A METHOD OF SAMPLING POPULATIONS OF PSYLLIDS LIVING ON THE LEAVES OF *EUCALYPTUS* TREES

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Abstract: Samples of leaves can be taken at random from the crown of a eucalypt tree by selecting — on the unbiased decision of a tossed coin or rolled die — foliage units within the crown, and twigs within each foliage unit. This has proved to be a sensitive method of estimating the relative abundance of psyllids living on *Eucalyptus* leaves.

The work described here was done in an attempt to devise a method of sampling natural populations of the psyllid *Cardiaspina densitexta* Taylor on pink gum (*Eucalyptus fasciculosa* F.v.M.) in South Australia.

The eggs of *C. densitexta,* and of all psyllids living on *Eucalyptus* leaves, are fixed to the surface of the leaf by a stalk driven into the tissue of the leaf by the ovipositing female. The nymphs settle and start feeding very soon after hatching, and in the case of *C. densitexta* and other lerp-forming species, usually do not move from the 1st feeding site until they have attained the winged adult stage. Nymphs that die from "natural causes" leave their lerps at the site of feeding, those killed by parasitoids remain as mummies beneath their lerps, those killed by predatory syrphids usually remain as withered husks beneath their lerps, and predation by birds results in characteristic damage to the lerps. All this means that the abundance of these insects and the survival of their eggs and nymphs can best be estimated by collecting samples of leaves bearing these immature stages.

Anyone who has taken samples of leaves or twigs from a tree will know how difficult it is to avoid bias; yet, it is essential to have a method that will give an unbiased sample. Examination of any eucalypt reveals that it grows by repeated bifurcation, starting with the stem and continuing to the ultimate twigs. It seemed that this growth form might lend itself to a truly random method of selecting leaves, by simply proceeding up through a tree, leaving to chance the choice of which direction to take at each bifurcation, and taking as the sample the ultimate twig arrived at in this way.

PRELIMINARY TRIAL

MATERIALS AND METHODS

To test this idea, 1 pink gum tree was selected. This tree had a single trunk and a sym-metrical crown which, with the aid of a stepladder, was fully accessible. It was carrying a moderate population of *C. densitexta,* apparently evenly distributed throughout the available foliage. The crown was visually divided into 4 sectors (NE, SE, SW, and NW) and

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TABLE 1.   Distribution of twigs between the upper and lower portions
of the crown of a pink gum tree.

	Lower	Upper	Total
Dead	20	24	44
Live	30	26	56
Total	50	50	100

2 halves (upper and lower). A sample of 100 twigs was collected from this tree. A twig was defined as the tip of a branch beyond the last bifurcation. It might bear anywhere from 3 to 12 leaves (usually 7 to 10), or it might be dead.

To choose any twig a start was made from the trunk of the tree. At each bifurcation a coin was tossed to decide whether to proceed to the left (heads) or to the right (tails). This process was repeated at each bifurcation until the ultimate twig was reached.

All live twigs were labelled according to sector and half, placed in individual plastic bags, and taken to the laboratory where all psyllids were counted and the total area of leaf per twig estimated with an air-flow planimeter (Jenkins 1959). My unpublished data show a highly significant correlation (R=0.86, Standard Deviation=3.04) between the areas of leaves obtained with an airflow planimeter and with conventional planimeter measurement of their sun prints.

Results

The distribution of samples between the 4 sectors was not random, being 40 NE, 14 SE, 15 SW and 31 NW, respectively; and gave a count of 71 on the north face and 29 on the south face, a highly significant (χ^2=11.91, p<0.001) deviation from randomness. This was due to the fact that the major branches arising from the trunk carried an unequal proportion of the foliage that made up the crown, and the 1st decision that led to a particular twig was made at this 1st bifurcation. Unequal bifurcation higher up the tree would also be expected to contribute to this bias.

The distribution of samples between the upper and lower halves did not differ significantly from that expected by chance (TABLE 1).

The proportion of dead to live twigs in the upper and the lower halves of the crown was not significantly different (χ^2=0.18) (TABLE 1), but there was a significantly greater proportion of dead twigs on the north face than on the south face (χ^2=5.45, p<0.05) (TABLE 2).

TABLE 2.   Distribution of twigs between the north and south faces
of the crown of a pink gum tree

	North face	South face	Total
Dead	37	7	44
Live	34	22	56
Total	71	29	100

TABLE 3.   Mean log ($\times$ + 1) psyllid/twig $\pm$ SE.

| | HEIGHT | | |
ORIENTATION	Lower half	Upper half	Mean
NE	2.35 $\pm$ 0.24	2.20 $\pm$ 0.16	2.25 $\pm$ 0.13
SE	2.02 $\pm$ 0.26	0.71 $\pm$ 0.26	1.37 $\pm$ 0.18
SW	0.55 $\pm$ 0.22	0.70 $\pm$ 0.26	0.61 $\pm$ 0.17
NW	1.89 $\pm$ 0.17	2.32 $\pm$ 0.33	1.98 $\pm$ 0.15
Mean	1.71	1.62	

Analysis revealed that there was no significant difference between the total area of the leaves on twigs taken from different parts of the tree. Leaf area was abandoned from this stage onward, however, as, although the regression was significant, only 12% of the variation of the number of psyllids per twig was accounted for by variation in leaf area per twig. Regarding the time and effort involved in obtaining leaf-areas and the fact that differences between leaf-areas per twig are not significant, it was considered more profitable to increase the number of replicates in the sampling and simply use total number of animals per twig as the basic sample unit.

As TABLE 3 illustrates, there was no significant difference between the number of psyllids per twig in the upper or lower sections of the crown, but numbers per twig in the different sectors were quite distinct, there being many more animals in the northern sectors than the southern ones; and in the eastern sectors than the western ones.

The greatest number of psyllids per sector and the greatest number of dead twigs per sector show essentially the same distribution in the crown of the tree.

MODIFICATION OF THE SAMPLING

MATERIALS AND METHODS

As a result of this preliminary experiment, the following system of sampling was devised and used to investigate between-tree variation. In order to avoid the bias caused by the unequal contribution of the major branches to different parts of the crown, and because it was desired to make a comparison between north and south faces of the tree in all future sampling, the crown was arbitrarily divided into north and south halves and equal numbers of twigs collected from each half, no cognizance being taken of the height of the sample within the crown. Low-growing and sucker shoots were ignored.

Because of the uneven number of branches and distribution of foliage among them, all large structural limbs were ignored and only the foliage-bearing units (Jacobs 1955) used. These units are quite distinct and can either be deliberately selected (and a twig selected within each in turn) so as to spread the sampling evenly over the whole north or south face, or they can be visually divided into right and left groups and sampled randomly with the selection process continuing to the ultimate twig. Where any difference between foliage units can be observed this latter method of selecting units *must* be used, in order to avoid personal bias. In random selection 10 or 15 decisions were usually needed to obtain each

twig in a heavily foliaged tree, and as few as 3 to 5 where there had been much defoliation and death. (When sampling was being done to collect live psyllids all dead twigs were ignored.)

Instead of the slow and tedious process of tossing a coin or consulting a table of random numbers, a 12 mm cube of "Perspex" was made. Three "L"s and three "R"s were engraved on its 6 faces, and it was placed in a small screw-topped jar which fitted comfortably into the palm of the hand. A quick twist of the wrist violently rattled the die in the jar, but it settled almost immediately, allowing a quick reading of left or right. (Eleven hundred test throws of this die to check for bias gave the result 546 "L" and 554 "R".) The other hand was free to grasp each bifurcation in turn, where necessary twisting it so that one branch was clearly to the left and one to the right of the observer before taking a decision from the die. When sampling twigs that were out of reach, the jar was held in front of the face so that it could be glanced at without losing sight of the position reached in the canopy.

Individual trees were selected at random by taking a decision "left" from the die, marching x paces (depending on the density of the stand), taking another decision from the die, and selecting the nearest tree on the side indicated.

This sounds a tedious and protracted business, but in practice is quite fast; the speed with which the die can be rolled and read sets the pace, and all samples are truly random.

Five samples were taken in the autumn from the north face of each of 20 trees in a uniform stand of pink gums carrying a moderate to light population of psyllids. In the following spring—by which time numbers were low—a sample of 10 twigs from each of 5 trees was taken in this stand, and a 3rd sample of 5 twigs from each of 5 trees was taken in the summer, by which time numbers were very low.

RESULTS

Counts of eggs and nymphs were analyzed separately. The ratios of the variance components for egg samples were very similar, and those for the nymphal samples were reasonably close, but different from those for egg samples. The means of these showed a 10:1 ratio for egg samples (0.35 between twigs within trees and 0.035 between trees) and a 4:1 ratio for nymph samples (0.085 between twigs and 0.02 between trees). This difference was due to a large reduction in the between-twig variance, presumably as a result of the nymphs spreading more evenly from the sites of oviposition before settling.

Considering the "cost structure" of the sampling, it was found that it took between 30 sec. and 2 min. to collect each twig, depending on the number of decisions and accessibility of

TABLE 4. Average sampling times.

SE (MEAN)	Eggs	Nymphs
0.1	6 trees × 6 twigs 1 hr 24 min.	2 trees × 4 twigs 14 min.
0.05	22 trees × 6 twigs 3 hr 18 min.	9 trees × 4 twigs 1 hr 3 min.

the foliage. A mean figure of 1 min. per twig was considered reasonable. The time to select each tree on a random basis (including time to divide north from south, and to sort out foliage units ready for sampling) took from 1 to 5 min., with a mean time of about 3 min. These were only rough estimates, no accurate timing of sampling having been done, but on this basis a sample of 20 twigs from each of 5 trees would take 1 hr 55 min., and 1 of 10 twigs from each of 10 trees, 2 hr 10 min. Experience proved these times to be about correct. On this basis it was possible to calculate the optimum number of twigs per tree from the formula:

$$n_1 = \sqrt{\frac{C_T \times \delta_W^2}{C_W \; \delta_T^2}}$$

where C_T="cost" per tree, C_W="cost" per twig and n_1=optimum number of twigs per tree.

Using the mean figures for the 2 variances, and the mean times for trees and twigs, and solving this separately for samples of eggs and nymphs gave:

       Eggs: n_1=5.48 (say 6) twigs per tree

       Nymphs: n_1=3.56 (say 4) twigs per tree

It was then possible to select the number of trees which must be sampled in each case to obtain any desired approximate standard error of the mean of the sample.

$$\text{From V (mean)} = \left(\frac{\delta_W^2}{n_1 \; n_2}\right) + \left(\frac{\delta_T^2}{n_2}\right)$$

where n_1 and n_2 are the number of twigs and number of trees, respectively, per sample.

For samples with a required standard error of 0.1 (in terms of logarithms to the base 10), n_2=5.5 trees for samples of eggs and 2.1 trees for samples of nymphs. For samples with a standard error of 0.05 the requirements would be 21.4 trees for eggs and 8.4 trees for nymphs.

From these figures the average time needed to collect samples at this degree of accuracy can be estimated for any selected standard error (TABLE 4).

This then seems to provide an adequate method of randomly sampling natural populations of *C. densitexta* when these insects are common, but may not be adequate when they are scarce unless an unduly great number of samples is taken.

DISCUSSION

This method of sampling was adopted and subsequently employed to measure changes in abundance in the 3 generations of this insect each year at a number of fixed sampling sites. These counts are still continuing and have enabled the monitoring of a widespread outbreak of *C. densitexta*. At the same time it has been possible to estimate the contribution of parasites, predators and "natural causes" to the mortality of each generation. The results of this work are yet to be published.

Other unpublished work using this sampling technique demonstrated that the proportion of dead twigs in the crown of a tree gave a parallel measure of the intensity of attack by *C. densitexta*. In the crown of a healthy tree which is not suffering attack, some twigs are dying all the time, but the damage done to leaves by nymphs of *C. densitexta* feeding on them increased the number of twigs dying in proportion to the increase in abundance of the insect, heavy and repeated attack eventually killing most or all of the crown of a tree.

LITERATURE CITED

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