Worker Environment Research. III. A Rapid Method for the Semi-Quantitative Determination of Some Dislodgable Pesticide Residues on Citrus Foliage

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Organophosphorus pesticides are used extensively in agricultural pest control, where the susceptibility of the organophosphorus (OP) compounds to undergo degradation to non-toxic products has led them to supplant the organochlorine pesticides in many applications. Their greater toxicity, however, requires that greater precautions be exercised to protect workers from overexposure. For example, after pesticide application to a crop, worker activity in the treated area must be restricted until the amounts of the pesticide and its toxic alteration products have declined to a predetermined acceptable level. This problem has led to the establishment of worker exclusion times or "re-entry intervals" for a number of crops and insecticides. They have been generally quite effective in reducing incidences of worker illnesses. An extensive discussion of the "re-entry problem" has been compiled (MILBY et al. 1975) and also presented in overview by SPEAR et al. (1975).

Citrus is a leading crop in the number of these reported poisoning episodes and the foliar dislodgable residues appear to be the main source of OP pesticides absorbed by workers. Currently the dislodgable residues are removed for quantitation by shaking standard leaf disc samples with an aqueous detergent solution (GUNTHER et al. 1974). The pesticide is then partitioned into hexane and subsequently quantitated by gas chromatography with a flame photometric or an alkali flame ionization detector. This approach is precise and sensitive but is not suited for use in the field. A rapid technique is desirable to enable an on-the-spot semi-quantitative determination of the level of toxic foliar dislodgable residues in order to assure safe working conditions for workers in the groves after public health professionals have established safe upper levels of dislodgable residues for each OP compound on the foliage.

We have developed a method for the rapid and convenient measurement in the field of dislodgable pesticide residues on citrus foliage. The method responds to phosphorothionates such as parathion, phosphorodithioates such as ethion and dioxathion, and phosphates such as paraoxon. The method consists of three steps:

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(1) Using a specially designed sampler (Fig. 1), transfer a known "area" of dislodgable pesticide residues from one or more leaves to a porous paper strip.
(2) Using paper chromatography, separate the sought compounds from dust and other material removed from the foliage.
(3) With suitable reagents, develop on the paper strip a color of an intensity proportional to the quantity of OP compounds removed.

Step (3) is based on the formation of a blue product when an OP compound and 4-(p-nitrobenzyl)pyridine (NBP) are heated together and then treated with base (GETZ and WATTS 1964, TURNER 1974). This reaction has been used for visualization of pesticides in paper and thin-layer chromatography (MEYER 1973).

The minimum detectability of the method herein described is ca. 0.05 µg of total OP residues/cm^2 of leaf surface sampled. This low foliar residue value is believed to be well below the level at which an effect is observed on human serum or red blood cell ChE activity, the present usual physiological index of human exposure to OP pesticides, but this assumption must be regarded as tentative until supporting evidence is obtained.

EXPERIMENTAL PROCEDURE

Sampling. Dislodgable pesticide residues are transferred to strips of chromatographic paper prepared as follows: cut Whatman No. 1 chromatographic paper into strips 1 x 9 cm. Lightly pencil-mark the strips along one edge at distances of 1.0, 1.5, and 2.25 cm from one end. Place 10 µl of a 5% solution of 4-(p-nitrobenzyl)pyridine*/ in acetone in the center of the strip at the 1.5-cm mark, and allow the acetone to evaporate. Strips prepared in this way are stable for at least two months at room temperature.

A prepared paper strip is so placed in the Dislodgable Residue Sampler (Fig. 1) that it can be reproducibly rubbed on the surface of a leaf or leaves as the sampler measures the distance over which the paper is rubbed. The jaws of the sampler (B and C)**/ are held open by an adjustable spring (E). A strip of treated paper (I) is attached to the fixed wheel (G), and wound around it and attached to the wire clip (H). The distance indicator wheel (F) is then set to zero cm. The leaf being sampled is placed between the jaws, which are then closed by means of the trigger (A); an adjustable spring (D) holds the jaws closed against the leaf. The leaf is then pulled through the jaws for the desired distance, indicated on the movable wheel; this process transfers the dislodgable residues on to the paper strip as a band 1 cm from the end of the strip and 1 cm wide. This band contains pesticide residues, dust, and some plant pig-

*/ Eastman Kodak Co., Rochester, New York 14650.
**/ Letters in parentheses refer to labeled parts in Fig. 1.

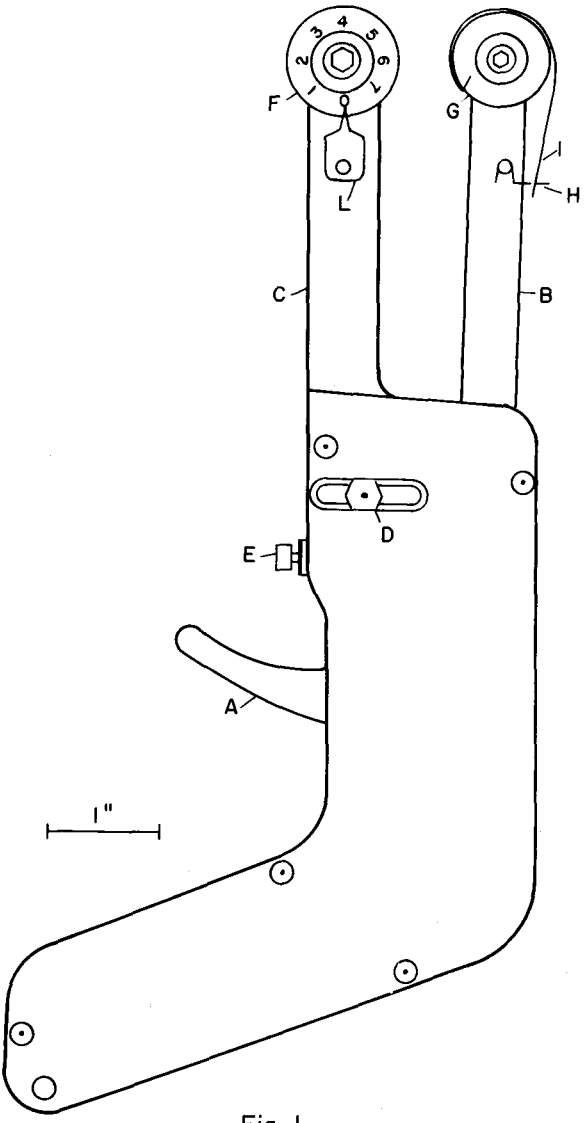

Fig. 1
DISLODGABLE RESIDUE SAMPLER

ments and waxes. The area of leaf sampled is 1 cm times the distance, generally 5 cm, the leaf is pulled through the sampler jaws.

Separation. The marked end of the paper strip is placed in a small vial filled 1 to 3 mm deep with a solution prepared by mixing 5 ml of water, 20 ml of acetone, and 0.25 g of potassium hydrogen phthalate; the vial is capped with a thin slotted cork or cap so the strip can be inserted and removed easily. The solution is allowed to rise until it reaches the 2.25-cm mark on the strip; it extracts the pesticide residues, in a rapid and efficient cleanup procedure, from the other material rubbed off the leaf. Pesticide residues and NBP move with the solvent front in a 1- to 2-mm band.

Color development and measurement. The paper strip is heated in a small portable battery-operated oven (Fig. 2) at 100°C for 5 min. It is then cooled and saturated with 5% Na_2CO_3 solution using a dropping bottle. A blue color, directly proportional to the pesticide residues transferred from the leaf to the paper develops immediately at the solvent front as a band 1 to 2 mm wide. As this color fades with time, it is necessary to compare the developed color to a series of standards within approximately 2 min. for accurate residue estimation. At present the amount of colored product on the paper is measured by placing known amounts of the chemical being measured on paper strips treated with NBP, developing the strips, and measuring the intensity of the colored band with a portable, battery-operated reflectometer (Fig. 2). The resulting calibration curve is used to determine the amount of OP compound removed from a leaf. We are currently developing a method for routine use in citrus groves in which the paper strip, after development of the color, is compared to a color chart prepared with stable colored materials to measure the concentration of OP compound.

DISCUSSION

No interferences resulting from the dust, plant pigments, and waxes removed from orange leaves have been encountered with the acetone-water solvent system used to separate the OP residues from the extraneous residues removed from the leaf. Work is in progress to develop a portable field kit which will be suitable for rapid measurement of dislodgable OP pesticide residues on citrus foliage, and also to develop rapid methods for sampling of residues on fruit and in soil, since these may also be sources for pesticide absorption by workers.

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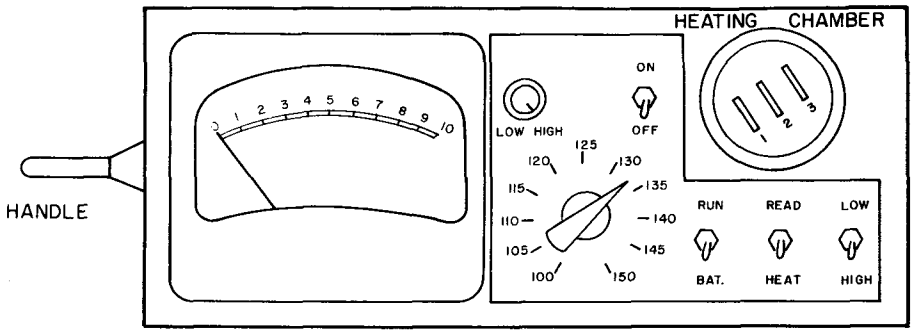
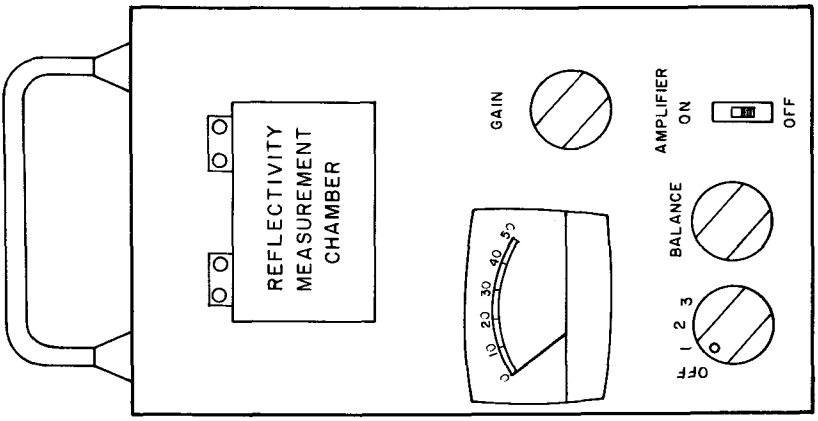


(a) OVEN



(b) REFLECTOMETER

Fig.2

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