SPITTLEBUG EGGS: IMPROVED EXTRACTION METHOD, LOCATION IN PASTURE, AND SUBSAMPLING FOR POPULATION ESTIMATES

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RESUMO

O exame de pastagens para contagem de ovos de cigarrinhas leva muito tempo, especialmente quando as amostras lavadas e secas têm grande quantidade de restos de plantas. Foi usado com sucesso o soprador de sementes para separar os restos das plantas e o solo. Obteve-se mais de 90% dos ovos em amostras, somente pelo exame de solo, e o tempo gasto foi reduzido a praticamente 1/2.

Cerca de metade dos ovos das cigarrinhas em pastagens de *Brachiaria decumbens* foram encontrados nas plantas, e o restante na área entre as plantas. O número de ovos encontrados entre as plantas mostrou uma tendência a aumentar com o aumento de restos de plantas.

O exame de 30 sub-amostras compreendendo 10% do volume da amostra inteira, foi eficiente para estimar o número de ovos de cigarrinhas na amostra toda, somente a uma densidade de ovos $\leq 356/m^2$ de pastagem. Este esquema de sub-amostragem pode ser usado para classificar as densidades de ovos em categorias como baixa, média ou alta.

INTRODUCTION

Information about spittlebug egg densities in various parts of a pasture would be useful for control methods such as use of fire in selective ares (MARTIN, 1983), and pasture management tactics where predictions about severity of the forthcoming infestation are necessary (NILAKHE, 1983). Spittlebug

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eggs are also sampled for various studies such as life table, modelling, detection of egg parasitism, diapause, etc.

To extract cercopid eggs from sugarcane fields, PICKLES (1946) washed the soil through a series of sieves and then examined the washed dried soil for eggs by spreading it in samll quantities on black paper. KING (1975) used a rotary sieve, hydrogen peroxide treatment, and flotation method. NILAKHE et alii (1984) used essentially the "Pickles method" to extract cercopid eggs from pastures of *Brachiaria decumbens* Stapf. Irrespective of the method used, examination of the material for eggs is a tedious process. Furthermore, presence of the plant debris lengthens the time spent in search for the eggs, reduces search efficiency and increases strain on the vision. Therefore, a method to remove plant debris from the pasture samples would be quite useful.

To expedite the process of examination of cercopid eggs from sugarcane fields, PICKLES (1946) examined five $2cm^3$ subsamples of the washed dried soil to estimate the number of eggs in an entire sample. He reported that the error involved in such an estimation was extremely small. However, he did not mention about the quantity of the washed dried soil in the entire soil sample, nor the values of the error involved.

Here, we report about the usefulness of a seed blower in extraction of spittlebug eggs from pasture, location of the eggs in pasture and on the use of subsampling to estimate the eggs numbers.

MATERIALS AND METHODS

Seed Blower and Egg Extraction

One of us (A.A. da SILVA) conceived the idea of using a common seed blower (Distributed by Seedburo Equipment Co., Chicago, Illinois, U.S.A.) for separation of plant debris and soil from pasture samples examined for spittlebug eggs. A pasture sample included the grass plants clipped to ca. 8 cm height from the soil, the associated plant debris and soil up to 2.5 cm depth from a designated area of the sample. The sample was then washed through a series of sieves and dried (NILAKHE et alii, 1984). Basically, the seed blower consists of a motor to blow air, a regulator to control the air velocity, and a cylinder with removable screen lids on both ends. A seed sample to clean is placed in the bottom chamber of the cylinder. The air flow blows the trash from the sample upwards where it is collected in the top chamber of the cylinder. After several trial and errors, we achieved the appropriate air velocity that allowed us to collect the majority of plant debris (>99%) from the washed dried pasture sample in the top cham-

ber of the cylinder, whereas the soil and most of the spittle bug eggs remained in the bottom chamber. The methodology used was as follows: about 7 ml of the washed dried pasture sample was placed in the cylinder. The machine was run for 17 seconds at the air velocity setting of 10. Heavier soil particles (without plant debris) that remained in the bottom chamber were removed and designated as the "first soil". The plant debris and the lighter soild particles collected in the top chamber are transferred to the bottom chamber. The machine was run again for 10 seconds at the air velocity setting of 9. The soil that remained in the bottom chamber is designated as the "second soil". The majority of plant debris (>99%) now appears in the top chamber. The plant debris was discarded and the first and second soil is examined for eggs by spreading it in small quantities on black paper.

To verify the usefulness of the seed blower, 3 tests were conducted using 12 samples each. In Tests 1 and 2, a sample consisted of 20 ml of washed dried pasture of *B. decumbens*. The sample was passed through the seed blower as described above and eggs in the first and the second soil and the plant debris were counted. The quantity of soil and plant debris was also measured. Test 3 was essentially the same as Tests 1 and 2, except that spittlebug eggs present naturally in the soil were removed and 18 spittlebug eggs were added to each of the 24 ml samples. After passing through the seed blower, eggs from the soil were counted, and the number of eggs in the plant debris were obtained by subtracting the number in the soil from 18.

Location of Spittlebug Eggs in Pasture

Test 1 - From the pasture of *B. decumbens*, 15 grass clumps of ca. 4 cm radius were chosen in such a way that no other grass plant was present within a 25 cm radius of the clump. At each of the 15 locations, a clump was clipped to a height of ca. 8 cm, then removed with the soil beneath taken to the depth of 2.5 cm. In a similar way, the soil and associated plant debris (mainly dried leaves and stems) in radius of 5-8, 9-12, and 13-16cm were removed and held separately. Since a grass clump is rarely a perfect circle, the sample designated as the "5-8"radius was an approximation. Each of the four portions collected at a location was washed, dried, passed through the seed blower, and the soil, free of the plant debris, was examined for spittlebug eggs as described in the previous section.

Test 2 - Grass clumps of ca. 4 cm radius were chosen in such a way that when the clump was located centrally, no other grass plant occoured in a 15 x 15 cm area. The clump and the associated soil were removed and held separately; then the remaining portion of the 15 x 15 cm area was removed. The details about obtaining sample and egg extraction were the same as in

Test 1. Fifteen grass clumps were chosen in the pasture with abundant plant debris ($\bar{x}$ dry weight 28.3±4.59g); and, another-15, were selected with less quantities of plant debris. ($\bar{x}$ of 4.4±0.59g). To obtain the dry weight, plant debris in 15x15 cm pasture, excluding the clump, was collected from closely-located grass clump similar to those used in the test.

TABLE 1. The results of the use of a seed blower to separate plant debris and soil from pasture samples to facilitate examination for spittlebug eggs[1].

Test	Quantity in ml±SE after passing through seed blower[2]			No. of spittlebug eggs±SE		
	First soil	Second soil	Plant debris	First soil	Second soil	Plant debris
1	12.7±0.12	2.2±0.30	5.1±0.26	0.66±0.28	2.08±0.48	0.16±0.11
2	12.7±0.20	2.4±0.17	4.9±0.21	0.42±0.19	2.83±0.46	0
3	14.9±0.35	4.4±0.25	4.7±0.13	4.67±0.91	11.42±1.05	1.92±0.34

[1] Soil from pastures of *B. decumbens* to a depth of 2.5 cm, and the above-ground portions of grass plants clipped at ca. 8 cm height were washed through a series of sieves and dried. In Tests 1 and 2, 20 ml and in Test 3, 24 ml of the washed dried soil was used. In Tests 1 and 2, eggs occuring naturally in the soil were counted; whereas, in Test 3, eggs present naturally were removed and 18 eggs/sample were added. For each test 12 samples were used.

[2] About 7 ml of the soil was placed in bottom chamber of the cylinder of the seed blower. After the first run of the machine, the soil that remained in the bottom chamber was called "First soil", and after the second run this was called "Second soil". Plant debris was collected in the top chamber. For details see the text.

SUBSAMPLING TO ESTIMATE EGG NUMBERS

For each of the 5 tests, the samples totalled 0.675m^2 of *B. decumbens* pasture. The details about obtaining and processing of the samples were given in the first section. We chose 0.675m^2 of the pasture on the assumption that at least 30 samples of 15 x 15 cm would be required for a reasonable estimate of spittlebug egg densities. The volume of the washed dried soil varied considerably among the tests (Table 2). No seed blower was used to remove plant debris from the soil. For Test 2, a pasture from Dourados was used; whereas, for the remaining

TABLE 2 - Subsampling to estimate the number of spittlebug eggs in the entire sample of the washed dried soil from pastures of *Brachiaria decumbens*, when the soil contained low (44/m² of the pasture), medium (356/m²), or high (844/m²) egg densities.

Repetition	Quantity of the washed dried soil in entire sample (ml)[1]	% of the total soil examined in 30 subsamples. In parenthesis ml of soil used/subsample	NO. OF EGGS ADDED TO THE SOIL IN THE ENTIRE SAMPLE AFTER REMOVAL OF THE NATURALLY OCCURING EGGS								
			30			240			570		
			Expected no. of egg/subsample	Observed no. of egg/subsample ±SE[2]	Confidence interval of 99%[3]	Expected no. of egg(s)/subsample	Observed no. of egg/subsample +SE	Confidence interval of 99%	Expected no. of egg/subsample	Observed no. of egg/subsample +SE	Confidence interval of 99%
						TEST 1:					
1	1072	9.0 (3.2)	0.09	0.37±0.11	0.06-0.68	0.72	0.70±0.17	0.23-1.17	1.79	1.13±0.15	0.72-1.53
2				0.30±0.10	0.03-0.57		0.67±0.14	0.28-1.06		1.13±0.21	0.55-1.71
3				0.37±0.11	0.06-0.68		0.87±0.15	0.46-1.28		0.83±0.18	0.33-1.33
						TEST 2:					
1	3033	9.9 (10.0)	0.10	0.27±0.11	-0.02-0.56	0.79	0.37±0.10	0.09-0.65	1.87	1.07±0.17	0.60-1.54
2				0.23±0.09	-0.02-0.48		0.53±0.13	0.16-0.90		1.00±0.16	0.56-1.44
2				0.03±0.03	-0.06-0.12		0.50±0.13	0.13-0.87		0.90±0.16	0.45-1.35
						TEST 3:					
1	600	20.0 (4.0)	0.20	0.47±0.10	0.18-0.76	1.60	1.27±0.15	0.85-1.69	3.80	2.53±0.35	1.57-3.49
2				0.47±0.13	0.10-0.84		1.67±0.18	1.19-2.15		3.60±0.30	2.77-4.43
3				0.33±0.11	0.02-0.64		1.00±0.15	0.58-1.42		2.87±0.30	2.04-3.70
4				0.60±0.16	0.17-1.03		1.37±0.19	0.85-1.89		3.27±0.45	2.02-4.52
5				0.60±0.13	0.23-0.97		1.43±0.29	0.63-2.23		3.60±0.42	2.44-4.76
6				0.43±0.10	0.14-0.72		1.00±0.22	0.39-1.61		3.10±0.35	2.14-4.06

CONTINUATION OF THE TABLE 2:

						TEST 4:					
1	600	20.0 (4.0)	0.20	0.17±0.07	-0.02-0.36	1.60	0.67±0.14	0.29-1.05	3.80	2.73±0.28	1.95-3.51
2				0.17±0.08	-0.06-0.40		1.10±0.26	0.37-1.83		2.43±0.37	1.42-3.44
3				0.13±0.06	-0.04-0.30		1.00±0.21	0.41-1.59		2.50±0.35	1.54-3.46
4				0.17±0.07	-0.02-0.36		0.63±0.12	0.29-0.97		1.87±0.24	1.21-2.53
5				0.10±0.06	-0.05-0.25		1.23±0.17	0.76-1.70		2.27±0.37	1.24-3.30
6				0.20±0.09	-0.04-0.44		1.03±0.22	0.43-1.63		2.63±0.25	1.95-3.31
						TEST 5:					
1	800	20.0 (5.3)	0.20	0.10±0.06	-0.05-0.25	1.60	0.63±0.13	0.27-0.09	3.80	1.80±0.24	1.14-2.46
2				0.17±0.07	-0.02-0.36		0.70±0.15	0.28-1.12		2.13±0.35	1.17-3.09
3				0.13±0.06	-0.04-0.30		0.50±0.13	0.15-0.85		1.53±0.24	0.87-2.19
4				0.17±0.07	-0.02-0.36		0.80±0.17	0.33-1.27		1.90±0.23	1.27-2.52
5				0.23±0.08	-0.01-0.45		0.80±0.22	0.21-1.39		1.77±0.27	1.03-2.51
6				0.17±0.07	-0.02-0.36		1.10±0.19	0.57-1.63		2.10±0.30	1.28-2.92

[1] Values are the actual quantities of the soil left after 0.675m^2 of the pasture was washed through a series of sieves. Quantities in tests 3-5 were rounded.

[2] After the eggs in 30 subsamples were counted, the soil and eggs from subsamples were added to the original sample. The soil was throughly mixed and then next batch of subsamples was drawn. In test 1, instead of 570, 600 eggs were used.

[3] Subsampling was considered effective in estimating no. of eggs in the sample, only in those cases where the confidence interval included the respective expected no. of egg(s).

tests pastures near Campo Grande were used. Spittlebug eggs present naturally in the washed dried soil were removed by examining the soil in small quantities on black paper. Then 30 spittlebug eggs (1/225 cm^2 of the pasture) were thoroughly mixed in the soil of each test. For Test 1, 30 subsamples of 3.2ml of the soil were drawn at random and were examined for the eggs. Afterwards, the soil and eggs from the subsamples were added to the original sample, the soil mixed thoroughly, and the next batch of the subsamples was drawn. Similarly, subsampling was done at egg densities of 240 (356/m^2) and 570 (844/m^2). The number of repetitions of the 30 subsamples, quantity of soil per subsample and percent of the total soil examined in the subsamples for each test are given in Table 2. To determine the effectiveness of subsampling in the estimation of the number of eggs in the sample, the 99% confidence interval was calculated (subsample $\bar{x} \pm t$ at 0.01 probability X SE). Subsampling was considered effective only in those cases when the interval included the respective expected number of eggs.

The work reported here was conducted during June-October 1983. The predominant spittlebug species in the sampling area were *Zulia entreriana* (Berg.) and *Deois flavopicta* Stal.

RESULTS AND DISCUSSION

SEED BLOWER AND EGG EXTRACTION

The quantity of soil remaining in the bottom chamber of the cylinder of the seed blower after the first and second runs of the machine, the quantity of plant debris separated from the soil and the respective number of eggs in each part of the sample are given in Table 1. Overall, 23.8% of all the eggs appeared in the "first soil", 67.6% in the "second soil" and 8.6% in the plant debris. In Tests 1 and 2, the plant debris was examined for eggs; however, we did not examine the eggs themselves. It is likely that these eggs were excessively light. Thus, even after disregarding the plant debris, one might still expect to recover > 90% of spittlebug eggs in a sample by examination of soil alone. This egg extraction efficiency is comparable to the 93% obtained by NILAKHE et alii (1984). By disregarding plant debris, the volume of the sample to be examined was reduced by one-fourth. Furthemore, the time spent in examination of the soil was reduced by about-one-half in comparison to soil containing plant debris (a sample not subjected to the seed blower). For example, it took an average of 30 min. to examine 20 ml soil for eggs, whereas when passed through the seed blower, the examination took an average of 15 min., and was also less tiring to the vision.

To verify the fate of light eggs, 20 hatched spittlebug eggs were placed in 20 ml of the washed dried egg-free soil, and subjected to the seed blower. This was done twice, and in both cases all of these eggs appeared in the upper chamber of the cylinder along with the plant debris.

The seed blower could be used to improve the search efficiency and to reduce the search time in egg extraction by the "flotation method" (KING, 1975). The washed dried soil sample could be subjected to the seed blower, then the soil excluding plant debris could be searched for eggs by the flotation technique. In absence of plant debris, the serach for eggs would be much easier. The used of seed blower in extraction of other insect eggs from soil should be investigated.

LOCATION OF SPITTLEBUG EGGS IN PASTURE

Test 1 - The mean number of eggs in the grass clump of ca. 4 cm radius was 4.36±1.76, in the adjacent area within the 5-8 cm radius 1.18±0.54, in the 9-12 cm radius 1.0±0.36; and 0.64±0.31 in the radius of 13-16 cm. The grass clump contained significantly greater number of eggs than the 3 surrounding areas ($P < 0.05$). No significant differences for eggs within the 3 areas were found ($P > 0.05$).

Test 2 - In samples with more plant debris, a greater number of eggs was found in the 15 x 15 cm area excluding the grass clump (4.5±1.53) than in the clump itself (4.0±1.02) ($P < 0.05$); whereas, in samples with less plant debris, a greater number of eggs was found in the grass clump (4.71±2.03) than in the remaining area of the 15 x 15cm (3.43±1.92) ($P < 0.05$).

In *B. decumbens* pastures, it is quite rare to find the grass clumps separated from one another by a distance of 25cm as in Test 1. In Test 2, grass clumps were separated from one another by 4 to 6 cm - a situation quite common in the pasture. These two tests were conducted during August and September. It is likely that occasional flooding due to excessive rain may have displaced the eggs somewhat. Nevertheless, the data indicated that the proportion of eggs laid in grass clumps and in areas between the clumps was influenced by the quantity of plant debris.

The nymphs eclosing from those eggs placed in the clump would probably readily find the preferred newly sprouted tillers and would not be much exposed to the sun. The nymphs hatching from the eggs placed between the clumps would have to move toward the clumps, and thus would be exposed longer to the sun rays and probably, also, to predators. When fire is used for control of spittlebugs (MARTIN, 1983), eggs present between the

clumps are probably more likely to be destroyed by burning than those within clumps.

Subsampling to Estimate Egg Numbers

Initially, we used ca. 10% or the total washed dried soil of the sample for 30 subsamples (Tests 1 and 2). At low egg density (44/m²), the subsample mean was higher than the respective expected mean in 5 of 6 cases, but in all the cases the confidence interval included the respective expected mean. At medium egg density (356/m²). the subsample mean exceede the expected mean in 1 of the 6 cases (Teste 1, Repetition 3) and the interval included the expected mean in 5 of 6 cases. However, at high egg density (844/m²), the subsample mean was consistently lower than the expected mean and the confidence interval never included the expected mean. Thus, based on these two tests, subsampling using 10% of the sample soil was considered adequate in estimating eggs in the sample at low and medium egg densities but at high. Therefore, we later increased the quantity of the soil used in subsampling 20% (Tests 3-5). At the low egg density, the intervals included the expected mean in all 18 instances; in 9 of the 18 instances at medium egg density; and, in 4 of 18 instances at the high egg density. Thus, by increasing the quantity of the soil used in subsampling from 10 to 20%, the instances of successfully estimating sample population at low egg density remained the same, at medium egg density the successful instances were reduced, and at high densities a slight improvement was obtained. Better results could probably be obtained by increasing the number of subsamples and/or increasing the quantity of soil used in subsamples.

The data presented here indicated that 30 subsamples comprising 10% of the total washed dried soil might adequately estimate the number of spittlebug eggs in the entire sample only at egg densities of $\leq$ 356/m² of the pasture. [It is assumed that the "washed dried soil" would be based on at least 30 samples of 15 x 15 cm pasture. More accurate sample numbers for certain degrees of precision are given elsewhere (NILAKHE *et alii*, 1984.] However, in Mato Grosso do Sul and in some other states, higher spittlebug egg densities do occur (NILAKHE *et alii*, 1984). Therefore, the present scheme could be used in classifying spittlebug egg densities in categories such a low, medium or high, either in different parts within a farm or between different farms, but is not recommended for accurate egg estimations.

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ABSTRACT

Examination of pasture samples for spittlebug eggs is very time consuming especially when the washed dried samples contain large amounts of plant debris. A seed blower was used successfully to separate the plant debris and the soil. Counting eggs from the soil alone accounted for > 90% of the eggs in the samples, and the egg search time was reduced by about one-half.

In pastures of *Brachiaria decumbens* Stapf, half of the spittlebug eggs were located in the grass clumps and the other half between the clumps. The number of eggs located between the clumps tended to increase with increased amounts of plant debris.

Examination of 30 subsamples, comprising 10% of the volume of the entire sample, was effective in estimating number of spittlebug eggs in the sample only at egg densities $\leq 356/m^2$ of the pasture. The subsampling could be used for classifying egg densities in categories such as low, medium or high.