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Comparison of Sampling Methods for Determination of Pesticide Residue on Leaf Surfaces

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Toxic materials present on plant surfaces routinely come under scrutiny for hazard assessment. For pesticides, loosely bound residues represent a source of exposure which may be toxic to workers (Gunther 1973). Methods have been developed to measure the amount of dislodgeable pesticide residue on leaf surfaces in order to estimate the potential amount of pesticide that could be transferred from leaf surfaces to workers (Iwata et al. 1977). Measurement of dislodgeable residues may be affected by a number of factors which include: the design used to sample the plant canopy; the chemical analytical method; and the method used to obtain samples from individual leaves. Suggestions have been made to use standardized techniques in each of these areas (Iwata et al. 1977). The plant canopy sampling and chemical methodology suggestions appear reasonable with respect to minimizing inherent sources of variation; sampling designs appear sufficient with respect to obtaining representative samples from a plant canopy and adoption of an effective standard chemical method minimizes potential biases in results between methods.

With respect to obtaining samples from a leaf, a specialized device called a leaf punch has been developed. This tool produces circular leaf punches that are collected in a glass jar which are then used for dislodgeable residue determination. A number of reports are available on the use of this method (Gunther et al. 1973; Iwata et al. 1977; Popendorf 1980 1985). Other methods have also been used to obtain samples. Whole leaf samples have been advocated as an appropriate sampling unit (Ware et al. 1975; Sava 1986). Also, in work with radioactive materials, a surface-wipe method has been used to assess surface contamination (Royster and Fish 1964).

Since there has been no systematic comparison of the results between leaf sampling methods, a study was conducted to determine the importance of residue sampling method.

MATERIALS AND METHODS

A 3x3x3 factorial design was used to compare the results from three

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leaf sampling methods for determining foliar pesticide residue. The sampling methods that were compared were: 1) leaf-punch where one punch was taken per leaf; 2) whole-leaf where the entire leaf was collected; and 3) surface-wipe where the entire leaf surface area was wiped with moistened cotton gauze. Samples were taken by three individuals (operators). The study was replicated three times, and provided three main factors of method, operator and trial. Within each trial, each operator obtained triplicate samples which provided a measure of sampling error. Each triplicate sample was a composite from 20 leaflets representing each of 20 plants.

The strawberry plant (Fragaria vesca L. 'Douglas') was selected as the experimental plant because three or more trifoliate leaves with 3 leaflets apiece are produced per plant. This allowed all three operators to sample the same leaf. Plants were grown outdoors in a soil medium consisting of two parts Pro-Mix®, a commercial soil-mix, and one part sand and under a shade screen to prevent heat stress. Sand was added to the mixture to enhance drainage.

For each trial, eighty plants were randomly selected and transported to greenhouse facilities of the Botany Department at the University of California, Davis. Plants that had fewer than three trifoliate leaves were rejected. Dead matter was removed from plants to eliminate any possible interference with the spray reaching the target leaf area.

Separate pesticide applications were made to different groups of 80 plants on August 5, 12, and 19, 1988. Spray applications were made using a linear spray chamber manufactured by O'Brien Industrial Equipment Company, Inc., San Francisco, California. The spray nozzle located inside the chamber was adjusted so that the height was 20 inches above the plant canopy. Captan in a wettable powder formulation (WP-50, 47% a.i.) was applied at a rate comparable to 2.5 lb a.i. per acre mixed in 200 gallons of water. The machine was calibrated to deliver the solution using a 6506 nozzle by adjusting the travel speed and pneumatic pressure. Plants were sprayed in groups of ten and eight sprays were made per trial. The application was made by a single pass of the spray nozzle across the spray chamber. After treatment, plants were held in a greenhouse and sampled 72 hr later to allow surfaces to dry. Plants were watered in irrigation trays during this drying period.

Three methods were used to sample strawberry leaves: 1) leaf-punch (Iwata et al. 1977) 2) whole-leaf (leaflet) collection (Sava 1986); and 3) leaf surface-wipe. For the leaf-punch method, a circular sample of tissue was punched from the central portion of the leaflet using a leaf punch, manufactured by Birkestrand Co., South El Monte, CA, outfitted with a 2.52 cm diameter die. The discs were collected in 0.12-L glass jars attached directly to the leaf punch. For the whole-leaf method, intact leaflets were collected. For the surface-wipe method, a 9 x 9 cm gauze pad, which served as a wiping base, was placed on an aluminum foil-covered table. The leaflet was placed on the wiping base and cotton gauze moistened with deionized water was passed 3 times over each side of the leaflet. Upon

completion, the moist gauze and wiping-base were collected in a glass jar. In addition, the wiped-leaflets from the surface-wipe method were also collected and analyzed for dislodgeable residues. Whole leaflets and gauze pads were collected in 0.95-L glass jars. The surface area of all leaf samples was measured after chemical analyses with a leaf surface area meter (Model LI-3100, Licor, Inc., Lincoln, NE).

A set of twenty plants, comprising the first subsample within a trial, was randomly selected from the sprayed plants. The first operator randomly removed a trifoliate leaf with petiole from one plant and selected one leaflet for sampling. The leaf was passed on to the next two operators who also obtained samples from the remaining leaflets. Each operator used a different method of obtaining leaf samples and the order of sampling was leaf-punch, whole-leaf, and then the surface-wipe method. This procedure was repeated until a total of 20 leaves had been sampled (each sample was comprised of 20 punches, leaflets, or 21 gauze pads (20 wipes + 1 wiping base)). The operators then rotated to a different sampling method and collected the appropriate sample from the next 20 leaves. Samples were stored on ice and transported to the laboratory for analysis by the CDFA, Chemistry Laboratory Services where they were extracted within 24 hr.

Captan residues were dislodged from the surface of leaf-punches by adding 50 mL of distilled water and 3-4 drops of 2% Sur-Ten solution to each jar containing punches. After the jars were rotated for 20 minutes, the aqueous 'strip' was decanted into a 500-mL separatory funnel. This procedure was repeated two more times and the extracts were combined. Twenty-to-thirty grams of sodium chloride were dissolved in the aqueous 'strip' and then 50 mL of ethyl acetate was added. After one minute of shaking, the ethyl acetate was drained into a 100-mL graduated cylinder. This was repeated and the combined extracts were adjusted to a final volume of 100 mL with ethyl acetate. A few grams of anhydrous sodium sulfate were added to the cylinder to remove residual moisture. The method used to dislodge residues from whole-leaves was similar to that used for leaf-punches with the exception that 100 mL of distilled water and 6-8 drops of 2% Sur-Ten solution was used and the 0.95-L jars were agitated using a gyratory shaker. Jars containing surface-wipe gauze pads were shaken with 300 mL of ethyl acetate for 30 min.

Captan residues in extracts were analyzed with a Hewlett-Packard 5880 gas chromatograph equipped with a 12 m in length 5% phenyl methylsilicone capillary column and electron-capture detector with isothermal conditions at 200° C.

In order to determine differences in results between trials, a full factorial Analysis of Variance (ANOVA) was conducted with the third-order interaction term between sampling method, operator and trial used to estimate experimental error. Three samples for each method were obtained by each operator within each trial and the residual from the factorial analysis was considered an estimate of sampling variation. Differences between means were measured using pairwise

t-tests (SAS Institute 1988). The dependent variable was the captan residue determined from each triplicate composite sample divided by the leaf area ($\mu g/cm^2$). For the leaf-punch method, an area of 200 cm^2 was assumed, which was the calculated area of the die in the leaf punch.

RESULTS AND DISCUSSION

Three significant effects ($p<0.05$) were measured in the complete factorial ANOVA (Table 1). Mean squares for the main effects of method and trial and for the interaction between them was significant when tested against the mean square for the third-order interaction term. In order to avoid complications presented by the significant method by trial interaction, separate ANOVA's were obtained for each method which tested for significant operator and trial effects (Table 2).

Table 1. Full factorial ANOVA for the effects of leaf sampling methodology, operator, and trial on the determination of dislodgeable residues of captan.

Source of Variation	Degrees of Freedom	Mean Square
Sampling Method (M)	2	30.0117**
Operator (O)	2	0.2168
Trial (T)	2	3.8959*
M x O	4	1.2391
M x T	4	2.2319*
O x T	4	0.1049
Error (M x O x T)	8	0.5642
Sampling Error	54	0.2274

* Significant at the p=0.05 level
** Significant at the p=0.01 level

Table 2. ANOVA by sampling method testing for significant differences in determination of dislodgeable captan residues between operators and trials.

		Sampling Method		
Source of Variation	Degrees of Freedom	Leaf-punch Mean Square	Whole-leaf Mean Square	Surface-wipe Mean Square
Operator (O)	2	1.6389*	0.7558	0.3003
Trial (T)	2	7.8343**	0.2179	0.3074
Error (O x T)	4	0.1436	0.7416	0.3479
Sampling Error	18	0.2433	0.2923	0.1465

*Significant at the p=0.05 level
**Significant at the p=0.01 level

Since the entire leaf was sampled in the whole-leaf and surface-wipe methods, the low variance between trials indicated that the amount of captan deposited per unit area was similar between trials.

Apparently, the spray apparatus had good precision with respect to providing uniform coverage between trials.

Significant effects were measured only in the ANOVA for the leaf-punch method where differences were measured between operators and between trials (see mean values, Table 3). The variance associated with the trial term was greatest for the leaf-punch method and its mean square term was 36 times larger than the trial mean square term for the whole-leaf method and 25 times larger than the trial mean square term for the surface-wipe method. In contrast, no significant effects were measured in separate analyses for whole-leaf and surface-wipe methods with only a small difference in the trial mean square term between methods.

Table 3. Amount of captan measured as dislodgeable residue for each operator in each sampling method and at each trial.

Sampling Method and Operator	Trial Number 1	2	3	Mean
Leaf-punch	----------µg/cm^2----------			
Operator 1	5.84±0.03[b]	4.32±0.77	4.61±0.24	4.92
2	5.19±0.25	3.40±0.83	4.24±0.41	4.28
3	6.34±0.62	4.19±0.45	4.72±0.16	5.09
Mean	5.79	3.97	4.53	
Whole-leaf[a]				
Operator 1	4.39±0.35	4.65±0.41	5.31±0.85	4.78
2	5.85±0.50	5.05±0.77	4.79±0.16	5.23
3	4.79±0.33	4.47±0.67	4.81±0.45	4.69
Mean	5.01	4.72	4.97	
Surface-wipe				
1. Residue from Analysis of Gauze Pads[a]				
Operator 1	3.33±0.49	2.77±0.08	2.44±0.35	2.85
2	3.13±0.39	2.87±0.20	2.93±0.32	2.98
3	2.99±0.22	3.59±0.77	3.03±0.14	3.21
Mean	3.15	3.08	2.80	
2. Residue from Wiped Leaves				
Operator 1	1.67±0.29	1.63±0.19	1.63±0.31	1.65
2	1.74±0.17	1.65±0.20	1.99±0.21	1.79
3	1.41±0.14	1.84±0.29	1.81±0.32	1.69
Mean	1.61	1.71	1.81	

[a] Data used in the comparison of methods in the full factorial Analysis of Variance in Table 1.

[b] Mean value ± standard deviation determined from three replicate samples.

Significant effects of trial and operator, measured for the leaf-punch method, may have been caused by differences in the pattern of captan deposited on leaf surfaces between trials and to biases in sampling produced between individuals. Both of these effects could

result from sampling only a portion of the leaf. A sampling method that utilizes the entire leaf would produce a more stable estimate of dislodgeable pesticide residue because those results would, essentially, represent an average over the entire leaf area. On the other hand, the distribution of pesticide on the leaf surface could only be determined from subsampling of entire leaves.

The overall estimate of dislodgeable residue from the surface-wipe method was 61% of the estimate from the whole-leaf method (3.01 $\mu g/cm^2$ and 4.90 $\mu g/cm^2$, respectively). Comparison between these results gave a highly significant difference for the effect of method (Table 4, I). However, leaves that had been previously wiped with gauze pads were also analyzed for dislodgeable residue. Those results averaged 1.71 $\mu g/cm^2$ (Table 3). Analysis of Variance using the combined data as the estimate of dislodgeable residue for the surface-wipe method indicated no significant difference from the estimate produced with the whole-leaf method; means of 4.72 $\mu g/cm^2$ or the combined surface-wipe analysis and 4.90 $\mu g/cm^2$ for the whole-leaf method (Table 4, II).

Table 4. Full factorial ANOVA comparing estimates of dislodgeable residue between: I. the whole-leaf method and only gauze pad results from the surface-wipe method; and II. between the whole-leaf method and combined gauze pad and wiped-leaf results from the surface-wipe method.

Source of Variation	Degrees of Freedom	Mean Square
I. Whole-leaf vs Gauze Pad Results from Surface-Wipe Method		
Sampling Method (M)	1	48.2876**
Operator (O)	2	0.3756
Trial (T)	2	0.2158
M x O	2	0.6805
M x T	2	0.3095
O x T	4	0.2772
Error (M x O x T)	4	0.8124
Sampling Error	36	0.2194
II. Whole-leaf vs Combined Gauze Pad and Leaf Results		
Sampling Method (M)	1	0.4505
Operator (O)	2	0.5917
Trial (T)	2	0.0834
M x O	2	0.5483
M x T	2	0.2166
O x T	4	0.3761
Error (M x O x T)	4	1.1354
Sampling Error	36	0.2638

**Significant at p=0.01

The ratio of residue captured by the gauze to the combined amount recovered was remarkably similar between operators when averaged

across trials: means ± standard deviation of 63.2±5.3% for operator 1, 62.4±3.3% for operator 2, and 65.5±4.2% for operator 3. If such consistency can be documented for other pesticides, the surface-wipe method may prove useful in determining the proportion of surface residue that may be transferable.

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