Extraction and Analysis of Oxamyl from Citrus Leaves

Oxamyl was extracted from treated citrus leaves by four successive rinsings with heated methanol or by shaking in methanol at room temperature and was analyzed directly by liquid chromatography. The material disappeared quickly from sprayed trees in the grove (probably washed off by rain); but under controlled conditions most was recovered after 3 weeks. This method should be useful in determining efficiencies of application procedures and studying further the mode of action of oxamyl.

The insecticide–nematicide oxamyl (methyl *N',N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate) has been reported to control nematodes on roots of citrus trees and other plant pests when applied as a foliar spray (Timmer, 1974, 1977; O'Bannon and Tomerlin, 1977; Radewald et al., 1973).

Little is known of its mode of action or stability under field conditions, likely due to lack of a simple method of analysis. Several methods of analysis have been reported: (a) gas chromatography, following hydrolysis to the oxime (Holt and Pease, 1976), (b) spectrophotometry, involving derivatization (Singhal et al., 1977), and (c) liquid chromatography after thorough cleanup (Thean et al., 1978). For routine analysis, we wanted a simple method that would avoid extensive cleanup or derivative formation (with their inherent possibilities of partial loss of the sample). Such a method could be used to determine the efficiency of spraying procedures or the residues of oxamyl on foliage after a period of time, or after rain, overhead irrigation, or spray application of other pesticides. This paper describes such a method.

MATERIALS AND METHODS

Vydate (E. I. DuPont de Nemours & Co. Inc.), which contains 24% oxamyl, was thoroughly mixed with the Atlas surfactant Span 20 (sorbitan monolaurate) at about 25 to 1, then diluted 2 to 25 with methanol. This mixture was applied by micropipet to leaves of 8- to 12-month-old citrus seedlings, *Citrus limon* (L.) Burm. f., at a rate of 25 μL (460 μg of oxamyl)/leaf. The methanol solvent dried rapidly and had no apparent adverse effect on the citrus leaves. The seedlings were kept in the laboratory at about 26–27 °C or in a growth chamber which averaged 27 °C and 60% relative humidity during the day and 21 °C and 80% at night.

We cut individual leaves into small pieces and extracted the oxamyl either by rinsing four times with 5-mL portions of heated (60 °C) methanol, which were combined and made up to 25 mL total volume, or by adding 20 mL of methanol to the cut leaves in a 50-mL Erlenmeyer flask, then stoppering the flasks and placing them on a New Brunswick gyrotory shaker at 150 rpm for 2 h at room temperature. Leaves from trees in a citrus grove sprayed with oxamyl (14 g in 9.5 L/tree) were analyzed, 10 leaves/sample, in 100 mL of methanol. The extracts were passed through 0.5-m Millipore filters (or their equivalent), and 10 μL of the filtrate was chromatographed directly without further cleanup.

Extracts were analyzed with a Waters Associates liquid chromatograph, μ-Bondapak C_{18} column, with an 80:20 mixture of water and methanol as solvent at 2 mL/min, and an LDC SpectroMonitor UV detector set at 212 nm. At the usual attenuation (×0.01), one division was equivalent to about 0.003 μg/10-μL sample. Standard samples of oxamyl and its oxime were obtained from the Biochemicals Department, DuPont de Nemours and Co. Inc., Wilmington, Del.

Table I. Oxamyl Extracted from Citrus Leaves Treated with 460 μg/Leaf, Averages of Ten Leaves Analyzed Individually

Method	% recov
Rinsing with 60 °C methanol	
Extract 1	81.5
Extract 2	11.9
Extract 3	1.5
Extract 4	0.6
Total	95.5
Range	87–103
Standard deviation	5.1
Shaking at room temperature	
30 min	81.4
1 h	85.2
2 h	99.2
Range (2-h extraction)	97–103
Standard deviation	2.2

Table II. Oxamyl Recovered by Rinsing from Leaves of a Tangelo Tree in a Citrus Grove Sprayed with 14 g in 9.5 L/Tree[a]

	Range, μg/leaf	Av, μg/leaf
Before spraying	ND[b]	ND
After 2 h	139–226	179[c]
After 3 days	157–258	187[c]
After 7 days (rain on 5th day)	ND	ND

[a] Five samples each time, ten leaves/sample. [b] ND = not detectable. [c] Approximately 448 and 467 ppm.

RESULTS AND DISCUSSION

The typical chart recordings of leaf extracts in Figure 1 show that chlorophyll and other plant material did not interfere with oxamyl (retention time 4.75 min), or with the oxime (retention time, 3.25 min). We did not detect the oxime in any of our experiments with treated leaves.

The percentage of oxamyl extracted from leaves by either procedure was satisfactory (Table I). In successive rinsings with heated methanol, the first averaged over 80% recovery and the fourth less than 1%. Shaking at room temperature required 2 h, but in practice was easier and afforded less chance of loss of sample. At room temperature, oxamyl was stable in the extract for at least 1 week.

A preliminary experiment on leaves taken from a sprayed tree in a citrus grove indicated that rainfall should be considered (Table II). Five 10-leaf samples taken before spray application showed that no oxamyl was present from any previous spray programs. Residues after 3 days were essentially the same as those 2 h after application. After 1 week, with rain (1.25 cm) occurring on the fifth day, no oxamyl remained. That oxamyl can be readily washed from leaves with water was confirmed in the laboratory. We allowed treated leaves to dry for 4 h, then dipped them into water. After extracting and analyzing the samples, we recovered 100, 4, and 1% of the oxamyl on leaves dipped for 0, 30, and 60 s, respectively.

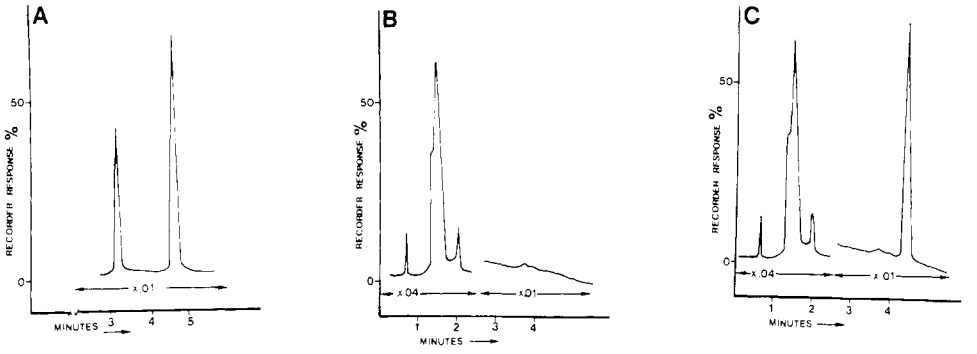

Figure 1. Chromatograms of (A) oxime (0.009 μg/μL; retention time, 3.25 min) and oxamyl (0.019 μg/mL; retention time, 4.75 min), (B) methanol extract of untreated citrus leaf, and (C) methanol extract of citrus leaf treated with 460 μg of oxamyl.

Table III. Percentage of Oxamyl Recovered by Shaking with Methanol from Leaves of Citrus Seedlings Treated with 460 μg of Oxamyl/Leaf[a]

	Lab			Growth chamber		
	Test	Range	Av	Test	Range	Av
1 day	1	94-101	97	1	88-92	89
	2	91-97	95	2	86-91	88
	3	94-101	97	3	100-101	100
1 week	1	94-101	98	1	95-104	100
	2	89-92	90	2	71-92	84
	3	91-101	97	3	88-98	92
2 weeks	1	91-106	97	1	84-99	93
	2	85-94	91	2	84-94	89
	3	91-106	97	3	63-87	77
3 weeks	1	91-94	92	1	60-98	81
	2	81-94	89	2	88-91	90
	3	85-88	86	3	54-84	72

[a] Three leaves per sample.

In experiments with treated plants kept in either the laboratory or growth chambers, the percentage of oxamyl recovered dropped slowly (Table III) and was lower for plants in the growth chamber. That difference indicates that the environment might affect breakdown or translocation of the material. It has been shown that oxamyl in water is degraded by light (Harvey, 1977). In our experiments, methanol extracts of oxamyl-treated leaves had peaks for recovery of oxamyl but none for recovery of oxime or other breakdown products. Grinding leaves in a Waring blender with ethyl acetate (similar to the procedure of Thean et al., 1978) increased recovery by only 0.2%. In addition, in experiments with rooted leaves (data not shown) the recovery was parallel to that of leaves on growing plants, and no oxamyl was detected in either the roots or soil. Possibly, however, some oxamyl was broken down into products which were not detected by our analytical procedure.

Although our tests indicate that the absorption of oxamyl by citrus leaves is minimal, field treatments for nematodes by foliar spraying were effective (O'Bannon and Tomerlin, 1977). For example, in a recent grove experiment conducted to control the burrowing nematode, *Radopholus similis* (Cobb) Thorne, on citrus, three oxamyl foliar sprays applied at 6-week intervals reduced, by 93%, nematode populations (as measured by nematode numbers 2 months after the last spray). In another grove, reduction was 79% even 4 months after the last treatment. Foliar sprays might be washed from the leaves by rain, irrigation, or other sprays and penetrate the soil. Our tests also do not rule out the possibility that oxamyl induces the production of a substance harmful to nematodes, which is then translocated to the roots. Only minimum radioactivity, however, was found in the roots of plants treated with radioactive oxamyl (Harvey, 1977).

The effects of light, humidity, temperature, variety, and age and condition of leaves on the mode of action of oxamyl should be investigated. For example, we found that oxamyl disappears more rapidly from old, soiled leaves than from new growth; however, here again, no other products were detected. The method of extraction and analysis presented in this paper might be useful for carrying out investigations with oxamyl and other pesticides.

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