A Method for Estimating Leaf Compartmentalization of Pesticides in Citrus

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A method for estimating surface, wax, and cellular pesticide residues of the citrus leaf is presented. Electron microscopy showed little alcohol penetration of the cell in a short surface strip. Methanol compared with the dislodgeable surface strip as a wax solvent. Chloroform was the most efficient wax solvent, penetrated to the cell, but left the cell membrane intact. Parathion in methanol did not partition into leaf wax or cell whereas 27% leaf partitioning occurred with the dislodgeable solvent. Low percentages of systemic acephate were removed with a methanol surface strip or a chloroform wax strip. The final method consists of a 1-min methanol surface strip, a 1-min chloroform wax strip, and a final analysis of penetrated residues.

The establishment of worker safety reentry times requires determination of dislodgeable pesticide residues on foliar surfaces (*Fed. Regist.*, 1975). The recommended technique for leaf surface residues consists of a soapy water leaf wash, followed by extraction of the wash for residue (Gunther et al., 1974; Iwata et al., 1977). Although the Gunther dislodgeable residue technique is generally in use, some investigators use organic solvents to extract "surface" pesticide residues from leaves (Cahill et al., 1975; Ware et al., 1975a,b; Staiff et al., 1975, 1977).

The dislodgeable surface-residue technique removes pesticide-laden particulate matter from the leaf surface with a soap solution (sodium dioctyl sulfosuccinate). Presumably extraction of the soap solution with an organic solvent extracts the pesticide both directly from particulates and from the soap solution. However, due to the 30% solubility of parathion in orange leaf wax (Okamura et al., 1977), the possibility exists that pesticides released from particulate matter in a water extraction could subsequently partition into the leaf wax. On the other hand, an organic solvent extraction could either remove pesticide from the wax or assist pesticide penetration into the interior of the leaf. Our purposes here were to design a leaf pesticide extraction procedure related to leaf chemistry which would remove surface residues with an organic solvent and allow separate extractions of the leaf wax and the internal cellular materials and to compare this method with the dislodgeable residue technique.

MATERIALS AND METHODS

Electron Microscopy. Leaf disks or whole leaves washed with soap solution, methanol, chloroform, benzene, hexane, and ethanol were used for transmission electron microscopy. Samples 2 mm square were cut from leaves of each treatment and fixed in 3% glutaraldehyde in 0.2 M potassium phosphate buffer, pH 7.2, for 1 h at room temperature. Postfixation was in 2% osmium tetraoxide in the same buffer for 2 h at room temperature. The samples were dehydrated in acetone and embedded in Spurr's (1969) plastic. After being sectioned on a LKB Huxley Ultra-Microtome with a diamond knife, they were stained with methanolic uranyl acetate (Stempak and Ward, 1964) and lead citrate (Reynolds, 1963). The sections were viewed on a Philips 201 electron microscope at 60 kV.

Epicuticular Wax. Whole leaves were matched so that all samples had the same area. Epicuticular waxes were extracted from 10 whole leaves or 40 2.5-cm leaf disks by shaking in 100 mL of benzene, chloroform, ethanol, hexane, methanol, methylene chloride, or dislodgeable solution at 350 rpm for 1 or 10 min. The dislodgeable solution was extracted 2 times with 20 mL of methylene chloride. Solvents were reduced to dryness on a rotary evaporator at 40 °C. Extractions were transferred to 0.6 mL of chloroform for thin-layer chromatographic (TLC) analyses. Samples were coded and analyzed blind.

Precoated 250-μm silica gel TLC plates (20 × 20 cm) were cleaned of any lipid components by developing in chloroform. Plates were dried in an oven at 105 °C for 1 h prior to use. Two plates, each scored into 18 developing lines, were spotted with 6 μL of the 21 samples along with lipid standards. Four sets were prepared for comparison of the intensities of up to 12 spots/sample. Plates were developed in hexane–diethyl ether (98:2) for 10 min after the solvent had reached the top of the plate. Plates were dried in the hood for 10 min and redeveloped for 17 cm in the same direction in hexane–diethyl ether–acetic acid (50:50:1). After being dried, each plate was sprayed evenly with 50% aqueous H_2SO_4 and charred in a muffle oven at 200 °C for 3 min. Spots were visually rated on a 10-point scale.

The sums of the spot intensities for each sample were determined. As chloroform-extracted samples gave the greatest sums, these were given a normalized extraction value of 100% for comparative purposes. Each of the three extraction procedures were compared separately. Each spot in the chloroform extraction was given a relative percent value based on its 0–10 intensity rating and the sum of these intensities. Each spot of the other six solvent extractions in the subset was assigned a relative percent extraction value based on their relative intensities. The mean relative percent values for the four determinations of each spot were calculated. These summarized values give wax extractability values of the solvent relative to those of chloroform.

Radioactive Experiment. Ethyl [1-^{14}C]parathion was from Amersham (CFA 0.380). The [^{14}C]parathion was diluted with nonradioactive parathion to a specific activity of 577 dpm/μg. The radiochemical purity of the diluted material was determined by reverse-phase high-pressure liquid chromatography (HPLC) on Zorbax-ODS (C-18; Du Pont), 80:20 acetonitrile–water, 2 mL/min. HPLC fractions were collected, reduced to dryness at 40 °C under gentle N_2, and counted in Aquasol (New England Nuclear) with external standard for quench correction on a Beckman LS-100 scintillation counter. Radiochemical purity of the diluted material was 100%.

Four hundred microliters of [^{14}C]parathion (0.104 μCi) was added to the first methanol extraction in one experiment and to the first dislodgeable extraction in another

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experiment. Leaf wax was subsequently removed with chloroform. A 3.0-mL aliquot of each separate extraction was applied to black combustion paper and combusted in an Ogg igniter according to Davidson and Oliverio (1968), and the radioactivity determined in Aquasol. The remaining leaf was homogenized in methylene chloride–acetone, 1:1, air-dried, and weighed, and a 100-mg aliquot used for combustion. Recovery of standard [^{14}C]parathion through the Ogg igniter was quantitative.

Residue Experiments. Experimental trees were greenhouse-reared 2-year-old Valencia orange on Carrizo rootstock in excellent horticultural condition. Trees were removed from the greenhouse and sprayed with ~1 gal of parathion emulsifiable concentrate or wettable powder (0.96 g/L active ingredient) by using a standard 3-gal Hudson sprayer, thoroughly wetting all leaf surfaces. Trees were dried for ~2 h and returned to the greenhouse for the duration of each experiment. In a separate experiment, acephate was applied in the greenhouse as a root drench at a concentration of 15 g of active ingredient (AI) (400 mL of water)$^{-1}$ tree^{-1}. Each treatment contained four trees and was replicated four times. Two untreated trees served as controls. Whole leaves were sampled at random by carefully picking 10 leaves by the petiole and placing them immediately into a leaf punch jar. Leaf disk samples were obtained by punching 40 leaf disks directly into a leaf punch jar with a 2.5-cm leaf punch.

Whole leaves were processed for dislodgeable residues by shaking 2 times in 100 mL of dislodgeable solution solution (0.08 mL of 70% sodium dioctyl sulfosuccinate-/1000 mL of H_2O) at 200 rpm for 10 min. Whole leaves were reversed end to end for the second shake. Leaf disk dislodgeable residues were obtained by shaking 2 times in 40 mL of dislodgeable solution (Gunther et al., 1974; Iwata et al., 1977). Parathion was extracted from the dislodgeable solution with two methylene chloride extractions (2 × 40 mL for whole leaves; 2 × 20 mL for disks). Separate samples of whole leaves and leaf disks were stripped in 100 mL of ethanol, methanol, or chloroform (depending on the experiment) at 350 rpm for 1 min. Extractions were reduced to dryness on a rotary evaporator at 40 °C. Parathion extracts were transferred into 10 mL of benzene and acephate extracts were transferred into 10 mL of methyl isobutyl ketone. Storage was at −20 °C in amber bottles over anhydrous sodium sulfate.

Parathion leaf cell residues were obtained by cutting leaves or leaf disks into small pieces and homogenizing in 50 mL of 1:1 CH_2Cl_2–acetone in an ice bath for 2 min. The extract was filtered through No. 41 Whatman filter paper and 20 mL of the filtrate was transferred to a 50-mL volumetric flask. This was reduced to dryness on a nitrogen evaporator at 40 °C; then 10 mL of benzene was added to the flask. Flasks were shaken for 1 min on a platform shaker at 50 rpm, 30 mL of 2% Na_2SO_4 was added, and flasks were shaken throughly by hand. Layers were allowed to separate. The benzene layer was brought to the neck of the flask with distilled water and transferred and stored as above. Acephate leaf body residue extraction has been previously described in detail (Nigg et al., 1979). Gas chromatographic conditions for parathion and paraoxon (Nigg et al., 1977) and acephate and methamidophos (Nigg et al., 1979) have been previously described. Whole leaf areas were determined according to Turrell (1961). Leaves and leaf disks were weighed prior to extraction or prior to leaf body extraction, depending on the experiment.

RESULTS AND DISCUSSION

A solvent might extract cellular incorporated pesticide if the solvent disrupted cellular integrity during the extraction. Fresh leaves washed in the dislodgeable solution showed no structural injury (Figure 1A). Frozen leaves washed in the dislodgeable solution had severely disrupted cells with broken walls and some removal of cell contents (Figure 1B). Methanol resulted in cell dehydration after 30 s with the denser contents being drawn toward the center of the cells (Figure 1C vs. Figure 1D). Cell membranes were quickly disorganized with chloroplast rupture by chloroform washing (Figure 1E). Although some epidermal cell contents were removed after 90-s washes in chloroform, most of the cell contents remained within the cell wall boundaries (Figure 1F).

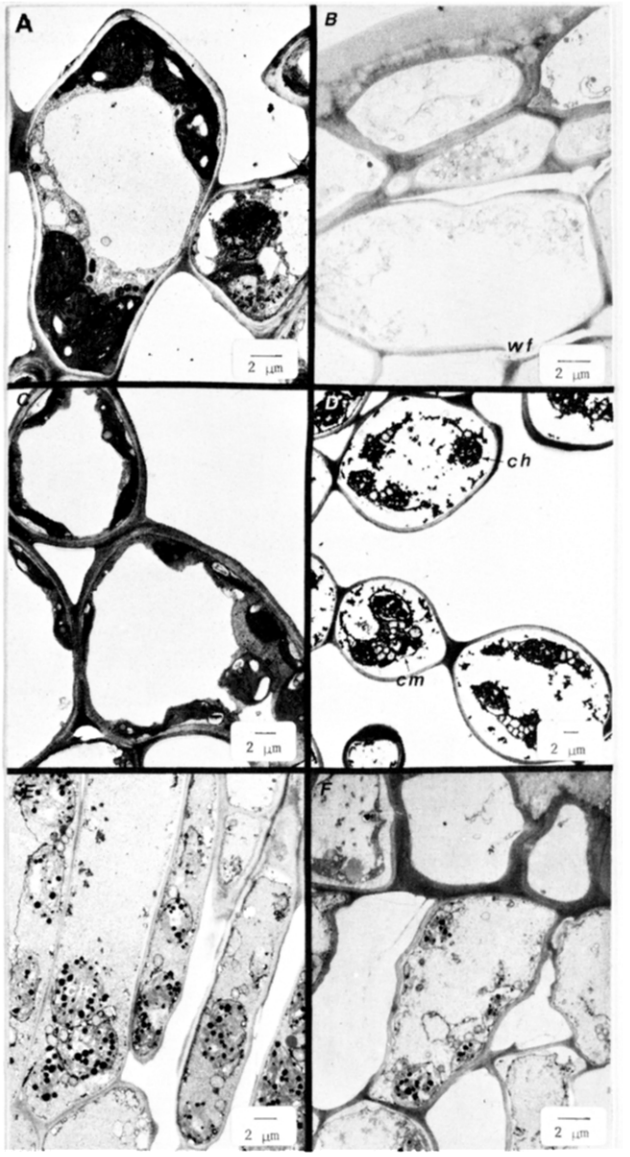

Figure 1. Parenchyma cells from whole leaves; unfrozen, dislodgeable (A), frozen, dislodgeable (B), washed in methanol 15 (C) and 30 s (D), and washed in chloroform 15 (E) and 90 s (F). Cell walls and contents were disrupted and contents partially removed after freezing (B). Membranes were disrupted and cell contents mixed by 30-s methanol (D) and 15- (E) and 90-s (F) chloroform washes: labels: wf, wall fracture; ch, chloroplast; cm, cell membrane.

The dislodgeable technique is unlikely to cause any internal leaf extraction unless the samples have been previously frozen. Freezing leaf disks did not affect the recovery of surface and penetrated residues of parathion, azinphosmethyl, and ethion for up to 90 days, but levels of omite were decreased (Gunther et al., 1974). As increasing amounts of pesticide penetrate into the leaf in-

Table I. Summations of Table II Intensities[a] of Spots of Three Methods of Extraction with Seven Solvent Systems

	methods				
solvent	1, whole leaves, 1 min	2, disks, 1 min	3, whole leaves, 10 min	$\bar{X}$	extractability
chloroform	41	38	43	41	100
methylene chloride	31	37	34	34	83
benzene	17	19	31	22	54
hexane	21	19	16	19	46
ethanol	13	13	19	15	37
methanol	7	7	11	8	20
soap	1	1	1	1	2
	131	134	155	140	

[a] Intensities of 12 spots rated on a 0 → 10 point scale.

terior, the potential for spurious data increases with frozen disks. Immediate extraction of dislodgeable residue samples is currently recommended (Gunther et al., 1977). Short washes with methanol and chloroform will cause some internal cell damage, particularly to the spongy mesophyll cells, and the tissue is easily reached by vapors of low surface tension liquids passing through the stomata on the lower surface of the leaves. Our short-term exposures did not appear to remove cell contents from the whole leaves.

Wax Extractability. Table I gives a summary of the mean sums of intensities of the 12 spots (lipids) obtained by charring the TLC plates containing the 21 extractions. A 10-min extraction of whole leaves gave a larger yield of material than either 1-min whole leaf or 1-min leaf disks (155 vs. 131 and 134, respectively). This greater extraction ability was manifested with benzene (31 vs. 17 and 19), ethanol (19 vs. 13 and 13), and methanol (11 vs. 7 and 7) as the solvent. Compared with whole leaf 1-min extractions, only methylene chloride gave any significant difference (31 vs. 37, respectively). Chloroform and methylene chloride ranked first and second, respectively (means of 41 and 34) and in their ability to extract total wax lipid. Compared by the three methods of extraction, chloroform was best on whole leaves for both 1- and 10-min extractions, whereas no distinct differences were observed with the disks. The other five solvents are listed in descending order of extraction strengths as determined by their mean spot intensities for the three extractive procedures.

In summary, we conclude that extraction of whole leaves for 10 min extracted the most lipid material. Whether all of the material is present in the epicuticular wax and not in the cutin is still to be examined.

Baker et al. (1975) described the lipids present in the epicuticular wax of citrus leaves. In orange leaves, hydrocarbons comprised 41.7%, primary alcohols 38.4%, and fatty acids 19.8% when the wax was extracted with chloroform. In Table II are tabulated the relative percents of the 12 lipid components of each of the 21 extraction samples. The intensities were not corrected for charring ability of the various components. When the samples were extracted with chloroform, the hydrocarbons (A), primary alcohols (F, G, and H), and free fatty acids (E) were 16.6, 44.7, and 4.9%, respectively. These differences between our values and those reported by Baker et al. (1975) may be due to (1) our use of an improved TLC solvent system

Table II. Extractability of Citrus Leaf Epicuticular Wax Components with Various Solvents[a]

lipid	A[b]	B	C	D	E[c]	F[d]	G[d]	H[d,e]	I	J[f]	K	L	
R_f	0.85	0.72	0.65	0.57	0.47	0.41	0.38	0.35	0.33	0.30	0.20	0.10	
R_f/R_f(Chol.)	2.8	2.4	2.2	1.9	1.6	1.4	1.3	1.2	1.1	1.0	0.7	0.3	
chloroform													
disk, 1 min	14.8	7.0	12.8	10.1	4.4	16.2	20.1	6.2	–	8.4	–	–	100.0
whole leaf, 1 min	19.5	6.3	7.4	6.2	4.2	14.9	19.9	8.7	–	9.9	3.0	–	100.0
whole leaf, 10 min	15.5	7.9	12.7	7.9	6.0	16.1	20.7	11.5	–	1.7	–	–	100.0
$\bar{X}$	16.6	7.1	11.0	8.1	4.9	15.7	20.2	8.8	–	6.7	1.0	–	100.1
methylene chloride													
disk, 1 min	11.4	6.8	9.2	9.2	3.6	14.9	20.4	6.9	–	9.0	0.4	–	91.8
whole leaf, 1 min	12.7	5.3	5.7	6.2	3.6	12.1	16.2	5.2	–	6.5	0.4	–	73.9
whole leaf, 10 min	15.6	4.2	9.1	7.4	9.2	15.7	6.3	8.1	–	0.7	–	–	76.3
$\bar{X}$	13.2	5.4	8.0	7.6	5.5	14.2	14.3	6.7	–	5.4	0.3	–	80.6
benzene													
disk, 1 min	8.7	3.7	4.0	2.2	1.7	12.9	11.1	2.0	–	3.6	0.4	–	50.3
whole leaf, 1 min	10.9	3.5	3.8	1.4	1.0	10.3	13.4	1.9	–	0.4	0.4	–	47.0
whole leaf, 10 min	11.2	4.4	5.6	5.1	5.7	10.0	14.0	7.2	–	6.7	4.4	–	74.3
$\bar{X}$	10.3	3.9	4.5	2.9	2.8	11.1	12.8	3.7	–	3.6	0.4	–	56.0
hexane													
disk, 1 min	14.6	2.9	3.4	–	1.1	16.8	9.1	–	–	1.6	–	–	49.5
whole leaf, 1 min	16.1	4.1	4.9	–	1.3	12.5	15.4	3.3	–	0.4	0.4	–	58.4
whole leaf, 10 min	9.3	2.7	5.4	–	1.0	14.2	4.2	2.0	–	–	–	–	38.8
$\bar{X}$	13.3	3.2	4.6	–	1.1	14.5	9.6	1.8	–	0.7	0.1	–	48.9
ethanol													
disk, 1 min	8.4	–	0.3	–	0.9	6.7	6.7	–	0.3	4.6	2.9	2.0	32.8
whole leaf, 1 min	9.7	1.4	3.3	0.5	0.6	7.5	5.1	0.8	–	2.7	0.3	–	31.9
whole leaf, 10 min	8.2	1.2	5.4	1.2	0.3	7.1	10.4	0.3	–	3.8	1.6	1.2	40.7
$\bar{X}$	8.8	0.9	3.0	0.6	0.6	7.1	7.4	0.4	0.1	3.7	1.6	1.1	35.3
methanol													
disk, 1 min	2.6	–	–	–	0.6	2.6	3.5	–	0.6	6.1	1.6	1.3	18.9
whole leaf, 1 min	3.9	–	–	–	0.3	3.7	3.7	3.4	–	1.5	–	–	16.5
whole leaf, 10 min	3.1	0.7	1.6	0.3	3.3	6.9	0.9	–	–	2.6	2.6	2.1	24.1
$\bar{X}$	3.2	0.2	0.5	0.1	1.4	4.4	2.7	1.1	0.2	3.4	1.4	1.1	19.7
soap													
disk, 1 min	2.3	–	–	–	–	0.3	–	–	0.3	–	–	–	2.9
	1.1	–	–	–	–	0.3	–	–	–	–	–	–	1.4
	1.2	–	0.3	–	0.3	0.3	–	–	–	–	–	–	2.1
	1.5	–	0.1	–	0.1	0.3	–	–	0.1	–	–	–	2.1

[a] Relative to chloroform (100%). [b] Alkanes. [c] Free fatty acids. [d] Alcohols. [e] C_{16}, C_{20}. [f] Free sterols.

Table III. Recovery of [^{14}C]Parathion by Scintillation and Gas-Liquid Chromatography (GLC) (230 668 dpm Added to First Extraction Step)

extraction	extraction step	total dpm	% of recovered	GLC, μg	% (400 μg)
methanol	MEOH	169 857 ± 19 836[a]	99.1 ± 0.2	387.0 ± 13.0	96.8 ± 3.3
	$CHCl_3$	40 ± 12	0.3 ± 0.1	0.3 ± 0.2	–
	CH_2Cl_2-acetone	958 ± 152	0.5 ± 0.1	ND	–
	leaves	407 ± 117	0.2 ± 0.1	ND	–
		171 662 ± 20 117		387.3 ± 13.2	
recovered		74.4 ± 8.7		96.8 ± 3.3	
dislodgeable	jar wash	475 ± 475	0.3 ± 0.4	ND	–
	dislog. solution	7170 ± 156	0.8 ± 0.2	ND	–
	dislog. extraction	109 401 ± 15 222	69.8 ± 3.6	265.9 ± 15.9	66.6 ± 4.0
	$CHCl_3$	23 574 ± 1 326	15.1 ± 0.6	48.7 ± 4.9	12.2 ± 1.2
	CH_2Cl_2-acetone	14 835 ± 3 432	9.6 ± 2.8	60.2 ± 2.2	15.1 ± 0.6
	leaves	6 779 ± 683	4.3 ± 0.2	–	–
		156 234 ± 14 236		374.7 ± 19.7	
recovered		67.7 ± 6.2		93.7 ± 4.9	

[a] Mean ± SD (three replicates).

(Schlotzhover et al., 1977), (2) releasing previously undetected components (compounds B, C, D, I, J, K, and L; 33.9%), or (3) the charring ability of the various lipid components. Compounds F, G, and H were determined to be primary alcohols by TLC with standards and by their retention times as acetates on GLC. The profile of the acetates was essentially that reported by Baker et al. (1975). Thus, the major wax component of ctirus is primary alcohols and not esters as reported by Schulman and Monselise (1970). This conclusion is supported by the results of Freeman et al. (1979) in Pineapple and navel oranges and Dancy tangerines. The percentage of each component, however, changes with leaf age (Freeman et al. (1979).

Comparing the three modes of extraction using chloroform, a 1-min extraction of whole leaves extracts 5% more alkanes and 5% less of compound C than the other two modes while the other components were extracted to about the same extent. Hexane also extracted more alkanes with a 1-min extraction than in a 10-min extraction (Table II). In its ability to extract alkanes, hexane ranked with methylene chloride; both were less suitable than chloroform. The other solvents showed decreasing ability to extract alkanes due to their increasing polarity. Compounds B and C (wax esters, ketones, or sterol esters, etc.) showed decreasing relative percent extractability relative to the increasing polarity of the solvent. Compound D again showed this constant decrease except with hexane. Where hexane was used for extraction, this lipid was not detected. Free fatty acids (E) were apparently extracted to the greatest extent when the whole leaf was extracted for 10 min. This difference in the three modes of extraction was observed for chloroform, methylene chloride, benzene, and methanol. Methylene chloride was best for extracting free fatty acids.

Primary alcohol F showed very little difference among the three modes of extraction. The extraction percentages paralleled those of alkanes, chloroform, hexane, methylene chloride, and benzene. Thus, it is very probable that these spots are very long chain linear alcohols. On the other hand, alcohol G followed the normal decreasing trend of the seven solvent systems. In general, alcohol H was more easily extracted by a 10-min whole leaf extraction than by the other modes. Free sterols (J) never accounted for more than 10% of the wax. Chloroform and methylene chloride were the best; ethanol, benzene, and methanol were intermediate, whereas hexane was a very poor solvent for extraction of this lipid. Lipids I, K, and L combined accounted for less than 5.2% of the total wax, ethanol being the best solvent for extracting these compounds.

For our purposes in this study, methanol was the solvent most nearly duplicating wax extractability compared to the dislodgeable method. However, methanol extracted ~8 times the epicuticular wax material compared to the dislodgeable soap solution (Table I).

Radiolabeled Studies. Table III summarizes these data for [^{14}C]parathion added to the methanol extraction. The [^{14}C]parathion was added to the methanol, leaves were then added, and the extraction was started. The same leaves were then surface extracted with chloroform and the remaining leaves homogenized. Each extract was analyzed for radioactivity and, excepting the leaf residue, by GLC.

The data indicated that essentially 100% of the radioactivity was recovered in the methanol extraction. Less than 1% of the recovered radiolabel appeared in the wax (chloroform extraction), in the leaf cell (CH_2CH_2–acetone), or in the remaining residue. GLC results are relatively identical with those of the radioactive analyses (Table III). However, only 70–85% of the radiolabel was recovered whereas GLC analyses indicated ~100% recovery. We have no explanation for this result.

The data for the dislodgeable technique are presented in Table III. Again, the trend for both radiolabel and GLC analyses is the same as well as the previously mentioned anomaly for total recovery of [^{14}C]parathion. However, the trends between methanol vs. dislodgeable extraction differ. About 70% of the recovered radiolabel was recovered in the dislodgeable extraction, 15% in the wax (chloroform), 10% from the leaf cell (CH_2Cl_2–acetone), and 4% in the remaining residue. GLC analyses of the dislodgeable extraction indicated 66% removed in the dislodgeable wash, 12% in the chloroform wax extraction, and 15% in the leaf cell extraction.

These data indicate that parathion released from a surface paraticulate in a soap wash could partition into the epicuticular wax and into the internal structure of the leaf. This may be due to the 20 min required for the soap extraction compared to the 1-min methanol extraction. We conclude that a short methanol wash would more accurately reflect surface residues than a soap wash which is subsequently extracted.

Results of an 8-day parathion experiment are summarized here. This experiment was designed to determine differences in recovery of surface pesticide with ethanol, chloroform, or the dislodgeable solution and to compare leaf disks with whole leaves. No significant differences (68% confidence level) in rates and initial concentrations were evident among the various solvents or between whole leaves compared to leaf disks except that chloroform did

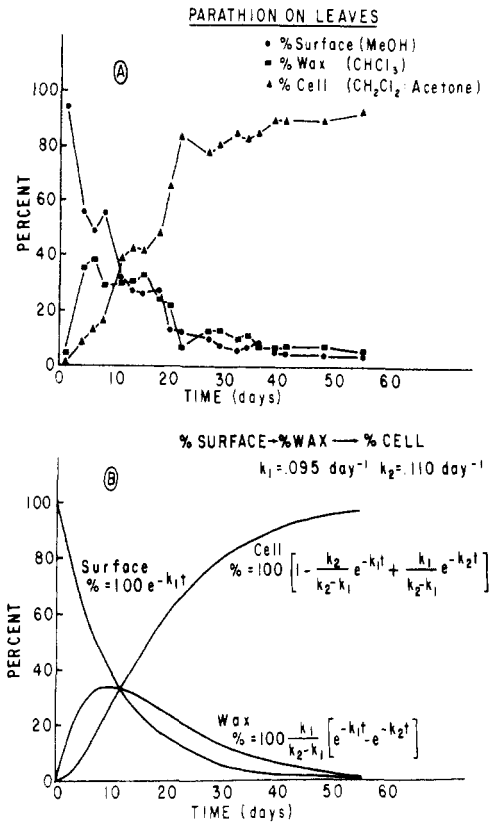

Figure 2. Leaf parathion content. (A) Percent parathion on the surface, in wax, and in the remaining leaf after a parathion (4E) 0.96 g/L (4 lb of AI/500 gal) application. (B) Kinetic representation.

remove more parathion initially with whole leaves.

Table IV presents data for a 42-day experiment with parathion. This experiment compared the dislodgeable method vs. a chloroform strip vs. total leaf residue. The rate of a disappearance and quantity of the dislodgeable residue were the same as those for chloform through day 7, as in the previous experiment. After day 10, the quantity of residue and rate of disappearance comparing chloroform to dislodgeable differed significantly at the 68% level and the overall rates of disappearance consequently differed significantly. The leaf cell residue analyses indicated that parathion or paraoxon must have penetrated into the leaf cells as paraoxon generally increased whereas for both chloroform and dislodgeable extracts paraoxon decreased.

The third parathion experiment is presented in Figure 2. These samples were progressively extracted: first a methanol surface strip, then a chloroform wax strip, and finally homogenization in methylene chloride–acetone. Figure 2A shows the percentage of parathion with time in each leaf component: surface, wax, and cells. The fitted curves and kinetics are in Figure 2B. These data resemble the decay of a parent nuclide into a radioactive daughter which then decays into a stable granddaughter, each process being strictly first-order:

$$\%\ \text{surface} \xrightarrow[k = 0.095\ \text{day}^{-1}]{} \%\ \text{wax} \xrightarrow[k = 0.011\ \text{day}^{-1}]{} \%\ \text{cells}$$

There was no difference between the rate of penetration into the epicuticular wax and the rate of penetration into the cell. Presenting these data as percentages makes it appear that no pesticide left the leaf or was metabolized. However, the absolute surface residue declined steadily. The wax residue peaked on day 4 and declined thereafter; the cellular residue reached ~2.8 ppm on day 4 and remained ~2.0 ppm throughout the experiment. Paraoxon

Table IV. Parathion (4E)[a] Leaf Stripping Dislodgeable vs. Chloroform

		day 0	day 3	day 7	day 10	day 14	day 21	day 28	day 35	day 42
Sur-ten dislodgeable, ng/cm²	parathion	3402.0 ± 778.3	1380.2 ± 322.6	546.8 ± 170.4	99.30 ± 74.55	78.34 ± 49.71	9.57 ± 7.53	2.96 ± 0.55	ND	ND
	paraoxon	60.84 ± 20.73	26.76 ± 21.27	7.33 ± 2.24	1.86 ± 1.41	1.55 ± 0.63	1.16 ± 0.84	ND	ND	ND
$CHCl_3$ dislodgeable, ng/cm²	parathion	3602.1 ± 1312.5	1306.6 ± 108.2	625.0 ± 179.5	347.1 ± 109.0	199.49 ± 69.25	105.58 ± 53.14	56.25 ± 8.51	34.25 ± 30.74	18.77 ± 10.90
	paraoxon	15.81 ± 7.38	7.04 ± 2.51	4.87 ± 0.82	2.73 ± 0.91	2.17 ± 1.74	3.29 ± 2.38	1.66 ± 0.29	1.07 ± 0.75	1.98 ± 1.31
CH_2Cl_2–acetone total, ppm	parathion	25.91 ± 6.45	129.2 ± 11.29	109.4 ± 46.33	75.03 ± 4.75	104.91 ± 31.23	62.64 ± 26.24	57.94 ± 13.09	69.05 ± 24.72	52.11 ± 36.45
	paraoxon	0.10 ± 0.02	1.72 ± 0.37	1.93 ± 0.82	2.08 ± 0.37	3.50 ± 1.06	2.78 ± 1.41	4.78 ± 2.19	3.35 ± 0.90	5.66 ± 3.34

[a] 1.92 g/L (8 lb of AI/500 gal). Statistical analyses (least-squares fit to C_0e^{-kt}, $t_0 = 0$ day) [data are given in the order solvent, C_0^{exp} (μg/cm²), R^2, k (day⁻¹), and hours]: (1) Sur-Ten, 3.4 ± 0.8, 0.98, 0.26 ± 0.02, and 7; (2) $CHCl_3$, 3.6 ± 1.3, 0.94, 0.12 ± 0.01, and 9.

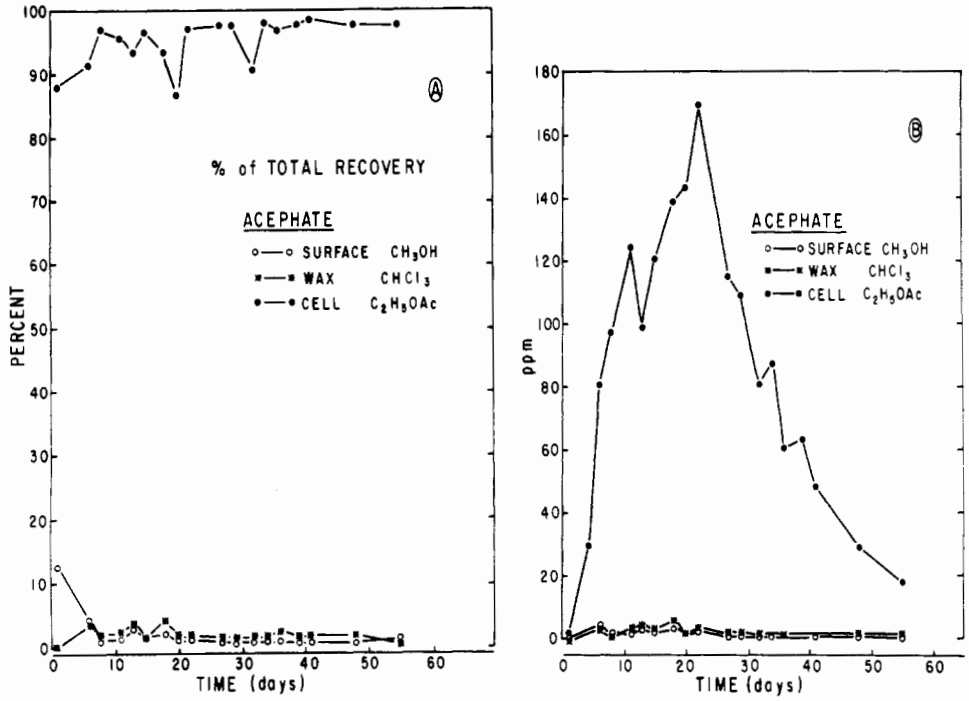

Figure 3. Leaf acephate content. (A) Percent acephate on the surface, in wax, and in the remaining leaf after application (15 g of AI/400 mL) to the roots of citrus seedlings. (B) Actual values in ppm.

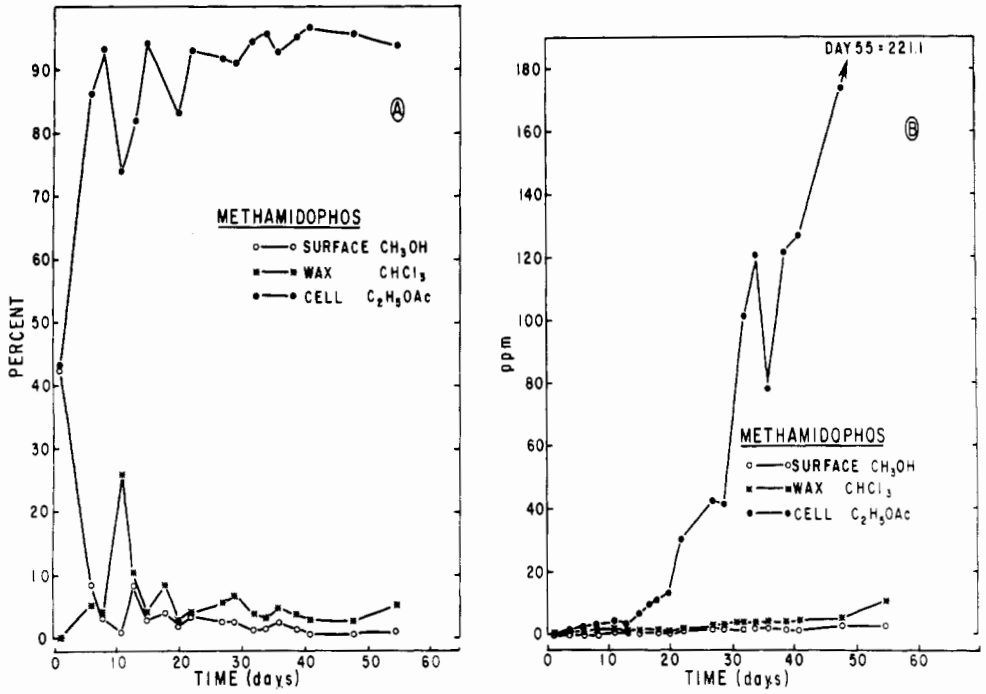

Figure 4. Leaf methamidophos content. (A) Percent methamidiphos on the surface, in wax, and in the remaining leaf after application (15 g of AI/400 mL) of acephate to the roots of citrus seedlings. (B) Actual values in ppm.

peaked on day 1 for the surface residue, and wax residue declined until day 20 and was not detected thereafter. The cellular paraoxon reached a peak on day 4 and remained relatively constant throughout the experiment.

Several conclusions are evident from the data. It was not possible to show a statistical difference between the dislodgeable and alcohol or chloroform surface residue strips up to 8 days after application. This appears to be due to the low percentage of parathion penetrated into the wax until about day 8 and because the coefficient of variation was generally 30–50%. This disagrees with unpublished results cited by Gunther et al. (1977). Further, a 1-min extraction with methanol showed little partitioning of pesticide into the leaf whereas ~27% of the parathion "removed" with the dislodgeable method partitioned into some portion of the leaf.

An interesting observation for these parathion data was that the rate of disappearance was strictly first order. In previous papers we presented a ln–ln representation of data for *field* data (Stamper et al., 1979; Nigg and Stamper, 1980). The greenhouse data presented here gave better fits to first order. This points again to the importance of knowing how environmental conditions affect the behavior of chemicals.

Figure 3 and 4 illustrate the residue data obtained after applying acephate (a systemic insecticide) to the roots of

citrus seedlings. The object here was to determine if cellular pesticide would be extracted with methanol or chloroform. Again, leaf samples were progressively extracted: first with methanol, then with chloroform, and finally homogenized in ethyl acetate. These data differed from data obtained from the direct leaf application of parathion. From Figure 3B, very little actual compound was recovered on day 1 and the percentages on day 1 (Figure 3A) mean little. By day 6, 92% of the acephate was recovered from cells, 3.6% from the wax, and 4.2% from the surface. On day 8, 97% of the acephate was cellular, 1.8% from the wax, and 1.4% from the surface. These percentages remained relatively constant after day 8 for 55 days. Acephate reached a peak on day 22 and declined thereafter. Its hydrolysis product, methamidophos, rose steadily in the cells until day 13 and then rose in direct proportion to acephate decline and was still rising on day 55 (Figure 4). The amount of methamidophos recovered on the surface increased from 0.01 to 2.4 ppm and in the wax from a "trace" to 12.3 ppm over 55 days (Figure 4B). At the peak quantity of cellular methamidophous (day 55), only 1% of the total residue was recovered from the surface (MeOH) and 5% from the wax ($CHCl_3$) (Figure 4A). If methanol and chloroform removed pesticide from within the leaf, a large percentage of acephate and methamidophos should have been removed in the methanol surface wash and chloroform wax extraction. Percentages were very low, well within experimental error. Electron microscopy indicated little penetration of the alcohols to the cellular level and good penetration of chloroform. Our short extraction times probably account for the low carry-over with both solvents.

Both the alcohol and dislodgeable methods have advantages for estimating surface residues. The dislodgeable method tends to remove less contaminating material for an electron capture detector than alcohol. Recovery of surface pesticides with alcohol generally requires cleanup prior to electron capture. Alcohol is fast, recovers water-soluble materials, and allows easy recovery of metabolites for formation of derivatives. The dislodgeable method is much slower and water-soluble metabolites may be unavailable for analysis.

We conclude that the alcohol–chloroform methodology presented here is accurate and fast for estimating surface residues and wax residues. Suitable solvents for remaining cellular residues will depend on each compound.

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