Worker Environment Research. II. Sampling and Processing Techniques for Determining Dislodgable Pesticide Residues on Leaf Surfaces

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A procedure has been previously reported (GUNTHER et al. 1973) for determining dislodgable pesticide residues on leaf surfaces as developed in the early stages of this new field of investigation of pesticides persisting in agricultural worker environments. Although the early procedure is satisfactory and will provide good data, shortening, simplification, and improvement of some of the laboratory steps have been achieved with additional field-program experience with several insecticides and are reported here to inform interested investigators. The step-by-step revised procedure is reproduced below, with separate supporting and supplemental discussions of major changes and operations.

EQUIPMENT CHANGES

Modified leaf punches. The equipment described by GUNTHER et al. (1973) has not been changed except that the citrus leaf punches were modified to permit using an 8-oz widemouthed (size 58 caps) collection jar (inner dimensions 5.8 x 11 cm) instead of the 4-oz one previously used to contain the leaf discs. The larger jars reduce the likelihood during the stripping procedure of occasional clumping of the discs that could cause incomplete recovery of dislodgable material.

Storage of leaf discs awaiting analysis. Leaf discs should not be stored frozen or in a refrigerator without proofs of non-deterioration of pesticide and of non-conversion of some of the dislodgable residues present into non-dislodgable residues (those residues tightly embedded or dissolved in leaf waxes or otherwise penetrated into subcuticular tissues).

REVISED LABORATORY PROCEDURE

Stripping dislodgable residues. The samples of 40 citrus leaf discs (2.54 cm diameter) or 200 grape leaf discs (1.8 cm diameter) are stripped in the 8-oz widemouthed jars using 100 ml of water each plus 4 drops of a 1:50 dilution of Sur-Ten wetting agent (American Cyanamid Co.). The jars containing leaf discs and stripping solution are shaken end-to-end on a reciprocating shaker at 200 cycles/min for 20 min. Each stripping "solution" is then poured into a 500-ml separatory funnel or a temporary holding bottle, retaining the leaf discs in the 8-oz jar. This procedure is

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repeated two more times, adding the succeeding strippings to the first. Care is taken to transfer all particulate matter in the aqueous strippings into the separatory funnel or holding bottle to avoid loss of pesticide mechanically entrained on the particles. The pesticide in this aqueous mixture is then transferred to an appropriate organic solvent as described by GUNTHER et al. (1973). The aqueous phase may now be filtered if desired (see next step).

Weight of particulate matter on discs. When the weight of dislodgable particulate matter is also to be determined, filter papers (9 cm) are pre-weighed and the organic solvent-extracted aqueous suspensions from above are filtered with careful transfer of all particulate matter onto the paper. Each paper is then oven-dried at 100°C, allowed to remain at room temperature and exposed to the air for several hours, and re-weighed.

Residues remaining after surface stripping (non-dislodgable residues). The discs are drained well to remove excess water after the last stripping, then transferred to a semi-micro Waring Blendor container. As adapted from BURKE (1971), 50 ml of acetonitrile-water (80:20 v/v) is added and the contents are blended for 2 min at high speed. The mixture is filtered with suction through Whatman No. 42 filter paper in a Büchner funnel and the recovered volume is measured. The entire acetonitrile-water solution is transferred to a 500-ml separatory funnel, 300 ml of water, 10 ml of saturated NaCl solution, and 50 ml of hexane (or other solvent) are added, and the funnel is shaken vigorously for one min. The aqueous layer is discarded after separation and the hexane solution is washed twice with 50 ml of water each time, discarding the washes. Each resulting extract is stored at 40°C over 1 teaspoonful of anhydrous Na_2SO_4. The sample equivalent of the volume recovered is calculated on the basis of the total theoretical volume of acetonitrile-water, assuming that the leaves are 80 percent water.

DISCUSSIONS AND PRECAUTIONS

Effect of refrigerated or frozen storage prior to residue recovery. Table I contains data for refrigerator storage (4°C) of leaf discs for 3 and 6 days. There was little, if any, effect on the recoveries of parathion, ethion, or azinphosmethyl. Omite, however, was recovered in very low yield after 6 days storage while after 3 days the recovery of the remainder was low although the value for the surface residue was normal. Discs stored for 6 days were limp but did not clump excessively during stripping.

Similar data for frozen storage (-10°C) recoveries are in Table II. The recovery of Omite residues was adversely affected after 6 days under both types of storage; there was no significant effect upon recoveries or migration of the other 3 pesticides after 90 days in frozen storage. After frozen storage, thawed leaf discs were limp but did not clump excessively during stripping.

TABLE I

Effect of refrigerator storage on residues on and in orange leaf discs.

Compound	Residue ($\mu g/cm^2$) after[a]					
	0 days		3 days		6 days	
	Surf.	Rem.	Surf.	Rem.	Surf.	Rem.
Parathion	1.2	0.8	1.3	1.0	1.1	0.5
Azinphosmethyl	1.2	0.2	1.4	0.3	1.3	0.2
Ethion	1.3	1.3	1.5	1.3	1.5	-
Omite	2.1	1.2	2.4	0.4	0.4	0.3

[a] Average values for triplicate samples.

TABLE II

Effect of frozen storage on residues on and in orange leaf discs.

Compound	Residue ($\mu g/cm^2$) after[a]							
	0 days		6 days		30 days		90 days	
	Surf.	Rem.	Surf.	Rem.	Surf.	Rem.	Surf.	Rem.
Parathion	1.2	0.8	1.3	0.9	1.2	0.7	1.1	0.6
Azinphosmethyl	1.2	0.2	1.4	0.3	1.4	0.3	1.1	0.4
Ethion	1.3	1.3	1.4	0.4	1.4	0.7	1.4	1.1
Omite	2.1	1.2	1.2	0.0	-	-	-	-

[a] Average values for triplicate samples.

To avoid unnecessary complications, storage of the leaf discs in any manner should be avoided, with immediate processing. If discs must be stored, preliminary tests should always be made to determine whether there may be an adverse storage effect, as shown for Omite and indicated for ethion, if this information has not been previously obtained.

Stripping conditions. The optimum stripping conditions were determined by using different shaking periods and shaking 1, 2, or 3 times. Table III includes data for parathion, azinphosmethyl, ethion, and Omite. Recoveries with the present 8-oz jars were not improved significantly by shaking for more than 20 min; shorter times might suffice for some compounds. The 20-min period has now been adopted as it gave a good margin of safety yet was not unduly long. Three shakings were consistently required for maximum recovery regardless of the duration of each.

Solute transfer. The number of equilibrations required for essentially complete transfer of a pesticide from the aqueous stripping mixture to an organic solvent varies with pesticide and solvent and the 3 equilibrations originally specified (GUNTHER et al. 1973) are often unnecessary. Parathion and phenthoate (Cidial®), for example, are recovered from their aqueous stripping mixtures in virtually 100% yield with one equilibration with 50 ml of hexane. The preferred solvent and the number of equilibrations required for satisfactory and reproducible recoveries

TABLE III

Effect of the number of strippings and the duration of each upon recovery of dislodgable residues from orange leaf discs.

Minutes for each stripping	$\mu g/cm^2$ recovered after 1 strip	2 strips	3 strips
	Parathion		
5	0.9±0.1	1.3±0.2	1.5[b]
10	0.7±0.0	1.1±0.2	1.2±0.2
20	0.6±0.0	1.2±0.2	1.6[b]
30[a]	0.9±0.1	0.9±0.1	1.4±0.2
60[a]	0.7±0.0	1.1±0.3	0.9±0.2
	Azinphosmethyl		
5	1.9±0.1	2.2±0.3	2.0±0.2
10	1.5±0.1	2.0±0.0	2.1±0.1
20	2.2±0.2	2.2±0.2	2.8±0.5
30[a]	2.1±0.2	2.0±0.5	2.7±0.3
60[a]	1.9±0.2	2.5±0.2	2.2±0.0
	Ethion		
5	1.9±0.9	2.6±0.2	2.7±0.2
10	2.6±0.9	3.0±0.6	3.4±0.5
20	2.8±0.3	3.0±0.2	3.4±0.4
30[a]	2.8±0.1	3.5±0.3	3.4±0.3
60[a]	2.4±0.0	3.0±0.2	3.0±0.1
	Omite		
5	2.1±0.1	4.1±0.3	4.0±0.7
10	2.7±0.5	3.3±0.4	3.3±0.4
20	2.6±0.1	3.3±0.5	4.3±0.6
30[a]	3.2±0.4	3.2±1.0	4.2±0.8
60[a]	3.0±0.4	3.8±0.4	4.3±0.2

[a] The first stripping was 60 min; the second and third were 30 min each.

[b] Average for duplicate samples. All other values derived from triplicate samples.

must be determined for each pesticide.

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