A Technique for Determining the Deposition of Heavy Metals in Pesticides

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Journal Series Paper 7058 of the North Carolina Agricultural Research Service, Raleigh.

This study was supported by CSRS Grant 701-15-56.

Accepted for publication 22 January 1985.

ABSTRACT

Travis, J. W., Sutton, T. B., and Skroch, W. A. 1985. A technique for determining the deposition of heavy metals in pesticides and foliar nutrient materials on apple leaves. Phytopathology 75: 783-785.

A technique is described for determining the deposition of pesticides and foliar nutrient materials on apple leaves. The technique is based on the application of a pesticide or foliar nutrient materials that contain heavy metals and the mineral analysis of the metal deposit by atomic absorption spectroscopy. The pesticide was metiram 80W (zinc, 14%) and the foliar nutrient materials were Sequestrene-formulated micronutrients (copper, 13.0%; iron, 10.0%; manganese, 12.0%; zinc, 14.2%). The amount of the compound deposited was calculated in micrograms (active ingredient) per square centimeter of leaf from the percent metal content of the compound and a leaf-dry-weight to surface-area regression equation. By using the technique, laboratory analyses of the deposit agreed closely with calculated deposit levels. The usefulness of the technique in studying pesticide deposition in apple trees depends upon the permanence of the tracer compound and upon the ease and reliability of deposition analysis.

Disease and insect control is among the most costly components of apple production expense in the United States (11). Because of the large number of pesticide applications made each season and the rising cost of equipment, labor, and materials, and because of environmental impact concerns, each application must be made as efficiently as possible. The distribution of pesticides deposited by air-blast sprayers within the tree canopy is variable (2,8,10,11). To study factors affecting deposition and to develop improved application techniques, it is necessary to determine the deposit dose and distribution on the leaves and fruit. Pesticide deposits can be determined by several methods. Gas chromatography is useful for measuring the amount of pesticide deposited initially and its degradation products over time (3,8). Cost and time required for analysis restrict the number of samples that can be analyzed with this procedure, and there are problems with extraction and volatilization of the pesticide (15). Colorimetry and fluorometry are also commonly used in pesticide analyses, but these methods have shortcomings (4–7,9,10,12–14,16).

This paper describes a rapid tracing technique based on the determination of heavy metal deposits by mineral analysis (1) for quantitatively measuring pesticide and nutrient deposits on apple leaves.

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MATERIALS AND METHODS

Analytical procedures. The pesticide and foliar nutrient compounds employed in this study all contained heavy metals. The pesticide was metiram 80W (zinc, 14%), and the foliar micronutrients were Sequestrene Copper (copper, 13.0%), Sequestrene 330 Fe (iron, 10.0%), Sequestrene Manganese (manganese, 12.0%), and Sequestrene Zinc (zinc, 14.2%). The mineral content of each of the micronutrient formulations was guaranteed by the supplier (Ciba-Geigy Corporation, Greensboro, NC).

Validation of the procedure. Standard solutions of several concentrations of each metal were analyzed and the results were compared to calculated estimates of the concentration.

Standard concentrations of 1,000 μg of metal per milliliter were pipetted in 0.1- and 0.2-ml volumes on five apples leaves with surface areas ranging from 20 to 30 cm^2. After the deposit had dried, leaves were analyzed individually as previously described. In addition, 10 groups of three leaves each were dipped into the same standard concentrations to determine deposition levels under "runoff" conditions. After the leaves were dipped they were tied to a nylon cord, suspended in a vertical position, and allowed to dry. Deposition of the metal contained in metiram or the foliar nutrient formulations applied to leaves was determined by using standard procedures (1) for foliar mineral analysis. Leaf samples were prepared for analysis by drying at 75 C for 48 hr and determining the dry weight. Dried leaves were ashed at 500 C for 8–12 hr, dissolved in HCl, dehydrated, and the residue was diluted to volume with water according to a standard procedure (1). The weight of metal in micrograms per gram of leaf tissue in each sample was determined on an atomic absorption spectrophotometer (model 306; Perkin-Elmer). Apple leaves obtained from the National Bureau of Standards (Office of Standard Reference Materials, Washington, DC), were analyzed to validate the laboratory procedure. The National Bureau of Standards provided information on the mineral content of the leaves along with an acceptable error range for each element being evaluated. If mineral levels obtained after laboratory analysis are within the acceptable error range, the laboratory procedure is considered acceptable. The "standard" apple leaves were analyzed routinely with the test leaves. This check on the procedure was used repeatedly to verify the accuracy of the mineral analysis technique being employed. When multiple compounds and therefore metals, were applied to the same leaves, the samples were analyzed by using the atomic absorption spectrophotometer with a different cathode lamp and wavelength setting for each metal. Calculation of the deposit of the compound in micrograms (active ingredient) per square centimeter of leaf surface was based on micrograms of the metal per gram of leaf tissue, dilution factors, and a leaf dry weight to surface area regression equation. The regression equation was derived from the surface area and dry weight of 100 three-leaf samples of Golden Delicious apple leaves. The leaves were collected from one orchard and ranged in age from young fully expanded leaves to mature leaves. Senescent leaves were not included. Leaf area was measured on one surface of the leaf. Leaf area of each sample was measured on an area meter (model LI-3000; Lamba Instruments Corp.) and the dry weight of each sample was determined after drying the leaves at 75 C. Leaf surface area was regressed on leaf dry weight, and the regression equation and the coefficient of determination were calculated.

TABLE 1. Micronutrient content of untreated Golden Delicious apple leaves

Mineral	Mean[a] (μg/g)	SD[b]	(μg/cm^2)[c]
Zinc	26	4	0.31
Manganese	40	4	0.42
Copper	35	3	0.48
Iron	108	13	1.29

[a]Based on 100 three-leaf samples.
[b]SD = standard deviation.
[c]These values represent the probable mean contribution of the background micronutrients in the apple leaves to deposition levels.

TABLE 2. Comparison of calculated and measured values for heavy metal contents of standard solutions of compounds used in spray material deposition tests

Compound and metal	Calculated value of standard (μg/g)[a]	Measured value of standard (μg/g) Mean[a,b]	SD
Metiram	0.70	0.70	0.0084
Zinc	0.14	0.15	0.0055
Sequestrene[c]			
Zinc	1.42	1.42	0.0114
	0.70	0.71	0.0084
	0.14	0.14	0.0045
Manganese	1.20	1.21	0.0130
	0.60	0.60	0.0055
	0.12	0.12	0.0084
Copper	1.30	1.31	0.0114
	0.65	0.65	0.0071
	0.13	0.13	0.0055
Iron	1.00	1.00	0.0114
	0.50	0.50	0.0084
	0.10	0.10	0.0071

[a]There was no difference between calculated and measured values of standard solutions ($P = 0.05$) according to Student's t-test.
[b]Averages based on measurements of 10 replicates.
[c]Sequestrene is the trademarked name of a chelating compound manufactured by Ciba-Geigy Corporation, Greensboro, NC.

RESULTS AND DISCUSSION

Analytical results. Untreated Golden Delicious apple leaves were analyzed for background levels of iron, zinc, manganese, and copper (Table 1). The untreated leaves were collected from the same orchard and were of the same age as the treated leaves. Except for iron, the mineral levels were low and did not interfere with deposition determinations. These background levels were insignificant compared to original application concentrations (1,000 μg/ml) and mean deposition levels (17). However, the background level of iron in apple leaves contributed noticeably to higher mineral levels (Table 1). When Sequestrene 330 Fe was applied, the mean background level of iron was subtracted from deposited iron values before calculation of the deposition level. After this adjustment, the maximum possible error of deposition due to leaf iron micronutrient content was 0.4 μg/cm^2 of leaf surface for any sample within three standard deviations of the mean. If micronutrient levels of iron are above 200 μg/g of apple leaf tissue or if deposition levels on the leaves are low (below 5 μg/cm^2) then materials containing iron cannot be used to determine deposit. This would also apply for any other heavy metal present in the leaf.

A wide range in leaf surface areas (84.21 ± 21.04 cm^2) and leaf dry weights (0.924 ± 0.29 g) occurred in the 100 three-leaf samples. Leaf surface area (in square centimeters) was related to leaf dry weight (wt) (in grams) by the equation: $\text{Area} = 102.7\ \text{wt} - 19.12\ \text{wt}^2$. The correlation of leaf sample dry weight to leaf surface area was good ($R^2 = 0.82$; $P = 0.01$) and the regression equation was a reliable estimator of leaf surface area.

Validation of the procedure. Standard solutions measured by atomic absorption spectroscopy agreed closely with calculations based on the percent metal content of the compounds (Table 2). In

TABLE 3. Comparison of calculated and measured deposits of known values of standard concentrations of heavy metals in a pesticide and in micronutrient solutions placed on apple leaves[a,b,c]

Volume (ml) of standard applied per leaf	Mean deposits (μg/cm^2) from: Sequestrene[d] Zinc Calc	Zinc Measured	Manganese Calc	Manganese Measured	Copper Calc	Copper Measured	Iron Calc	Iron Measured	Metiram Zinc Calc	Metiram Zinc Measured
0.1	1.93	1.86	1.68	1.70	2.00	1.92	1.72	1.78	2.32	2.34
0.2	4.10	4.10	2.00	1.92	4.22	4.24	3.50	3.62	4.58	4.60

[a]Five replications of one-leaf samples.
[b]There were no significant differences in calculated or measured deposits ($P = 0.05$) within each volume of the standard according to Student's t-test.
[c]All metal deposit values were multiplied by a constant to convert them to micrograms of metiram per square centimeter of leaf.
[d]Sequestrene is the trademarked name for a chelating compound manufactured by Ciba-Geigy Corporation, Greensboro, NC.

TABLE 4. Deposit of heavy metal tracers in a pesticide and in Sequestrene[a]-formulated micronutrient solutions placed on apple leaves under simulated runoff application

Compound and metal	(μg a.i./cm^2)[b]	
	Mean	SD
Metiram		
Zinc	31.8	5.0
Sequestrene		
Zinc	27.5	3.4
Manganese	26.5	4.5
Copper	29.5	5.3
Iron	298.8	3.0

[a] Sequestrene is the trademarked name for a chelating compound manufactured by Ciba-Geigy Corporation, Greensboro, NC.

[b] Means and SD determined from 10 replicates of three leaves each. Leaves were dipped into concentrations equivalent to 1,680 μg a.i. of metiram per milliliter and measurements of heavy metal deposits were multiplied by a constant value to convert them to micrograms of metiram per square centimeter.

all cases, calculated and measured values were not signficantly different. Metal chelates were not cross contaminated with the other metals.

When known concentrations of the metals were deposited on the apple leaves in 0.1 and 0.2 ml of water, there was no difference between the measured determinations of mean deposit on one-leaf replicates (Table 3). Measured deposition was also not different from the calculated estimates of deposition.

When leaves were dipped in solutions with concentrations of compounds equivalent to 1,680 μg of metiram per milliliter, the mean deposit of zinc ranged from 26.5 to 31.8 (μg/cm^2) and the standard deviation from 3.0 to 5.3 μg/cm^2 (Table 4). All values were multiplied by a constant to relate to the active ingredient metiram 80W. Under uniform conditions of application, the apple leaves did not retain the metals equally. Some observed factors that influenced retention were leaf curling, leaf hair density, and leaf shape.

The technique described here is a rapid method to quantitatively determine heavy metal pesticide or foliar nutrient deposition on apple leaves. Any heavy metal that is a normal constituent of a pesticide or a micronutrient can be used to determine deposit. The micronutrient materials have the advantage of being highly soluble in water and can be applied with orchard airblast sprayers. With this technique, several heavy metals can be applied to the same tree at different times. Thus, differences between application treatments can be observed on the same tree.

Although this technique was developed to study pesticide deposition on apple foliage, it could be used on any plant to trace pesticide, growth regulator, micronutrient or herbicide deposition, if a metal is contained either in the material being studied or is applied along with the material of interest.

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