Establishing Dislodgeable Pesticide Residues on Leaf Surfaces

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Some pruners, thinners, and pickers have been made ill by working with some plants too soon after commercial applications of some organophosphate insecticides, notably parathion, azinphosmethyl, ethion, and dioxathion in citrus groves and parathion in grape vineyards, peach orchards, and cotton and tobacco fields. Considering that there are more than 300,000 such workers in California, the about 20 authenticated incidents of such illnesses involving about 500 persons (with no deaths) since 1949 (BAILEY et al., 1972) represent a remarkable safety record. Nonetheless, most of these incidents involved worker reentry after the legally prescribed safe waiting intervals before harvest, and clearly may be the result of some circumstance or combination of circumstances not adequately controlled by existing requirements underlying legal uses of these and perhaps other cholinesterase-inhibiting insecticides. That the circumstances leading to overexposure by a few workers are unusual is attested by the few illnesses compared to the huge number of long-term annual exposures by these 300,000 workers and by the completely random occurrences of illnesses both geographically and seasonally in California; also, many years have been devoid of reported worker illnesses.

So-called minimum intervals for picker reentry into tree fruit orchards are based upon insecticide deposits and residues on and in the fruits (Federal Food, Drug, and Cosmetic Act) for consumer protection against excessive residues in foodstuffs. Recent findings (GUNTHER, 1969, CARMAN et al., 1972) that citrus foliage may accept and retain much greater deposits and residues longer than the fruit suggest the foliage may be very important in consideration of worker reentry into insecticide-treated orchards,

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for they are exposed to much greater areas of foliage than of fruits. An average 20-year-old orange tree, for example, has about 100,000 leaves averaging about 50 cm^2 total surface each, or 5,000,000 cm^2 of exposed leaf surface; this tree will bear about 1,200 fruits averaging 250 cm^2 surface area each, or 300,000 cm^2 of exposed fruit surface. The citrus leaf:fruit area ratio is thus 17:1. An average 10-year-old peach tree also has about 100,000 leaves averaging about 40 cm^2 total surface each, or 4,000,000 cm^2 of exposed leaf surface; a freestone peach tree will have about 500 fruits of 150 cm^2 each, or 75,000 cm^2 of exposed fruit surface; the corresponding figures for cling peaches are 1,000 fruits and 150,000 cm^2. The peach leaf:fruit area ratios are, therefore, 53:1 for freestones and 28:1 for clings.

Despite the well-established fact that most organophosphate deposits (material initially laid down by the spray) penetrate rather quickly (usually hours to days) into clean leaf surfaces, thus becoming unavailable residues, dust and other detritis including the solid components of WP formulations may mediate this migration into waxy and other subsurface layers of the foliage by strong sorbtive action. Insecticides thus retained on such surfaces for long periods are properly "residues" rather than "deposits" (GUNTHER and BLINN, 1955). Incorporated onto and into particulate matter, such residues are, therefore, transferable to workers via dislodgement into air from worker activity and transferable directly to skin and clothing, unquestionably in large amounts over an eight-hour day. Airborne material represents both inhalation and oral exposures, while transfers of dislodgeable material to skin and clothing represent dermal exposures.

Involvement of insecticide actually in the vapor phase is being established by standard techniques. The role of "dislodgeable residues" is also under investigation, including use of the special determinative techniques herein reported. These "dislodgeable residue" techniques may be of interest as a guide to everyone working with insecticide-treated crops where pickers, especially, are in frequent contact with treated leafy material, as artichokes, strawberries, tobacco, cotton, lettuce, tomatoes, etc. and most tree-fruit and vine crops.

Requirements

Any technique to evaluate total dislodgeable residues must accommodate statistical size and appropriate field representation requirements, be as simple as possible yet provide adequate reproducibility and standardizability, and be adaptable to a variety of pesticides and types of foliage. Results should be expressable both as ppm and as $\mu g/cm^2$ for eventual comparison of fruit vs. leaves and of worker residue hazard vs. consumer residue hazard with edible foliage. This requirement means that both surface areas and weights of the samples must be easily ascertained, for large numbers of samples will be involved, as established at Riverside for dioxathion, parathion, and phosphamidon on citrus foliage and at Davis for parathion on peach and grape foliage. Leaf discs meet this requirement: the leaf punches are of known aperture and the discs are easily weighed; discs also can be obtained from any desired part of a leaf, to emphasize the characteristic tip concentration of deposit from full-coverage applications with the present purpose, for example. Measuring areas of large numbers of entire leaves is difficult.

Reproducibly removing the dislodgeable residue (for analysis) from the statistically adequate numbers of discs clearly involves a mechanical dislodgement via thorough water washing rather than "stripping" with an organic solvent: the latter not only will carry external insecticide into the leaf tissue but will also extract field-penetrated insecticide.

The so-called "stripped" discs can then be processed to determine field-penetrated residues, if this value is desired.

Leaf-Punching Procedure

Experiences with dioxathion, parathion, and phosphamidon indicate the adequacy of 40 2.5-cm (4.9 cm^2 single surface) leaf discs per sample for citrus foliage and 200 1.8-cm (2.5 cm^2 single surface) leaf discs for parathion on grape or peach foliage.

The two types of leaf punches we have developed are shown in figure 1; they are both based upon the basic design of SMITH and LITTLE (1954). The citrus-leaf punch[1/] has a diameter of 2.5 cm and is designed to cut discs from thick xeromorphic leaves. The peach- and grape-leaf punch[2/], with a diameter of 1.8 cm, is for thin mesomorphic leaves;

[1/] Not yet available commercially.
[2/] Available from Norman Willett, 1807 Oleander Place, Davis, 95615.

this punch rotates 1/8 turn at the completion of the stroke to shear the tissues and has an ejector for pushing the severed disc into the jar. Both punches have stroke-activated counters. The punches must be strongly concave to avoid disturbance of the surface material.

Sampling--citrus foliage. Forty discs weigh from 6-8 g and have a total surface area of 392 cm^2. Discs are punched at 45° intervals around eight trees at shoulder height, with five discs/tree as follows to afford five leaves/sampling position CARMAN, 1971):

Tree 1	0°	45°	90°	135°	180°
Tree 2	45°	90°	135°	180°	225°
Tree 3	90°	135°	180°	225°	270°
Tree 4	135°	180°	225°	270°	315°
Tree 5	180°	225°	270°	315°	0°
Tree 6	225°	270°	315°	0°	45°
Tree 7	270°	315°	0°	45°	90°
Tree 8	315°	0°	45°	90°	135°

Three field replicates are used and variability among field replicates averaged ±15% for dislodgeable residues and ±25% for penetrated residues of dioxathion, parathion, and phosphamidon.

Stripping of Dislodgeable Residues

Citrus. Weigh jar and sample (jar previously tared). Add 50 ml of water and two drops of a 1:50 dilution of Sur-Ten wetting agent (American Cyanamid Co.), and shake vigorously on a reciprocating shaker for 60 min. Decant the liquid into a 500-ml separatory funnel, add another 50 ml of water and two drops of wetting agent solution to the discs and shake 30 min. Combine this surface stripping suspension ("strip") with the first, then add 25 ml of water to the discs, shake by hand for five sec, and combine this liquid with the other two decantates. Add to this aqueous "strip" 50 ml of hexane (chloroform for phosphamidon) and shake 20 sec; transfer the organic solvent phase into a sample storage bottle. Repeat this extraction twice more, combining the three extracts; add 20 g of anhydrous Na_2SO_4, cap the bottle with an aluminum foil liner, and store at 4°C.

For penetrated insecticide (phosphamidon and dioxathion) shake as much water as possible from the stripped leaf discs, transfer them to a micro Waring blender, add ten g of anhydrous Na_2SO_4 and 100 ml of hexane, rinsing sample jar, also, and blend three

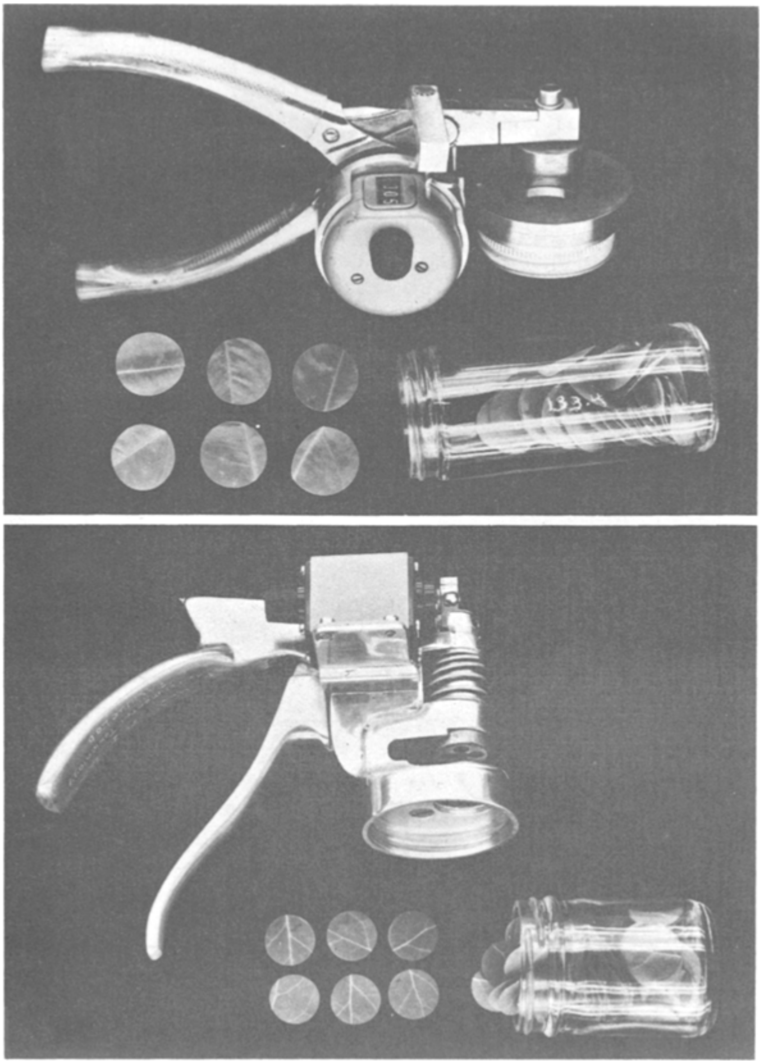

Fig. 1--Leaf punches used for sampling foliage. Upper photograph is the punch used for citrus (xeromorphic) leaves. Below is the one used for peach and grape (mesomorphic) leaves.

min at high speed (use chloroform for phosphamidon). Decant the solvent through a No. 1 Whatman fluted filter paper into a sample storage bottle, retaining as much leaf material as possible in the blender jar. Reblend the leaf material 30 sec at high speed with another 100 ml of solvent, and filter the extract through the same filter into the storage bottle. Add 20 g of anhydrous Na_2SO_4, cap the bottle with an aluminum foil liner, and store at 4°C. For parathion, blend the leaf discs for two min at high speed with 50 ml of acetonitrile containing 20% water. Filter the solution, measure the volume recovered (or take an aliquot), transfer it to a

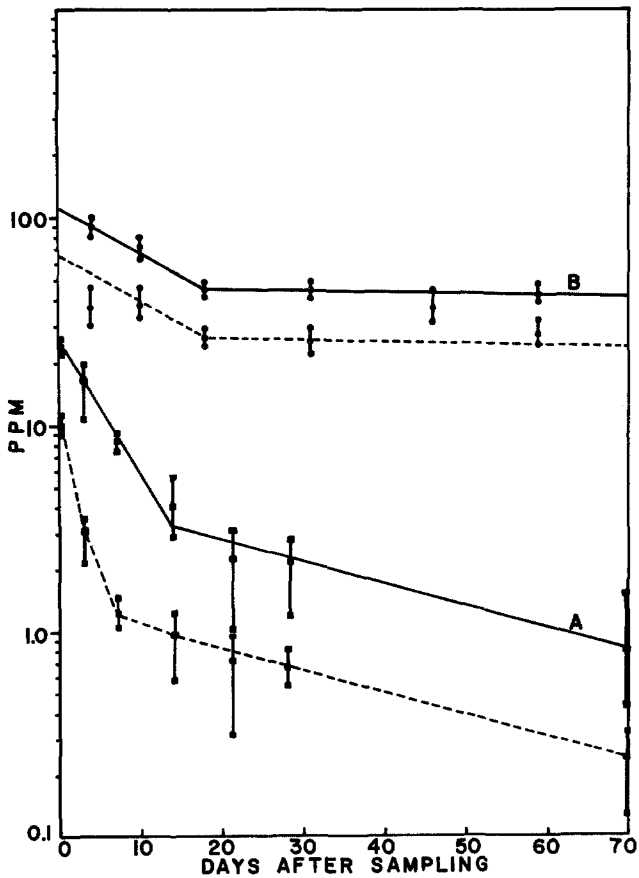

Fig. 2--A = residues of parathion on peach leaves following application of one lb A.I./A of encapsulated parathion. B = dioxathion residues on orange leaves after application of five lb A.I./A of an emulsion concentrate formulation. ——— = surface residue. — — — = remaining residue.

500-ml separatory funnel, add 50 ml of hexane and shake vigorously for 30 sec. Add 300 ml of water and five ml of saturated NaCl solution and mix vigorously for 30 sec. Allow the layers to separate and discard the aqueous phase. Wash the hexane twice by shaking with 50-ml portions of water, discarding the water each time. Drain the hexane into a sample storage bottle containing 20 g of anhydrous Na_2SO_4 and store at 4°C.

Peaches. Follow the same procedure for surface stripping except that the 200 weighed leaf discs are transferred to a glass-stoppered 500-ml Erlenmyer flask for stripping. For surface strips 200-ml, 200-ml, and 25-ml portions of the wetting agent solution are used, with eventual transfer of hexane-soluble materials with 200-ml portions of solvent in a 1,000-ml separatory funnel.

For penetrated material, also double the volumes of solvent and of Na_2SO_4.

Discussion

Figure 2 shows typical persistence curves for dioxathion dislodgeable and penetrated residues from orange leaves and for parathion from peach leaves to demonstrate variability among replicates as well as credibility of total data.

Different insecticides and different plants may require different wetting agents and different organic solvents. In other applications, the recoveries at each repeated stripping and extracting stage should be checked for maximum recovery commensurate with a reasonable number of operations using actual field-tested samples as substrates. Size of stripping container and speed and vigor of machine shaking must be adjusted to achieve minimum clumping of discs; clumped discs are not completely stripped.

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