et al. (1970). Voss described an enzymatic procedure for the detection of monocrotophos and its metabolites. For this study an analytical procedure employing both the gas chromatography and the enzymatic inhibition method was chosen. This work reports the distribution and dissipation of monocrotophos in the leaves and in the fruit of orange and lemon trees.

MATERIAL AND METHODS

A. Field application

The experiment was carried out in a citrus grove in the coastal plain of Israel. Plots of orange trees and lemon trees were sprayed on the 7th of July 1970 with 0.08% or 0.16% monocrotophos. On the 19th of October other orange trees in the same plot were sprayed with 0.08% or 0.16% monocrotophos. Each treatment was one replica of five trees. The insecticide was sprayed with spray guns at a rate of 160 liter per hectare. Rain first occurred in December, more than a month after the autumn application. Sprinklers were used during the summer and were adjusted to scatter the water above the ground without wetting the leaves.

B. Sampling

Samples consisting of 15 fruits (3 kg) and 100 leaves were taken for the chemical analysis at predetermined times. Samples were taken at random from the plot, and were stored at -16°C until analyzed.

C. Method of analysis

(1) Chemical analysis - Three fruits were sliced into small pieces and a 250 gr samples of this was taken and macerated twice with acetone (2:1 and 1:1 v/w). An equivalent of 100 gr of the extract was transferred to a separatory funnel, fifty ml water added and the extract shaken with three portions of chloroform (200,100 and 100ml).The chloroform layer was drawn through anhydrous sodium sulfate and concentrated on a steam bath to 3-4 ml. The extract was then transferred to a chromatographic column (30 cm long and 19mm I.D.)

filled with a mixture of 8 gr activated carbon (Nuchar C-190-N) and 4 gr of Celite (Hyflosupercel). The sample was eluted with 220 ml chloroform, concentrated to 3 ml on a steam bath and the remaining residues dissolved in three portions of 40 ml acetone. The sample was concentrated to 1 ml, and 10 microliter were injected into the gas chromatograph. For leaves, the sample size was 25 gr (approximately 25 leaves). The leaves were extracted with acetone in a blender. One hundred ml of water was added to the blender, followed by three successive acetone extractions (150, 100 and 100 ml).

(2) Gas liquid chromatography - A MicroTek 2000 R gas chromatograph equipped with a KCl coated stacked thermionic flame detector was used in this study. The chromatographic column (pyrex 4 feet long and 4 mm inside diameter) contained 1% Epon 1001 on 100 to 120 mesh Gas Chrom Q. The column temperature was 180°C. Carrier gas was nitrogen at a flow rate of 80 ml/min.

(3) Cholinesterase inhibition method - Each sample was first analysed on the gas chromatograph, then concentrated on a steam bath and quantitatively transferred into hexane with three successive washings. The final volume of the hexans washings was adjusted to 50 ml. The hexane phase was extracted in a separatory funnel with 50 ml of distilled water, and the aqueous phase was assayed by the cholinesterase inhibition method of Voss (1969).

RESULTS AND DISCUSSION

Method of analysis

Monocrotophos residues were determined by two different analytical procedures - gas chromatography and cholinesterase inhibition method. Gas chromatography served as a specific technique for the detection of the parent compound. The cholinesterase inhibition method served as a general technique for the detection of monocrotophos and any other possible biologically active metabolites.

The detection and determination of monocrotophos on the gas chromatograph is simple and requires little clean up. It involves extraction with acetone, partitioning into chloroform and elution through activated carbon (Nuchar C-190-N)

and celite (Hyflosupercel) column. The use of gas chromatography, with a thermionic flame detector, enabled detection of nanogram quantities of monocrotophos without the interference of additional peaks (Fig. 1). The lower limit of detection was 0.05 ppm for residues in the fruit and 0.2ppm for residues in the leaves. The chromatographic column, 1% Epon 1001 on 100/120 mesh Gas Chrom Q, proved very useful, although it was not very stable.

Residues in citrus leaves

The commercial formulation of monocrotophos-Nuvacron 40 E.C. was applied to one group of the orange trees in the middle of the summer and to another part in the autumn, just one month before the beginning of harvesting. Samples of orange leaves were taken seven times throughout the first three months after application. Leaves and fruit samples were taken separately from the lower and the upper parts of the trees.

Residues of monocrotophos in orange leaves were found to decrease from 180 ppm to 1-2 ppm within 60 days after spraying. No significant difference in the levels of residues was found in samples drawn separately from the lower and upper parts of the trees (Table 1).

A distinct difference in the level of residues was observed when the trees were sprayed with the higher concentration of Nuvacron. Application with a two fold concentration, namely 0.16% a.i. instead of 0.08% a.i. monocrotophos, resulted in twice as high residues in the leaves, and this difference was still measurable 60 days after spraying (Table 1). The residues of monocrotophos dissipated much faster in lemon trees than in oranges (Table 1).

Citrus leaves were analyzed for monocrotophos residues by gas chromatography and by the cholinesterase inhibition method. The results by either method were almost identical (Fig. 2). The presence of any biologically active metabolite in any measurable quantities should have been detected by the enzymatic assay. However, the similarity in the results obtained from gas chromatography and from the enzymatic assay indicates that biologically active metabolites of monocrotophos were not present, or occurred only in very minute quantities.

Residues in citrus fruits

Samples of orange fruits that were sprayed in July were collected 90 to 120 days after spraying and analysed by gas chromatography. No detectable residues of monocrotophos were found in the fruits. The limit of detection in the above analysis was 0.05 ppm.

When monocrotophos was sprayed in October the residues in the whole fruit decreased from 2.2 ppm (4 days after spraying) to 0.7 ppm 60 days after spraying (Table 2). There was a rapid decrease in the amount of monocrotophos residues during the first 7 days after spraying. However, during the next 7 weeks, the residues remained at almost constant levels indicating a minimal dissipation rate.

Spraying monocrotophos at double the concentration, i.e. 0.16% instead of 0.08% a.i. resulted in much higher residues throughout (Table 2).

A distinct difference in the pattern of dissipation of monocrotophos was observed between the rind and pulp of the fruit. There was a definite decrease in the level of monocrotophos detected in the rind (Table 3). However, the residue levels of monocrotophos in the pulp were almost the same 21 or 60 days after spraying.

Dissipation of monocrotophos from lemon fruits was much faster than on orange fruits. Lemon trees were sprayed in the summer at a stage when both small and ripe fruits were present on the trees. Residues in the fruits were 2.7 ppm one day after spraying and decreased to 0.06 ppm after 40 days (Table 2). Application of a double concentration i.e. 0.16% instead of 0.08%, resulted in much higher residue levels.

Table 1: Residues of monocrotophos in orange and lemon leaves

DAYS AFTER SPRAYING	LEMON LEAVES sprayed on 7.7.70		ORANGE LEAVES sprayed on 7.7.70				ORANGE LEAVES sprayed on 19.10.70	
	0.08% monocrotophos		0.08% monocrotophos		0.16% monocrotophos		0.16% monocrotophos	
	Lower Leaves	Upper Leaves	Lower Leaves	Upper Leaves	Lower Leaves	Upper Leaves	Lower Leaves	Upper Leaves
	Residues of monocrotophos in ppm							
1	280	300	150	200	210	270	-	-
4	-	-	-	-	-	-	105	130
7	87	88	44	50	80	76	79	130
14	37	25	15	17	27	34	39	42
21	5.5	5.1	3.5	6.1	23	24	20	26
40	0.7	0.3	1.4	2.1	3.4	2.9	1.5	1.9
60	0.2	0.2	1.4	1.5	2.7	1.4	0.2	0.9
control	0.3	0.2	0.2	0.3	-	-	0.2	0.2

Table 2: Residues of monocrotophos in citrus fruits (whole fruit).

DAYS AFTER SPRAYING	LEMON FRUITS sprayed on 7.7.70		ORANGE FRUITS sprayed on 19.10.70		
	0.08% monocrotophos	0.16% monocrotophos	0.08% monocrtophos	0.16% monocrotophos	
	Residues of monocrotophos in ppm				
				Lower fruit	Upper fruit
1	2.7	-	-	-	-
4	-	-	2.2	-	-
7	2.4	-	0.9	-	-
14	1.5	3.9	0.8	-	-
21	1.2	-	0.6	2.8	4.5
40	0.06	0.3	0.7	-	-
60	0.02	-	0.9	0.7	1.8
90	0.02	-	-	-	-
control	0.05	0.05	0.05	-	-

Table 3: Residues of monocrotophos in citrus fruits (pulp and rind).

DAYS AFTER SPRAYING	LEMON FRUITS sprayed on 7.7.70				ORANGE FRUITS sprayed on 19.10.70					
	0.08% monocrotophos		0.16% monocrotophos		0.08% monocrotophos		0.16% monocrotophos			
	pulp	rind	pulp	rind	pulp	rind	pulp	rind	Pulp	rind
	Residues of monocrotophos in ppm									
							Lower fruit		Upper fruit	
14	0.6	2.9	0.4	8.5	-	-	-	-	-	-
21	-	-	-	-	0.1	1.9	0.5	8.1	1.9	11.3
60	-	-	-	-	0.8	1.0	0.6	1.0	1.1	3.7
control	0.05	0.05	0.05	0.05	0.05	0.05	-	-	-	-

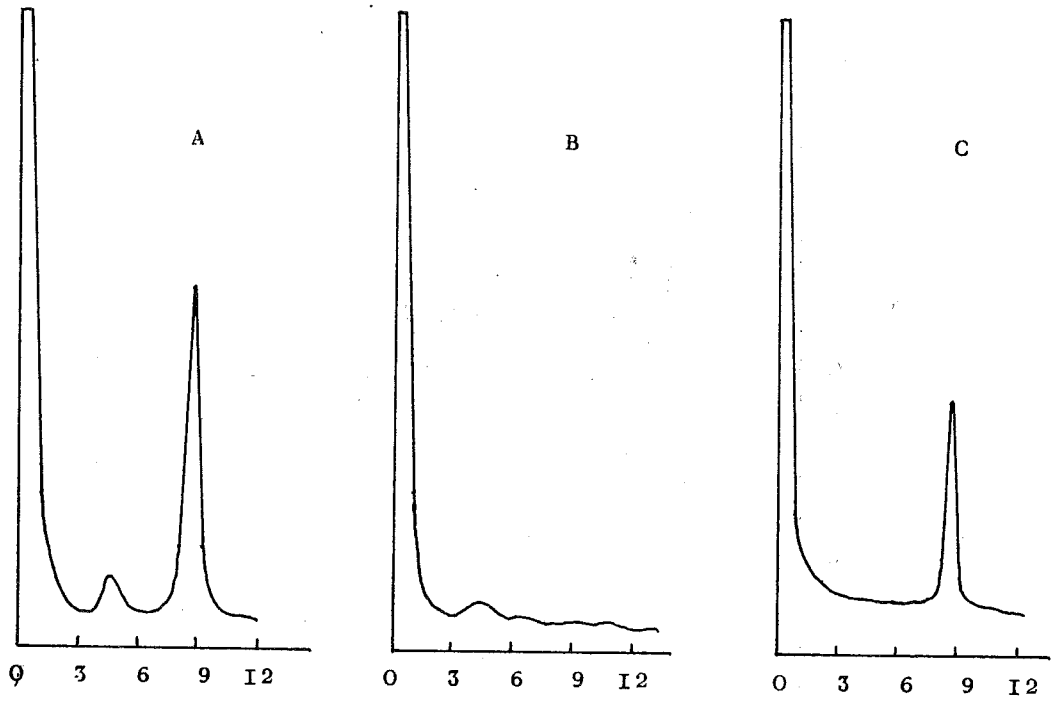

Fig. 1. Chromatograms of nuvacron obtained on stacked thermionic detector. A. Orange extract 60 days after spraying. B. Untreated orange extract. C. Nuvacron 30 nanogram.

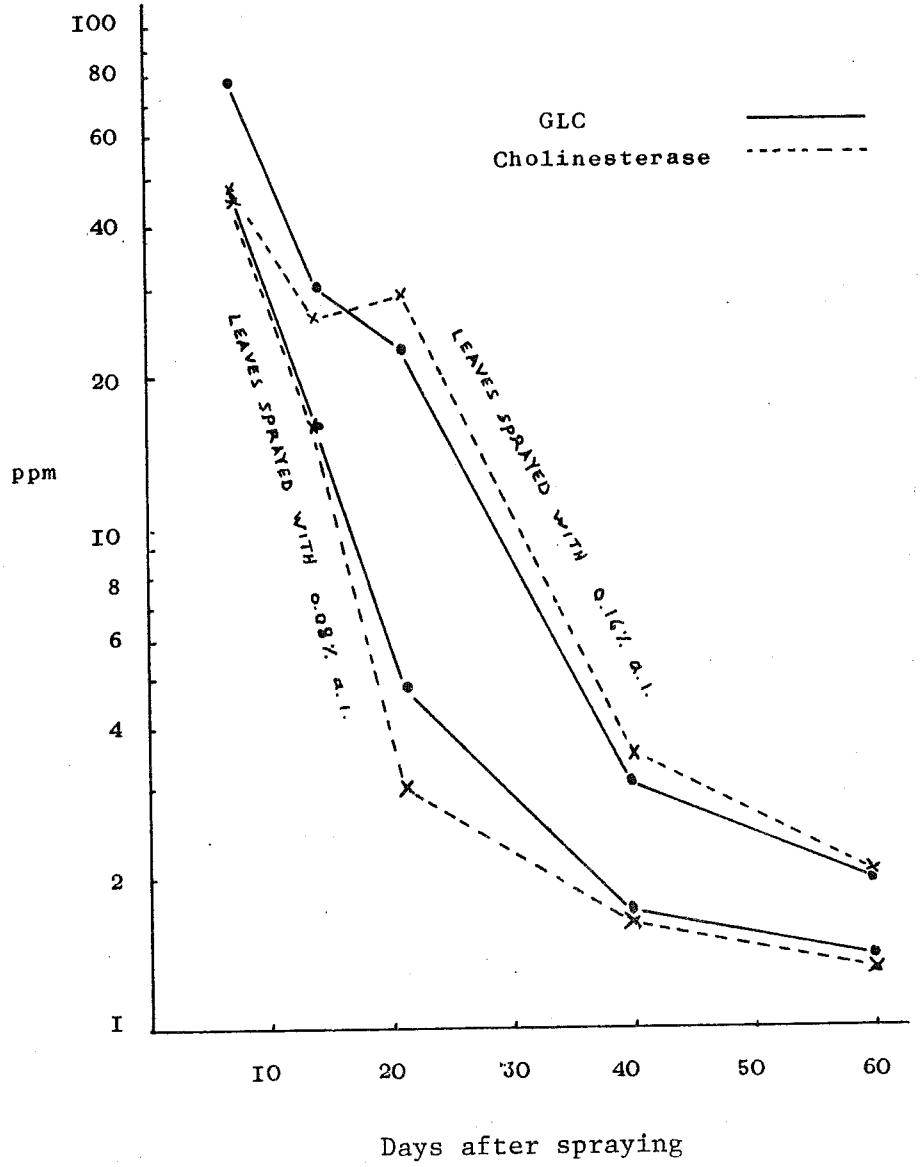

Fig. 2. Decline of monocrotophos in orange leaves as determined by GLC and cholinesterase inhibition test.

REFERENCES

Voss, G. and Dittrich, V. 1967. The translocation of insecticidal enolphosphates in plants. *Z. angew.Entomol.* 59:430-442.

Menzer, R.E. and Casida, J.E. 1965. Insecticide metabolism, nature of toxic metabolites formed in mammals, insects and plants from monocrotophos and its n-methyl analog. *J. Agr. Food Chem.* 13:102-112.

Giang, B.Y. and Beckman, H. 1969. Thin layer chromatographic determination of Bidrin, Azodrin and their metabolites. *J. Agr. Food Chem.* 17:63-69.

Bowman, M.C. and Beroza, M. 1967. Gas chromatographic analysis of 3-Hydroxy-N-methyl-cis-crotonamide dimethyl phosphate (Azodrin). *J. Agr. Food Chem.* 15:465-468.

Westlake, W.E., Gunther, F.A. and Jeppson, L.R. 1970. Persistence of Azodrin residues on and in Valencia oranges in lab. processed citrus pulp cattle feed. *J. Agr. Food Chem.* 18:864-865.

Voss, G. 1969. Cholinesterase inhibition autoanalysis of insecticidal organophosphates and carbamates. *J. AoAC* 52:1027-1034.