INSECTICIDE RESIDUES ON PINE BARK: INFLUENCE OF TREES, SAMPLE VOLUME AND SIZE ON VARIABILITY[1]

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ABSTRACT

Residues of carbaryl (Sevimol®) in ponderosa pine bark were quantified by gas chromatography and the major sources of experimental error were determined. Quantification of carbaryl residues was significantly affected by between-tree variation and disc size (bark volume). Variation between trees had the greatest effect on residue estimates. Mean carbaryl residues for all trees ranged from 241 µg/g of bark to 1418 µg/g of bark. Nonsignificant tree-disc size interactions indicated that residue levels were fairly consistent within trees. Prediction curves were constructed to project levels of precision for combinations of discs and trees.

Key Words: Gas chromatography, variance-component model, prediction curves, bark beetle control, pesticide safety.

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INTRODUCTION

Quantification of insecticide residues in or on forest substrates is essential in assessing the efficacy and safety of a pesticide. To develop pest management strategies involving chemicals, managers should use data that are "residue-representative" (Gunther 1980). However, insecticide residue data are exceedingly variable. Application irregularities and factors that influence residue deposit, distribution or disappearance affect variability (Ebeling 1963). In addition, quantification of insecticide residues is influenced by inherent variability in plant structure, physiology and experimental procedures.

When using residue data in efficacy evaluations, precision and accuracy are two important concepts which must be considered. Precision refers to the variability of sample estimates from their mean, and accuracy refers to the variability of sample estimates from the population mean (Fowler 1979). Investigators should know how closely the insecticide concentrations they report in a sample represent the true concentration. Therefore, accuracy becomes the most important concept, and accuracy relies on a precise estimate of the true mean. Although investigators connot reduce the inherent variability within biological systems, they can increase the precision of the estimated parameter.

In earlier studies, Page (1983) and Page and Pieper (in preparation) described the residue decay curves of insecticides sprayed on bark of ponderosa pine, *Pinus ponderosa* Dougl. ex Laws. They attempted to reduce insecticide residue variation within and between trees, by repeated sampling of the same trees at multiple

[1] This publication reports research involving insecticides. It does not contain specific recommendations for their use, nor does it imply that uses described here have been registered. All use of insecticides must be registered by the appropriate state and/or federal agencies before they can be recommended.

heights over time. The estimates of insecticide deposits on bark were so variable that a four-fold difference in sample mean residues between the lowest and highest spray concentrations was not statistically significant.

This paper reports the results of a study designed to identify the major sources of variation in the quantification of insecticide residues on pine bark. The results provide researchers and managers a technique to allocate more effectively effort spent in gathering samples (i.e. more trees sampled or more samples per tree) and laboratory analyses, and to obtain the most precise estimate of insecticide residues with limited or fixed resources.

MATERIALS AND METHODS

Insecticide was applied and deposits were collected at the UDSA Forest Service's Institute of Forest Genetics, in Placerville, California. The site, at approximately 825 m in elevation, is an arboretum that is relatively flat, with evenly-spaced ponderosa pines of approximately 30 to 50 cm diameter-at-breast-height (DBH). Test trees were randomly selected from a pool of numbers corresponding to tree tag numbers.

Carbaryl (Sevimol®) spray (2% wt/wt in water) was applied to the boles of 10 trees with a Maruyama® power sprayer (model MS253-ECR) at a pressure of 30 kg/cm^2. The insecticide was applied to the entire bole of each tree to a height of *ca.* 4.0 m. The spray was applied to the point of runoff or *ca.* 1.0 liter/m^2. Trees were sprayed in the morning from 2 hr past sunrise to *ca.* 1130 am. Ambient air temperature never exceeded 25°C.

Bark samples were collected 1 day after insecticide application with either a hole punch (3.18 cm diameter) or a hole saw (5.7, 7.6 or 10.2 cm diameter). On each tree, eight bands or circumferences were marked at least 15 cm apart between 1.0 m above the ground up to 4.0 m. On each band five points were labeled at least 10 cm apart. Thus on each tree 10 replicates of each of four disc sizes were randomly assigned to these 40 points. Each bark sample included both bark and cambial tissue. All replicates of each disc size were pooled as one sample and placed in labelled, plastic bags, immediately frozen with dry ice, returned to the laboratory in Berkeley, California, then stored at -20°C.

Samples were processed for residue analysis within 30 days of collection, by modifiying the method of Page (1983) to include an aluminum oxide cleanup step. Each pooled sample was crushed in a steel blender and a bark subsample 2.5 g removed. The subsample was pulverized in ethyl acetate, centrifuged, and then poured into a 10-ml disposable glass pipette containing 3 g of aluminum oxide (Neutral 200). A 1-ml aliquot of the clear liquid was diluted as appropriate for analysis by gas-liquid chromatography.

Carbaryl residues were determined with a Hewlett-Packard model 5790A gas chromatograph equipped with a nitrogen-phosphorus detector. The glass column (61 cm long, 6.4 mm i.d.) was packed with 2% OV-101 on 100/120 mesh Chromsorb-WHP. GLC operating temperatures were: oven 160°C; injection port 235°C; detector 300°C. Gas flow rates were: helium 25 ml/min; hydrogen, 3 ml/min; air, 80 ml/min. Chromatograms were automatically quantified with a Hewlett-Packard 3390A integrator by using external standards injected prior to sample analysis.

To establish the efficiency of extraction, control bark (2.5 g) was fortified with 1500 µg/g of technical grade carbaryl. The acetone in which the carbaryl was

dissloved was allowed to evaporate and bark samples were processed as described earlier. Mean percent recovery was 92% ± 5%. In addition, four external standards, 0.1, 1.0, 10, 100 μg/ml, were injected into the gas chromatograph to determine the lower limit and linear range of detectability. The gas chromatograph was calibrated daily with carbaryl standard solutions. Each sample was injected three times and averaged to estimate residues. Standard solutions were used every third injection to monitor changes in detector response.

We used a randomized complete-block design with 10 blocks (trees), 4 treatments (bark disc size: 3.2, 5.7, 7.6, 10.2 cm diameter), and 10 replications. The response variable was μg carbaryl per g bark. Data were subjected to analysis of variance. Means of treatments with significant differences were separated at the alpha = 0.05 level by Tukey's procedure (Steel and Torrie 1960).

We partitioned the error according to the following mixed model:

$$Y_{ijk} = \mu + D_i + T_j + E_{ij} + S_{ijk}$$

where μ = the true population mean, D_i = disc size (fixed), T_j = tree or block (random), E_{ij} = tree-disc interaction, and S_{ijk} = sampling error. The error associated with the random effect (tree) was partitioned by Henderson's method (Searle 1971). This variance component was used to estimate sample size for future experiments.

RESULTS AND DISCUSSION

Residue analysis studies rarely include evaluation of the various components that may influence residue estimates. In this study, it is axiomatic that residues were variable. Carbaryl recoveries are listed in Table 1. The lower limit of detector response was 0.1 μg/ml with a linear range of 1000. The overall average carbaryl residue level for all disc sizes and all 10 trees was 639 ± 433 μg/g. Differences among disc size were the least variable component (Table 2, Fig. 1). Analysis of

Table 1. Carbaryl residues on pine bark (μg/g) for four sample sizes (volumes) on each of ten trees.*

	Size of discs (cm)				
Trees	3.18	5.22	7.62	10.16	Tree Mean†
1	756(452)	958(224)	756(522)	563(185)	763(390)b
2	683(246)	670(291)	764(276)	670(288)	697(268)bc
3	973(465)	1292(222)	1418(398)	1408(226)	1273(378)a
4	383(193)	511(185)	533(235)	523(143)	488(194)cd
5	560(339)	581(135)	664(254)	637(189)	611(236)bc
6	293(360)	241(240)	444(475)	317(209)	324(323)d
7	250(123)	307(130)	389(186)	320(249)	316(179)d
8	757(326)	670(470)	886(629)	756(740)	767(547)b
9	567(357)	596(285)	612(310)	610(371)	596(319)bc
10	400(480)	484(453)	596(596)	748(450)	557(473)bcd
Disc Mean†	562(402)b	631(401)ab	706(475)a	655(442)ab	

* Means and (standard deviations) of each tree by disc size combination.
† Means for disc size or trees, followed by the same letter, are not significantly different at the alpha = 0.05 level by Tukey's procedure.

our data by Tukey's LSD, however, showed that carbaryl residues were affected by disc size or bark volume. We assumed that increases in disc size would increase the precision (or reduce variation) in estimates of insecticide residues. The

Table 2. Analysis of variance of carbaryl residues on ponderosa pine bark.

Source	df	SS	MS	F†	EMS
Tree	9	26890064	2987785	24.69***	$\sigma^2 + 10\sigma^2_{TD} + 40\sigma^2_T$
Disc	3	1068825	356275	2.94*	$\sigma^2 + 10\sigma TD + Q$
T X D	27	2609582	96651	0.79	$\sigma^2 + 10\sigma^2 TD$
Error	360	44230192	122862		σ^2
Total	390	74798656			

† * indicates F value exceeds the tabular value for the 0.05 level; *** indicates F value exceeds the tabular value for the 0.005 level.

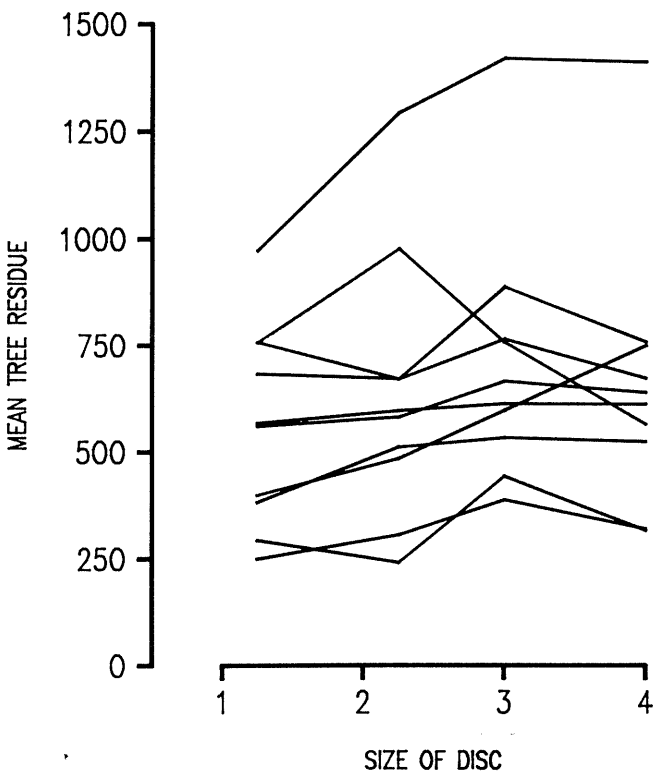

Fig. 1. Carbaryl residues (μg/g bark) for each of four disc sizes (volumes) from each of ten ponderosa pine trees sprayed with a 2.0% suspension of carbaryl. Each point represents the average residue from 10 bark discs.

differences between the 3.18-cm and 7.62-cm discs were statistically significant but not so for the 3.18-cm and 10.16-cm. These data do not clearly indicate that disc size increased precision.

Variation between trees had the greatest effect on residue estimates. Mean carbaryl residues for each tree ranged from 241 ± 240 μg/g to 1418 ± 398 μg/g (Table 1, Fig. 1). These differences between trees are probably due to variation in bark topography and bark thickness. More insecticide will adsorb to bark with greater surface area per cm^2 of bark (more flakes and convolutions). Also, thick bark should dilute insecticide concentration by virtue of its greater volume per cm^2 of bark surface. Variation in spray volume, in our opinion, was not a significant contributor to residue variation because trees were sprayed until to the point of runoff. Our analyses did not indicate statistically significant tree by disc-size interactions. Lack of statistical significance of this statistic indicates residue levels were fairly consistent within trees. Within-tree residues did not differ statistically by disc size.

The application of statistical methods to biological experimentation occasionally causes uneasiness for many biologists. This concern invariably results from suggested increase in the number of observations or increase in the size of a given sample. Biologists usually do not have *a priori* measurements of the variance of a population, thus experiments are often designed without adequate sampling to achieve the desired level of precision. For sampling protocols to provide the precision desired, the components of variation that affect the expression of the population mean should be examined.

We evaluated two components that contribute to experimental error and constructed prediction curves to project the level of precision ($\pm$ 2 standard errors) for combinations of these factors: number of discs and number of trees (Fig. 2). Our data were gathered from a combination of 10 discs/tree and 10 trees. If we had reduced the number of discs/tree to four, precision ($\pm$ 2 standard errors) would have decreased from $\pm$ 180 μg/g to $\pm$ 200 μg/g: a decrease of only 11% with a 60% decrease in the number of samples processed. However, the precision would have increased to $\pm$ 125 μg/g by reducing the disc/tree to two and increasing the number of trees to 40: an increase in the precision of 31% with a 20% decrease in the number of samples processed.

Between-tree variation was the largest component of experimental error. Researchers who conduct experiments to determine the levels of insecticide residues on ponderosa pine bark would be well advised to minimize the number of bark discs and maximize the number of trees sampled to increase the precision of the estimate of the population mean. In an earlier study (Page et al. 1985), we evaluated carbaryl dissipation on the bark of lodgepole pine in Colorado, 12 and 16 months after treatment, by sampling two discs per tree from 200 trees. This sampling regime provided a precise estimate of mean carbaryl residues in a population of approximately 10,000 trees.

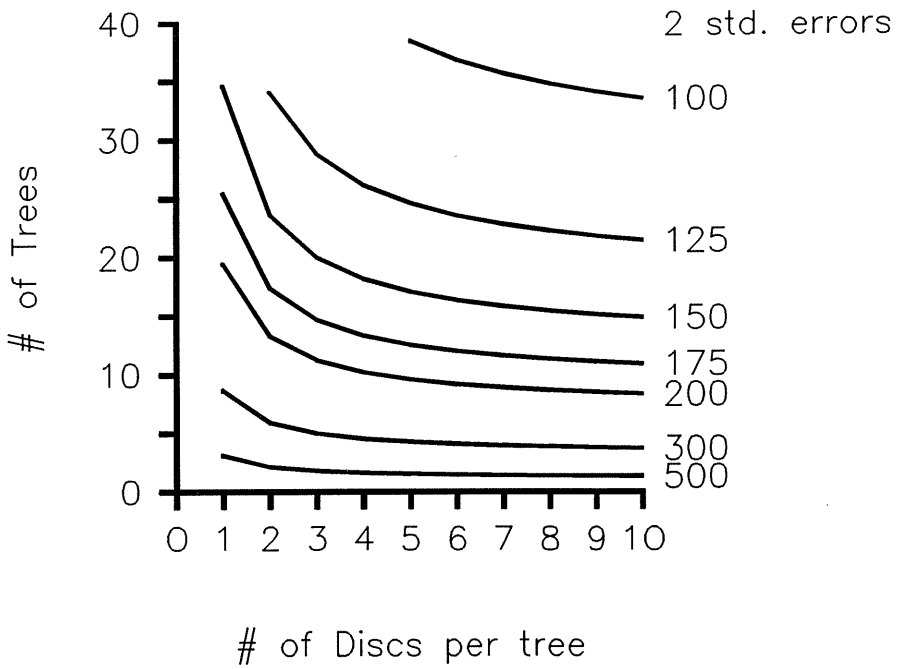

Fig. 2. Variation (2 standard errors in μg of carbaryl/g pine bark) associated with combinations of number of discs and trees sampled.

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