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Simple Method for Assessing Acephate and Methamidophos Residues in Plant Tissues

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Gas-liquid chromatography (GLC) is a useful technique in assessing insecticide residues in plant tissue. By correlating the amount of residue with data on insect mortality, it is possible to judge the persistence of contact and systemic insecticides. The translocation of systemic insecticides in plants can also be determined by GLC without measuring insect mortality.

Acephate (O,S-dimethyl acetylphosphoramidothioate) has been studied as one of the more environmentally-safe insecticides for use against forest insects (RICHMOND et al. 1977, ARMSTRONG and NIGAM 1975, HOPEWELL 1975, HOPEWELL and NIGAM 1974). The probable systemic properties of acephate make it particularly suitable for the purposes of our study.

We have developed a simple, rapid method for extraction and GLC determinations of acephate and methamidophos, its metabolite, in various plant tissues and in litter and duff from the forest floor. The method described reduces contamination, cost and time because disposable, inexpensive glassware is used and the column cleanup step is eliminated.

METHODS AND MATERIALS

Several common types of forest plant tissue were fortified with known solutions of acephate (Orthene® 99.8% Analytical Standard)[1] and methamidophos (Monitor® 99.6% Analytical Standard).[1] Concentrations used ranged from 0.5 to 30 ppm. Just before homogenization a small volume of insecticide was added to each sample tissue. Samples were processed for residue analysis immediately after addition of the chemical.

Plant material was classified as hard tissue (cones and bark) or soft tissue (foliage, needles, litter, duff, and grass). Before insecticide was added, hard tissue samples were first pulverized in dry ice using a Hobart Food Chopper. Except for pulverization of hard tissue samples, the process of residue analysis was identical for both hard and soft tissues.

Five g of sample were combined in a 10-dram stoppered shell vial (29 x 85 mm) containing 18 g of sodium sulfate for every 5 g of tissue. Twenty mL of ethyl acetate were added to the vial for every 5 g of

[1] Ortho Division, Chevron Chemical Company

0007-4861/79/0022-0512 $01.00

tissue, and the contents homogenized using a Brinkman Polytron.

The homogenate was centrifuged for 5 min in the shell vial at 1000 rpm at -10°C. The supernatant was decanted into a disposable screw top (22 mm) vial containing 1 g of activated charcoal (Nuchar C 190-N) per 20 mL of supernatant. The charcoal and supernatant were thoroughly mixed by shaking on a vortex shaker, then centrifuged again. A 4-mL aliquot per 20 mL of supernatant was removed and evaporated to exactly 1 mL under a stream of dry nitrogen at 40°C. After evaporation, samples were ready for analysis.

In some instances the amount of insecticide on the surface of a sample must be determined. In such cases, the sample (5 g) is washed 3 times by shaking in a covered Mason jar with 200 mL of water. The water is reduced to 100 mL using *in vacuo* distillation (Buchi Rotavapor). Three hundred mL of ethyl acetate were added to the 100 mL of water in a 950-mL Mason jar. Three hundred g of sodium sulfate were added to the mixture while stirring with an overhead mixer (Lourds, Model 18-309X) for 5 min. The solution was decanted through 300 g of sodium sulfate in a 15-cm glass funnel filled with a piece of filter paper (Whatman #3). The filtrate was collected in a 1000-mL r.b. flask. A 200 mL ethyl acetate rinse was added to the Mason jar and stirred for 5 min. The solution was decanted through the sodium sulfate funnel and the same procedure repeated once more. The resultant 700 mL of ethyl acetate was concentrated by *in vacuo* distillation and transferred to a volumetric flask.

To analyze for residues we used a Varian gas-chromatograph equipped with an alkali flame ionization detector and a 183-cm by 2-mm glass column packed with 1% Reoplex 400 coated on 100/120 mesh Gas Chrom Q. Operating temperatures were: column 175°, injector at 210°, and detector at 210°C. Carrier gas was helium at 50 mL/min. The detector was maintained with flow rates of 36 mL/min hydrogen and 235 mL/min air. The GLC analysis for acephate also detects any methamidophos that is present. Control tissue was analyzed for the presence of interfering materials. Each sample was analyzed 3 times and the average was taken.

RESULTS AND DISCUSSION

For our work, a recovery of 75% with a S.D. no greater than 10% of the mean is the lower limit of acceptability. Mean % recovery from all material tested was 87% (S.D. 8.8) for acephate and 77% (S.D. 6.3) for methamidophos (Tables 1 and 2). In most cases we achieved acceptable recovery with a small S.D., (less than 10% of the mean). Some of the data fell outside our limits of acceptability. This was probably the result of variations in the chromatographic techniques used by various workers performing the actual analysis or, in some cases, insufficient replication.

TABLE 1

Percent recovery and standard deviation of acephate from several substrates fortified at various levels.

Substrate	Fortification level (ppm)	No. of samples	% Average recovery	S.D.(%)
1. Needles *(Pinus ponderosa)*	30	5	86.4	9.4
	5	5	94	20
2. Leaves *(Glycine max)*	30	5	78.3	2.5
	10	5	82.3	5.6
	3	5	96.5	11
3. Bark *(Pinus taeda)*	10	2	92.	0.4
	5	6	104	11.8
4. Buds *(Pseudotsuga menziesii)*	30	3	83.2	9.9
	20	3	80.6	4
	10	3	95	4
5. Needles *(Pseudotsuga menziesii)*	10	5	101	15.4
	5	9	93	9
	3	5	84.4	12.2
	1	5	86.2	13
	0.5	9	94	29
6. Cones *(Pseudotsuga menziesii)*	4	13	98.5	8.7
7. Needles *(Abies gradis)*	1	3	96.3	3.51
	5	5	78.	3.51
8. Leaves *(Symphoricarpos* spp.*)*	1	3	96	5.86
	5	3	78	1.53
	10	4	77	3.56
9. Leaves *(Poa* spp.*)*	10	4	77	6.65
10. Leaves *(Alnus* spp.*)*	10	4	80	3.32
11. Litter and duff	10	3	72	1.73
12. Cones *(Pinus sylvestris)*	40	6	77	1.4
	20	6	84	1.6
	4	6	82.3	3.2

Total mean % recovery for acephate = 87%; S.D. = 8.8

TABLE 2

Percent recovery and standard deviation of methamidophos from several substrates fortified at various levels.

1. Buds *(Pseudotsuga menziesii)*	1	3	85	2.6
	5	3	76	3.2
2. Needles *(Pseudotsuga menziesii)*	5	8	71	3.4
3. Cones *(Pseudotsuga menziesii)*	0.5	13	84.14	6.1
4. Cones *(Pinus sylvestris)*	20	6	71	1.2
	4	6	82.3	4.2
	2	2	72	2.0

Total mean % recovery for methamidophos = 77.3%; S.D. = 6.3

No interference from other materials was found in the control samples. Figure 1 shows a typical GLC scan of methamidophos and acephate extracted from Douglas-fir buds. Although we did not establish a minimum detectable level for this method, we achieved reliable results at 0.5 ppm for both insecticides with a sample size of only 5 g. If lower levels of sensitivity are required, then a method which uses a column cleanup step may be necessary (LEARY 1971, 1974). Such a method would increase analysis time and cost.

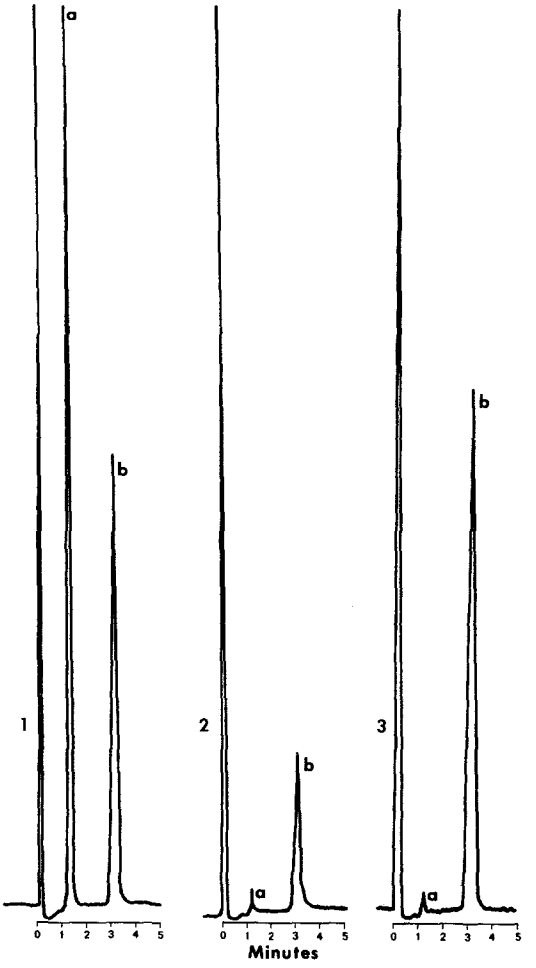

Figure 1. Representative chromatograms of *acephate* and *methamidophos*. Scan 1. -- the reference standard; a. *methamidophos* (5 ng), b. *acephate* (10 ng); Scan 2. -- Samples extracted from Douglas-fir buds; a. *methamidophos* (<0.1 ng), b. *acephate* (3.5 ng); Scan 3 -- Samples extracted from Douglas-fir buds; a. *methamidophos* (<0.1 ng), b. *acephate* (11.6 ng). (All injections were 1 microliter.)

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