SAMPLING PLANS FOR SPITTLEBUG EGGS IN PASTURES OF BRACHIARIA DECUMBENS[1]

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ABSTRACT - A sampling study of spittlebug eggs in pastures of *Brachiaria decumbens* was conducted by using a sample unit of 15 x 15 cm. The number of samples required for a certain level of precision was inversely proportional to population density. A crude estimate of number of samples necessary for 10, 15 and 20% of precision were 133,59 and 33, respectively. A study of sampling variation showed that differences between plots were much more important than the block differences; therefore plot to plot variation must be considered while sampling spittlebug eggs. The distribution pattern of numbers of eggs per 225 cm^2 of pasture fitted the negative binomial series. The sequential sampling plan presented here would reduce the sampling time over the conventional (fixed sample numbers) sampling.

Index terms: *Zulia entreriana, Deois flavopicta*, Cercopidae, sampling efficiency, sequential sampling.

PLANOS DE AMOSTRAGEM DE OVOS DE CIGARRINHAS EM PASTAGENS DE BRACHIARIA DECUMBENS

RESUMO - Foi conduzido um estudo sobre amostragem de ovos de cigarrinhas em pastagens de *Brachiaria decumbens* com o uso de uma unidade de amostragem de 15 x 15 cm. O número de amostras necessárias para um certo nível de precisão foi inversamente relacionado à densidade da população. Uma estimativa grosseira mostrou a necessidade de 133 amostras para se obter um nível de 10% de precisão, 59 para 15% e 33 para 20%. Um estudo sobre a variação na amostragem mostrou que a variação entre as parcelas foi mais importante do que entre os blocos; e assim sendo, a variação entre parcelas deve ser considerada na amostragem de ovos de cigarrinhas. O número de ovos por 225 cm^2 de área de pastagens mostrou uma distribuição do tipo binomial negativa. Um plano de amostragem tipo sequencial, apresentado no presente trabalho, reduziria o tempo gasto na amostragem em comparação à amostragem convencional onde o número de amostras é fixo.

Termos para indexação: *Zulia entreriana, Deois flavopicta,* Cercopidae, amostragem sequencial, eficiência da amostragem.

INTRODUCTION

In Brazil, the majority of spittlebug damaging pastures belong to genera *Zulia, Deois* and *Mahanarva*. They suck sap and inject toxins which result in whitish streaks on leaves. Under severe attack the leaves turn yellow and growth of plants in curtailed drastically. The extent of loss to the cattle industry was summarized by Domingues & Santos (1975), Ramos (1976) and Pacheco (1981).

There is a great interest in knowing the level of spittlebug egg populations in various parts of a pasture. This information would be useful in control methods such as use of fire in selective areas (Martin 1983) and certain pasture management tactics (Nilakhe 1983a). Eggs of spittlebugs are also sampled for various studies such as life table, modelling, detection of extent of parasitism, diapause etc.

In the state of Mato Grosso do Sul, the most predominant spittlebugs damaging pastures belong to species *Zulia entreriana* (Berg.) and *Deois flavopicta* Stal (Valério & Oliveira 1982). These spittlebugs oviposit primarily in soil and ground trash around plants and some eggs are found on plants parts also (Domingues & Santos 1975, Pacheco 1981, and Martin[6]). While sampling for spittlebug eggs, it is a common practice to include

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in a sample the soil up to a depth of ca. 3 cm (Cosenza 1981, Pacheco 1981).

Information on methodology of separation of spittlebug eggs from soil is available (Cosenza 1981, Pacheco 1981); however, no information on improving egg sampling efficiency is available. King (1975) reported on the number of soil samples necessary for 10 and 20% level of precision (proportion of standard error to mean expressed as a percentage) but his work dealt with the sugarcane froghopper, *Aeneolamia varia saccharina* (Dist.) in sugarcane fields.

Herein a report is made about a study of numbers of sample necessary for various levels of precision and on choosing of sampling sites in a sampling area. Also included is the information on distribution pattern of eggs and sequential sampling plan.

MATERIALS AND METHODS

The sampling was done during the dry season of 1982 and 83 (Table 1) and, since the majority of spittlebugs during the wet seasons were adults of *Z. entreriana* and *D. flavopicta*, it was assumed that the eggs sampled belonged to these two species. Names of the 14 fields of *Brachiaria decumbens* Stapf. used in the study are given in Table 1. Fields "CNPGC" and "W.B. Martins" were located near Campo Grande, and the rest in the region of Dourados. Each field had an area of 100 x 100 m divided in four equal blocks. Each block was further divided by means of a 2 x 2 grid into four equal plots, thus giving a total of 16 plots per field and a field was sampled just once. At each sampling, three areas of 15 x 15 cm of the pasture were selected at random per plot by throwing the square frame. A sample consisted of the above-ground portions of grass plants clipped at ca. 8 cm height and soil inside the square up to 2.5 cm depth.

In the laboratory, the sample was emptied in a 30 mesh sieve which was placed above a 60 mesh sieve. The sample was washed with a stream of water while breaking soil clods and separating plants parts until the water passing through sieves was almost clear. Thereafter, the remains in the 60 mesh sieve were transferred for drying in a greenhouse. The dried sample was carefully examined for eggs by spreading it in small quantities on black paper (Pickles 1931). The possibility of eggs passing through the finer sieve while washing is slim, since the sieve openings of 0.25 mm are smaller than the avg

TABLE 1. Mean number of spittlebug eggs in 15 cm x 15 cm area in pastures of *Brachiaria decumbens*, sampling variability and estimates of number of samples for three levels of precision.

Sampling date	Field name/ location	$\bar{x} \pm$ S.E.[1]	CV[2]	RV[3]	No. of samples required for S.E. of[4]		
					10	15	20
19 May 83	UEPAE	0.21 ± 0.08	262	38.5	686	305	172
5 May 83	Catalan	1.02 ± 0.41	276	39.9	762	339	190
10 May 83	CNPGC	1.19 ± 0.28	161	23.2	259	115	65
24 Aug 82	Florestal	1.46 ± 0.19	89	13.0	79	35	20
20 May 83	W.B. Martins	2.88 ± 0.38	93	13.3	135	60	34
29 Jul 82	Catalan	6.69 ± 1.30	135	19.4	182	81	46
5 Aug 82	Machado	8.02 ± 1.17	99	14.6	98	44	25
11 Aug 82	CNPGC	8.85 ± 1.84	144	20.8	207	92	52
6 Jul 82	Catalan	9.77 ± 2.06	146	21.0	213	95	53
10 Jul 82	CNPGC	9.92 ± 1.09	76	11.0	58	26	15
2 Sep 82	V. Alegre	16.81 ± 2.59	107	15.4	115	51	29
14 Jul 82	CNPGC	21.00 ± 2.11	70	10.1	49	22	11
14 Jul 82	Continental	24.17 ± 2.16	62	8.9	38	17	10
8 Aug 82	Continental	26.27 ± 3.10	82	11.8	67	30	17

[1] Mean based on 48 samples; S.E. = Standard Error

[2] CV = (Std. dev./$\bar{x}$) X 100.

[3] RV = (S.E./$\bar{x}$) X 100.

[4] No. of samples required for S.E. of certain probability (P) = $(CV/P)^2$.

width of 0.31 mm of *Z. entreriana* eggs (Ramos 1976) and 0.42 mm of *D. flavopicta* (Cosenza 1981).

The distribution pattern of spittlebug eggs within a field was tested for conformity to the Poisson distribution by calculating the index of dispersion (I_D). I_D is approximately distributed as χ^2 with n - 1 degrees of freedom (Southwood 1978). I_D values between limits of 0.95 and 0.05 for 47 degrees of freedom were considered as conforming to the Poisson distribution. Values of the negative binomial parameter (k) were determined as $k = \bar{x}^2/(s^2 - \bar{x})$ (Southwood 1978). Calculation of "common k" was done using procedures given by Bliss & Owen (1958), Southwood (1978) and Rudd (1980). The methodology for development of sequential sampling plan was reported earlier (Nilakhe et al. 1982, Nilakhe 1983b).

RESULTS

Table 1 shows that a good representation of spittlebug egg densities was found in this study. The mean number of eggs per 225 cm^2 of pasture varied from 0.21 to 26.27. In fact, it was difficult to find fields with low egg densities (< 2)/225 cm^2. Thus fields chosen in the 1983 sampling were those where almost no spittlebug adults were seen during the previous wet season.

Values of the relative variation (RV) exceeded 20% in five of the 14 cases, which indicated that the sampling efficiency was generally good. The highest RV values (38.5 and 39.9) were obtained for the lowest population densities (0.21 and 1.02 eggs). Table 1 also gives the number of samples required for the three levels of precision. The sampling plan illustrated graphically (Fig. 1) shows that the numbers of samples required to examine for a certain level of standard error was inversely proportional to the population density.

It can be observed from Fig. 1, that it is extremely difficult to use just one particular number of samples for all levels of population. Still there is a need for such an estimation. Therefore, a crude estimate of number of samples for a given level of error was obtained by using the following formula given by Rudd (1980),

$$n = \frac{k + \bar{x}}{c^2 k \bar{x}},$$

where, n = number of samples, c = standard error equal to fraction of the mean ($\bar{x}$), and k = negative binomial parameter (in our case common k of 1.41). Using this formula, the number of samples for three levels of precision (10,15 and 20%) for spittlebug egg densities of 0.5, 1, 2, 8, 20 and 25/225 cm^2 of pasture were calculated (Since the sampling efficiency is generally poorer at lower than at higher egg densities, more representation to lower densities was given). The number of samples for 20% level of precision for the above 6 egg densities were 68, 43, 30, 21, 19 and 18,

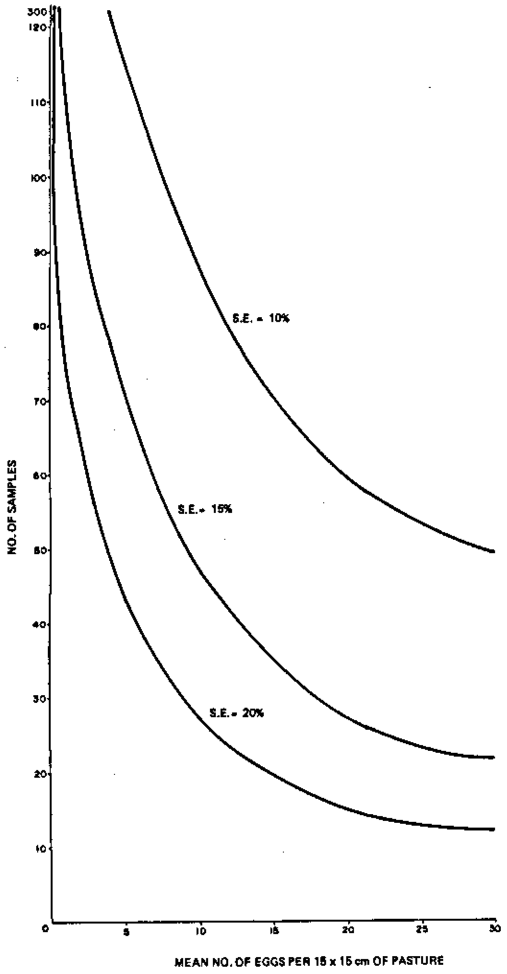

FIG. 1. Relation between population density and number of samples necessary for three levels of precision for spittlebug eggs in pastures of *Brachiaria decumbens*.

respectively, with a mean of 33; for 15% level of precision 120, 76, 54, 37, 34 and 33, with a mean of 59, and for 10% level of precision were 271, 171, 121, 84, 76 and 75, with a mean of 133. Thus, sample numbers of 33, 59 and 133 could be used to give precision levels of 20, 15 and 10%, respectively.

To study the importance of variation between blocks and plots within blocks, the egg counts were subjected to an analysis of variance. Because the spatial pattern of spittlebug eggs within a field conformed to the negative binomial distribution (discussed in the next paragraph), variance was stabilized by transforming the data using log (x + 1), where x is the observed count. The analysis of variance using one of the sets of data is illustrated in Table 2. In the 14 sets of data, blocks were significant 7 times, whereas differences between plots were significant in 11 cases.

Table 3 gives variance and values for index of dispersion (I_D) and negative binomial parameter (k). Values of I_D for the egg counts indicated that only in one of 14 instances (24 Aug., 1982 sampling), the Poisson model satisfied the distribution; for the same sampling, k value of 8.88 was obtained which also indicated agreement with the Poisson (Southwood 1978). With the exception of 24 Aug. 1982 sampling, values of k were about 2 or less indicating the negative binomial distribution. A contagious distribution of eggs was also supported by the variance mean ratios, since in all instances the ratio was greater than one (Tables 1 and 3).

A nonsignificant correlation coefficient (r = -0.01) for the egg mean and k values indicated that k is independent of density, and therefore calculation for a common k would be justified (Rudd 1980). A common k of 1.41 was obtained. Using this value of common k, 4 and 10 spittlebug eggs/225 cm^2 of pasture as the lower and upper density limit and $\alpha = \beta = 0.20$, a sequential sampling plan was developed (Fig. 2).

TABLE 2. Analysis of variance of counts of spittlebug eggs, CNPGC, July 10, 1982.

Source of variation	df	SS	MS	F
Blocks	3	0.095	0.032	0.48
Plots within blocks	12	3.544	0.295	4.47[1]
Residual	32	2.125	0.066	

[1] Significant at 1% level of probability

TABLE 3. Variance (s^2), departure of distribution from randomness (Index of dispersion - I_D), and measure of clumping (k) for spittlebug eggs[1].

Sampling date	s^2	I_D[2]	k
19 May 83	0.3	67	0.49
5 May 83	7.9	366	0.13
10 May 83	3.7	146	0.57
24 Aug 82	1.7	55	8.88
20 May 83	7.1	116	1.96
29 Jul 82	81.2	570	0.59
5 Aug 82	65.7	385	1.11
11 Aug 82	162.8	865	0.48
6 Jul 82	202.8	976	0.49
10 Jul 82	57.2	271	2.08
2 Sep 82	322.6	902	0.92
14 Jul 82	207.1	464	2.36
14 Jul 82	223.1	434	2.94
8 Aug 82	462.3	827	1.58

[1] Means are given in Table 1.

[2] I_D value of 55 was the only one that agreed with Poisson distribution.

DISCUSSION

There are many more studies on eggs of the sugarcane froghopper in sugarcane fields (King 1975) than on spittlebug eggs in pastures. The same author developed a sieve and flotation system for extraction of the froghopper eggs. He reported extraction efficiency of 76%, whereas Pickles (1931), who examined washed dried soil from the cane fields on black paper, reported 100% extraction efficiency. The Pickles method was essentially used in this work, but our egg extraction efficiency averaged 93%; the discrepancy was apparently, because our samples contained a greater quantity of plant debris than samples from sugarcane fields.

For sampling of spittlebug nymphs, Nilakhe (1983b) found a sample unit of 25 x 25 cm to be more efficient than 50 x 50 cm or 1 x 1 m units. However, for spittlebug egg sampling, a smaller sample unit (15 x 15 cm) was chosen, because

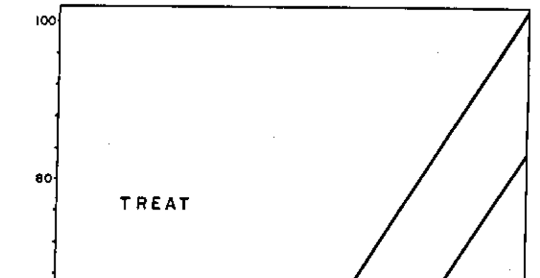

FIG. 2. Sequential sampling plan for spittlebug eggs in pastures of *Brachiaria decumbens* using a common k of 1.43, slope b of 6.16, and 4 and 10 eggs per sample as lower and upper density limits, and α and β = 0.20.

experience showed that the egg densities per unit area are much higher than for nymphs. The sampling was done during dry season; during the wet season it is likely that egg densities may not be as high. The aggregation pattern of spittlebug eggs was comparable to that of nymphs (Nilakhe 1983b); similar finding was reported for the sugarcane froghopper eggs (Evans 1972, King 1975).

For most intensive sampling programs, relative variation of 10% or less is considered adequate, whereas for extensive sampling, 20 to 25% may be adequate (Pedigo et al. 1972, Hilhouse & Pitre 1974, Southwood 1978, Nilakhe & Chalfant 1982). It is hoped that sample numbers 133,59 and 33 would provide the precision levels of 10,15 and 20%, respectively. Clearly, examination of so many samples would not be necessary at higher egg densities. However, in absence of prior information on the densities, using the suggested sample numbers would act as a safeguard against the lower densities.

The use of a sample unit of 15 x 15 cm may also reduce time spent in examination of soil for eggs. For example, Martin (unpublished data) examined 20 samples of *B. decumbens* pastures with a sample unit of 25 x 25 cm. In three fields, he obtained egg densities of 8.8, 25.9 and 37.6 with RV values of 10,20 and 25%, respectively. In this work it was found that 33 samples of 15 x 15 cm units would also give RV of ca. 20% or less. However, the pasture examined for eggs would be 0.74 m^2 or 40% less than for 20 samples of 25 x 25 cm. To estimate spittlebug eggs in. *B. decumbens* pasture, Pacheco (1981) examined 510 samples of 20 cm^2 grass area (5 cm diameter sampler). Studies are needed to compare sampling efficiency of sample units such as 20 and 225 cm^2.

Analysis of variance of egg counts showed that plot to plot variation was much more important than variation between blocks. Similar results were reported for spittlebug nymphs (Nilakhe 1983b) and for white grubs, *Phyllophaga* spp. (Guppy & Harcourt 1973). Thus, for sampling of spittlebug eggs, dividing a sampling field in equal-sized plots (e.g. 16) and taking a certain number of sample per plot (e.g 2 for 20% level of precision) would be useful.

Pickles (1946) used egg sampling as a tool to predict the imminent infestation of *A. varia saccharina* in sugarcane fields. A similar methodology could be used for spittlebugs in pastures. To reduce population of diapausing spittlebug eggs, Martin (1983) recommended control-burn of each pasture every one to four years. Thus, knowing egg densities in various areas of a pasture would help decide those areas that need burning. A sequential sampling plan presented here could be used for such a purpose. The egg densities chosen in development of this plan (Fig. 2) were only for an example. By using information presented here, plans with different densities and α and β levels could be developed easily.

The calculation of average sample number (ASN) curve is necessary to determine the feasibility of the sequential sampling plan. The ASN curve would show the average number of eggs which need to be examined before making a decision at different spittlebug egg densities. Obviously, it would be necessary to examine the maximum numbers of samples when the cumulative egg counts keep falling especially in the middle of the two decision lines. The calculation of maximum ASN for the plan in Fig. 2 gave a value of 13. The sequential sampling plan could be used in the following manner: Collect and wash thrirteen, 15 x 15 cm samples as described in materials and methods. Begin examination of samples for eggs. If in the very first sample 14 eggs are found, a decision to treat (e.g., burning or a certain other management tactic) is made. Absence of eggs in the first two samples would indicate a decision not to treat. Continue examinig samples for eggs until a decision is reached. In rare cases, the cumulative egg count of 13 samples may still fall in the continue sampling zone. In such a situation, if the count is closer to the upper limit decision line, then decision to treat is made, and otherwise. Even if it became necessary to examine all 13 samples, the time to obtain and examine the samples would be ca. two mandays, however, in majority of situations a decision could be reached within a day's work.

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