COMPARISON OF SAMPLING METHODS FOR DETERMINATION OF PESTICIDE RESIDUE ON LEAF SURFACES

by

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INTRODUCTION

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 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; and Popendorf, 1980 and 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

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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

Experimental Design

A 3x3x3 factorial design was used to compare the results from three leaf sampling methods for determining foliar pesticide residue. Samples were taken by three individuals (operators) and the study was replicated three times, providing 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 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. Dislodgeable analyses also were conducted on the previously wiped leaves.

Plant Material

The strawberry plant (Fragaria vesca L. 'Douglas') was selected as the experimental plant for two reasons. Captan use on strawberries has come under intense investigation by the California Department of Food and Agriculture (CDFA) in regard to the safety of strawberry pickers, making this an important combination of crop and pesticide. Also, 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 transferred from flats into 4-inch pots and grown in a soil medium consisting of two parts Pro-Mix®, a commercial soil-mix, and one part

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sand.   Sand was added to the mixture to enhance drainage.   Plants were grown outdoors under a shade screen to prevent heat stress.

Application of Captan

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.

Pesticide applications were made 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 a 200 gallon solution.   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 hours later, allowing surfaces to dry. Plants were watered in irrigation trays during this drying period.

Sampling Methods

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. So., El Monte, CA, outfitted with a 2.52 cm diameter die. The discs were collected in 0.12 liter glass jars attached directly to the leaf punch. For the whole-leaf method, intact leaflets were collected. For the surface-wipe method, a 9 cm 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 liter 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, Nebraska).

A set of twenty plants, comprising the first subsample, was randomly selected from the sprayed plants. The first operator 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 hours.

Chemical Analyses

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 liter jars were agitated using a gyratory shaker. Jars containing surface-wipe gauze pads were shaken with 300 ml ethyl acetate for 30 minutes.

Captan residues in extracts were analyzed with a Hewlett-Packard 5880 gas chromatograph using a 12M % phenyl methylsilicone capillary column and electron capture detector with isothermal conditions at 200° C (see Appendix I for complete details).

Data Analysis

Data for each replicate sample obtained within each trial are given in Appendix II. The 3x3x3 design consisted of three main effects, namely method, operator, and trial. In order to determine differences in results between trials, a full factorial Analysis of Variance (ANOVA) was conducted with the third-order interaction term 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).

In the primary analysis, the dependent variable was the captan residue determined from each triplicate sample divided by the leaf area ($\mu g/cm^2$). For whole-leaf and surface-wipe methods, leaf areas were measured after residues were dislodged for chemical analysis. 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. Actual areas of the leaf-punch samples were also measured after extraction of residue from the leaf surface. Results based on the actual measurements were compared to those derived from the calculated area of 200 cm^2.

Additional tests were conducted to examine the relationship between the whole-leaf and surface-wipe methods: results from the whole-leaf method were compared to the combined results from the surface-wipe method for gauze pads and previously wiped-leaves.

RESULTS AND DISCUSSION

Comparison of Sampling Methods

A complete factorial ANOVA indicated three significant ($p<.05$) effects (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).

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.

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.

Table 1. Full factorial ANOVA for the effects of leaf sampling methodology, operator, and trial on the determination of dislodgeable residues of Captan from the leaves of strawberry plants.

Source of Variation	Degrees of Freedom	Mean Square	F value	p>F
Sampling Method (M)	2	30.0117	53.20	0.0001
Operator (O)	2	0.2168	0.38	0.6929
Trial (T)	2	3.8959	6.91	0.0181
M x O	4	1.2391	2.20	0.1596
M x T	4	2.2319	3.96	0.0465
O x T	4	0.1049	0.19	0.9392
Error (M x O x T)	8	0.5642		
Sampling Error	54	0.2274		

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

Source of Variation	D.F.[b]	Sampling Method							
		Leaf-punch				Whole-leaf[a]		Surface-wipe[a]	
		Calculated Area[a]		Measured area					
		Mean Square	p>F	Mean Square	p>F	Mean Square	p>F	Mean Square	p>F
Operator (O)	2	1.6389	0.022	1.8086	0.018	0.7558	0.44	0.3003	0.49
Trial (T)	2	7.8343	0.001	7.1154	0.001	0.2179	0.76	0.3074	0.48
Error (O x T)	4	0.1436		0.1407		0.7416		0.3479	
Sampling Error	18	0.2433		0.3327		0.2923		0.1465	

[a] Methods compared in the full factorial Analysis of Variance in Table 1.

[b] Degrees of Freedom.

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
	$\mu g/cm^2$			
Leaf-punch				
1. Based on Calculated Surface Area[a]				
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	
2. Based on Measured Surface Area				
Operator 1	6.37 ± 0.16	4.94 ± 0.69	5.30 ± 0.30	5.54
2	5.71 ± 0.33	3.93 ± 1.03	4.88 ± 0.47	4.84
3	6.85 ± 0.91	4.77 ± 0.44	5.42 ± 0.10	5.68
Mean	6.31	4.55	5.20	
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 deposition on leaves 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.

Leaf-Punch Method: Calculated vs Measured Leaf Surface Area

Surface area measurements used in the calculation of dislodgeable residue for the leaf-punch method are usually based on the diameter of the die in the leaf punch. The leaf punch used in this study produced a circular disk with a surface area of 5 cm^2/side giving a total area for top and bottom leaf surfaces of 10 cm^2. Twenty leaflets were punched per sample, so a surface area of 200 cm^2 was expected per sample. In practice, a leaf may not be fully inserted into the punch, resulting in less than the expected area or it may become folded-over onto itself when inserted into the punch, resulting in a larger than expected sample.

For completeness, all leaf punch samples were saved after dislodgeable residue extraction and surface area measured with a surface area meter. The overall average of the measured surface area for a 20 punch sample was

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177.4 cm^2, which was 88.7% of the expected area of 200 cm^2. A comparison between estimates of dislodgeable residue based either on calculated or measured surface area indicated higher values for data based on measured surface area because of consistently lower surface area measurements for each sample (Table 3). However, conclusions for the separate ANOVA of the leaf-punch method were the same whether data were based on measured surface area or on calculated surface area: variation between trials was still large with significant effects of operator and trial (Tables 2 and 3). Measurements of the actual sampled surface area affected the magnitude of the dislodgeable estimate but it had no affect on variation produced by the leaf-punch method.

Comparison of Whole-Leaf and Surface-Wipe Methods

The overall estimate of dislodgeable residue from the surface-wipe method was 39% lower than the estimate from the whole-leaf method (3.01 µg/cm^2 and 4.90 µg/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 µg/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 µg/cm^2 for the combined surface-wipe analysis and 4.90 µg/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	F value	p>F
I. Whole-leaf vs Gauze Pad Results from Surface-Wipe Method				
Sampling Method (M)	1	48.2876	59.44	0.0015
Operator (O)	2	0.3756	0.46	0.6597
Trial (T)	2	0.2158	0.27	0.7792
M x O	2	0.6805	0.84	0.4968
M x T	2	0.3095	0.38	0.7056
O x T	4	0.2772	0.34	0.8388
Error (M x O x T)	4	0.8124		
Sampling Error	36	0.2194		
II. Whole-leaf vs Combined Gauze Pad and Leaf Results from Surface-Wipe Method				
Sampling Method (M)	1	0.4505	0.40	0.5630
Operator (O)	2	0.5917	0.52	0.6293
Trial (T)	2	0.0834	0.07	0.9304
M x O	2	0.5483	0.48	0.6489
M x T	2	0.2166	0.19	0.8334
O x T	4	0.3761	0.33	0.8451
Error (M x O x T)	4	1.1354		
Sampling Error	36	0.2638		

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.

Comparison of Leaf Area Between Trials.

Additional ANOVA's were conducted to determine if sampled leaf area differed between trials. The results indicated a significant difference in sampled leaf area between trials for each method (Table 5). For the whole-leaf and surface-wipe methods, leaf surface area decreased from the first to the last trial. Changes in size may have reflected differences in the age of sampled leaves between trials. Owing to the consistency in results for the whole-leaf method, leaf area had little effect on the estimate of dislodgeable residue measured per unit area between trials. Comparison of measured leaf area for the leaf-punch method also indicated a significant difference in leaf area between trials; sample size in trial 1 was larger than in trials 2 and 3 (Table 5). As previously noted, results of the ANOVA were similar whether the dislodgeable estimate was based on calculated or measured surface area. Thus, differences between trials and operators for the leaf-punch method must have been attributed to sources of variation other than sample size.

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Table 5. ANOVA by sampling method testing for significant differences in sample size between operators and between trials.

Source of Variation	D.F.[a]	Sampling Method					
		Leaf-punch		Whole-leaf		Surface-wipe	
		Mean Square	p>F	Mean Square	p>F	Mean Square	p>F
Operator (O)	2	10.3321	0.11	2383.36	0.29	774.48	0.80
Trial (T)	2	270.7389	0.001	44641.42	0.004	50153.22	0.01
Error (O x T)	4	2.7044		1405.53		3344.42	
Sampling Error	18	22.1392		977.57		1591.31	
Mean Values (cm^2)							
Trial 1		183.72		611.33		610.27	
2		174.34		533.08		522.50	
3		174.11		470.77		461.78	
LSD for p<0.05[b]		2.15		49.07		75.69	

[a] Degrees of Freedom.

[b] Least significant difference to determine a difference between 2 means.

CONCLUSIONS

1. Results for the leaf-punch method were more variable across operators and across trials than for whole-leaf or surface-wipe sampling methods. Since samples for the leaf-punch method were obtained from only a portion of the leaf, those results could reflect the pattern of captan residue on leaves which would provide an additional source of variation not detected in whole leaf sampling.

2. Results from a method where the surface of leaves were wiped with a gauze moistened with water (surface-wipe) were lower than from whole-leaf collections indicating that only a portion of the total residue was sampled. However, when remaining dislodgeable residue from the wiped-leaves was included in the comparison, the difference between methods was insignificant. The portion of total residue captured by gauze pads alone was remarkably similar between operators (approximately 63% of total residue) indicating possible use of this method in determining amount of residue readily transferred to workers. Surface-wipe sampling may be advantageous in situations where chemical stability during storage is questionable because samples could be immediately frozen or chemically preserved at the time of collection.

3. Consistent results between trials for methods that obtained samples from the entire leaf indicated that application rates of captan were relatively uniform between trials. Differences between trials with the leaf-punch method could have reflected changes in the pattern of deposition which could have been due to different drying conditions or,

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perhaps, different leaf surface characteristics between trials. A significant difference in leaf area was measured between trials indicating potential differences in leaf characteristics.

4. Other issues not addressed in this study may also need further consideration. In cases where leaves are much larger, the issue of obtaining a representative sample of leaves without obtaining a sample that is physically too large to extract efficiently can become a problem. Also, sample storage and transportation of large whole-leaf samples may be more easily handled using leaf-punch or surface-wipe samples. These concerns may play a role in the eventual selection of an appropriate sampling methodology.

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APPENDIX I

Chemical Analyses

CALIFORNIA DEPT. OF FOOD & AGRIC.   Original Date:1/12/89
CHEMISTRY LABORATORY SERVICES   Supercedes: NEW
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Captan Analysis for Sampling Methods Evaluation

SCOPE:
        A determination of captan residues that are dislodgeable from leaf
punches and whole leaves and extractable from leaf wipes.

PRINCIPLE:
        Captan residues are dislodged from leaf surfaces by shaking in water
containing a few drops of 2% Sur-Ten solution and then partitioned into ethyl
acetate. The captan residues on leaf wipes are extracted directly with ethyl
acetate. The extracts are analyzed by gas chromatography.

REAGENTS AND EQUIPMENT:
Captan analytical standard
Distilled water
Ethyl acetate, nanograde
NaCl
Na_2SO_4, anhydrous
Sur-Ten solution, 2%
Graduated cylinders
Separatory funnels
Gyratory shaker
Rotators
Gas chromatograph equipped with an electron capture detector

ANALYSIS:
A. Extraction

    1. Leaf Punches
            Add 50 ml distilled water and 3-4 drops of 2% Sur-Ten solution
    to each sample jar containing the punches. Rotate the jars for 20 min
    and decant the aqueous "strip" into a 500-ml separatory funnel. Repeat
    the procedure two more times combining the extracts.
            Add 20-30 g NaCl to the aqueous "strip" in the funnel and shake
    to dissolve. Then add 50 ml ethyl acetate and shake for one minute.
    Drain the ethyl acetate layer into a 100-ml graduated cylinder. Return
    the aqueous solution to the funnel and repeat the extraction. Combine
    the extracts and adjust the volume to 100 ml with ethyl acetate. Add a
    few grams of anhydrous Na_2SO_4 to the cylinder to remove residual
    moisture.

I: Captan, 1/12/89, MdV

2. Whole Leaves

    The whole leaves contained in mason jars are shaken with 100 ml distilled water and 6-8 drops of 2% Sur-Ten solution. This procedure is done twice plus a final rinsing with 15-20 ml distilled water. The extracts are combined in a separatory funnel. Extraction with ethyl acetate follows the same procedure as that for leaf punches.

3. Leaf Wipes

    The wipes contained in mason jars are shaken with 300 ml ethyl acetate for 30 min.

B. Chromatography

    The ethyl acetate extracts are analyzed for captan in a Hewlett-Packard 5880 gas chromatograph using a 12M 5% phenyl methylsilicone capillary column and an electron capture detector. Isothermal conditions at $200^{\circ}C$ are used.

CALCULATIONS

    The amounts of captan present in the samples are determined from a linear regression curve of peak height vs. concentration derived from captan analytical standards.

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APPENDIX II

Raw data

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TRIAL 1

SAMPLING METHOD	OPERATOR	R	CAPTAN RECOVERED (ug)	MEASURED LEAF AREA (sq cm)
LEAF-PUNCH *	1	1	1170.73	178.74
		2	1172.08	187.22
		3	1159.75	184.50
	2	1	1095.18	180.76
		2	997.75	184.52
		3	1022.23	180.12
	3	1	1158.17	189.84
		2	1245.70	189.20
		3	1402.85	178.58
WHOLE-LEAF	1	1	2347.50	548.94
		2	2512.91	611.10
		3	2921.86	610.98
	2	1	4143.05	645.02
		2	3315.83	591.28
		3	3535.56	639.50
	3	1	2797.67	629.00
		2	2995.63	587.44
		3	3085.20	638.70
SURFACE-WIPE GAUZE PADS	1	1	2369.90	617.46
		2	1829.13	638.12
		3	2273.32	690.58

*
    CALCULATED LEAF AREA FOR THE LEAF-PUNCH METHOD WAS 200 sq cm.

TRIAL 1

SAMPLING METHOD	OPERATOR	R	CAPTAN RECOVERED (ug)	MEASURED LEAF AREA (sq cm)
GAUZE PADS	2	1	1885.55	595.44
		2	2078.87	593.88
		3	1742.52	641.16
	3	1	1509.08	506.54
		2	1920.30	596.26
		3	1705.44	613.00
WIPED-LEAVES	1	1	1048.86	617.46
		2	877.76	638.12
		3	1346.09	690.58
	2	1	942.74	595.44
		2	1019.17	593.88
		3	1225.73	641.16
	3	1	639.02	506.54
		2	853.84	596.26
		3	949.62	613.00

TRIAL 2

SAMPLING METHOD	OPERATOR	R	CAPTAN RECOVERED (ug)	MEASURED LEAF AREA (sq cm)
LEAF-PUNCH	1	1	1011.51	178.24
		2	704.65	163.76
		3	873.18	179.94
	2	1	825.91	169.08
		2	717.17	178.40
		3	499.30	174.20
	3	1	859.77	177.62
		2	739.04	171.62
		3	916.55	177.20
WHOLE-LEAF	1	1	2467.97	525.12
		2	2231.47	528.04
		3	2861.02	567.86
	2	1	3228.45	557.00
		2	2485.61	582.96
		3	2810.27	552.36
	3	1	2576.81	491.64
		2	2110.29	510.28
		3	1940.27	482.44
SURFACE-WIPE GAUZE PADS	1	1	1290.73	464.24
		2	1488.16	523.66
		3	1346.34	503.10

TRIAL 2

SAMPLING METHOD	OPERATOR	R	CAPTAN RECOVERED (ug)	MEASURED LEAF AREA (sq cm)
GAUZE PADS	2	1	1441.24	521.12
		2	1432.55	520.60
		3	1832.77	591.20
	3	1	2229.42	558.58
		2	1406.96	518.78
		3	2044.99	501.24
WIPED-LEAVES	1	1	825.47	464.24
		2	893.31	523.66
		3	713.63	503.10
	2	1	827.71	521.12
		2	771.50	520.60
		3	1106.94	591.20
	3	1	1207.45	558.58
		2	833.12	518.78
		3	885.01	501.24

TRIAL 3

SAMPLING METHOD	OPERATOR	R	CAPTAN RECOVERED (ug)	MEASURED LEAF AREA (sq cm)
LEAF-PUNCH	1	1	967.85	172.36
		2	872.31	173.60
		3	928.38	176.36
	2	1	932.76	175.44
		2	844.71	171.20
		3	768.29	175.18
	3	1	951.67	174.10
		2	910.36	171.62
		3	972.84	177.12
WHOLE-LEAF	1	1	2350.57	468.46
		2	2235.24	481.30
		3	3034.52	484.52
	2	1	2112.73	439.48
		2	2422.52	523.76
		3	2318.93	469.30
	3	1	2379.04	446.94
		2	2310.68	515.30
		3	1880.43	407.90
SURFACE-WIPE GAUZE PADS	1	1	1132.80	397.96
		2	1101.09	494.04
		3	946.71	421.32

TRIAL 3

SAMPLING METHOD	OPERATOR	R	CAPTAN RECOVERED (ug)	MEASURED LEAF AREA (sq cm)
GAUZE PADS	2	1	1416.90	491.10
		2	1188.68	451.50
		3	1543.35	472.06
	3	1	1272.66	436.02
		2	1704.68	533.40
		3	1368.37	458.60
WIPED-LEAVES	1	1	509.64	397.96
		2	931.65	494.04
		3	723.62	421.32
	2	1	955.23	491.10
		2	814.75	451.50
		3	1046.02	472.06
	3	1	875.57	436.02
		2	768.48	533.40
		3	907.17	458.60