Sampling unit and optimal sample size for the detection of *Aeneolamia albofasciata* (Lallemand) eggs in sugarcane

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ABSTRACT

Objective: To compare the efficiency of a 30×30×5 cm iron sampling frame (Frame30) with a smaller 15×15×5 cm one (Frame15), as sampling method for spittlebug eggs [(*Aenolamia albofasciata* (Lallemand)] that reduces the amount of soil removed, optimizes time used, and reduces the sampling effort.

Design/methodology/approach: Mean, variance, coefficient of variation, sampling effort, spatial arrangement, and sample size were determined with both sampling frames. Forty systematic soil samples were obtained using each frame in two plots planted with the variety MEX 69-290, and two others with MEX 91-662. Each soil sample was mixed and homogenized to obtain a subsample of 250 g, from which eggs were extracted by decantation in saline solution.

Results: Both frames estimated different numbers of eggs in the four plots ($\bar{x} \pm s$) (Frame15: 2.71±1.71; 3.49±1.81; 2.74±2.08; 4.44±2.22; Frame30: 4.42±3.58; 6.65±3.92; 4.40±3.45; 7.84±4.54). Significant differences were found between sampling frames ($P<0.0001$) and between plots ($P<0.0001$), but not in the plot-sampling frame interaction ($P=0.1509$). The optimal sample size (accuracy 0.1) was smaller with Frame15 (40, 27, 57 and 25), compared to Frame30 (65, 34, 61 and 34). Both frames estimated a conglomerated spatial arrangement of eggs using three methods.

Limitations on study/implications: This study suggests changing the sampling frame used in Veracruz, Mexico, for a smaller, more efficient one.

Findings/conclusions: Frame15 reduced by 75% the soil removed, provided more accurate population estimates, and simplified field and laboratory management, compared with Frame30.

Keywords: spotted spittlebug, eggs, sampling metal frame, systematic sampling.

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INTRODUCTION

The spotted spittlebug or salivazo [*Aeneolamia albofasciata* (Lallemand)] is one of the pests that most affects sugarcane production (*Saccharum officinarum*) in the Gulf of Mexico region (García-García *et al.*, 2006) and the coastal zone of the Pacific Ocean (Parada-

Domínguez *et al.*, 2019), within an altitudinal strip comprising 10 to 1700 masl (Alatorre-Rosas and Hernández-Rosas, 2015). The nymphs, which secrete a protecting foamy substance (Obando *et al.*, 2013), feed from adventitious roots, main roots, stem and basal bracts (Hernández-Rosas and Figueroa-Rodríguez, 2011), while winged adults suck sage from the underside of the leaves, at the same time that they emit enzymes that block the vascular conducts and provoke linear chlorotic spots, as well as longitudinal necrosis (Badilla, 2002).

The preferred substrate for oviposition of the spotted spittlebug eggs is the soil around the variant of sugarcane, between the roots, at few centimeters of depth (Thompson and León, 2005). The eggs can enter or exit diapause depending on the moisture (Hernández-Rosas and Figueroa-Rodríguez, 2011; Badilla, 2002). This biological stage does not cause harm, but its quantification (León-Hernández *et al.*, 2014; Ramos-Hernández *et al.*, 2018) allows estimating the population density of nymphs and adults (Auad *et al.*, 2011), for which the application of methods for biological control (Bautista-Galvéz and González-Cortés, 2005; Parada-Domínguez *et al.*, 2019), mechanical, trapping, and chemical control (Badilla, 2002; García-García *et al.*, 2006; Ortiz-Laurel *et al.*, 2014) are necessary.

In sugar mills of the central zone of the state of Veracruz, iron frames measuring 30×30×5 cm are used (Frame30) for soil extraction, necessary to count the spittlebug eggs; however, their use requires removing a large volume of soil (4500 cm^3 or 6 kg), which represents greater sampling effort for the operator.

This study compares the number of spotted spittlebug eggs, the optimal sample size, the indicators of spatial distribution, and the sampling effort between soil samples collected with the Frame30 (30×30×5 cm), versus a smaller frame (Frame15, 15×15×5 cm), in sugarcane plots of the varieties MEX 69-290 and MEX 91-662, during three sampling events.

MATERIALS AND METHODS

The soil samples used to count spotted spittlebug eggs were taken from four sugarcane commercial plots located in two municipalities of the state of Veracruz, Mexico: plots 1 (Lat. 19.332350, Long. −96.369933) and 2 (19.338927, −96.353470) in El Salmoral, municipality of La Antigua, grown with the variety MEX 69-290, and plots 3 (19.314149, −96.355744) and 4 (19.305975, −96.368308) in La Víbora, municipality of Paso de Ovejas, grown with the variety MEX 91-662. The physical and chemical characteristics of the soil from the four plots were obtained through the analysis methods indicated in the NOM-021-RECNAT (2001) during the three sampling dates: July 23, August 11, and August 30 in 2012.

A surface of 1.0 ha was selected in each plot, surrounded by an edge of 6 m of crop. To achieve high accuracy (0.1), 40 systematic sampling events were conducted per plot (Villanueva-Jimenez *et al.*, 1993; Southwood and Henderson, 2000). In each sampling point, two sampling frames were used (15×15×5 and 30×30×5 cm), made from laminated iron beams with 3.2×50.8 mm thickness. The frames were buried to soil level, opposite to the variant and on the same furrow.

The analysis was performed in the Plant Health Laboratory of Colegio de Postgraduados, *Campus* Veracruz, where each sample was weighed and homogenized to obtain a subsample of 250 g of soil (± 208.33 cm^3). Next, each subsample was mixed with a saturated NaCl solution (70%) and was left resting for 30 min; then, it was sifted (30, 40 and 60 nets) and washed with running water. The sieve content from 60 nets was mixed inside a separation funnel of 500 mL with saline solution (NaCl, 30%) and left resting for 10 min. The largest particles and the saline solution were kept in the bottom, while the spittlebug eggs floated in the supernatant. The eggs and the content from washes of the decantation funnel performed with a pipette with water were retained in a 7×10 cm organza fabric. The fabric was placed on a paper filter circle inside a Petri dish; 10 mL of distilled water was added to moisten the soil particles dragged with the eggs. The total number of eggs from each subsample was counted, with the help of a Stemi DV4 Carl Zeiss® stereoscopic microscope.

The optimal sample size was calculated with: $\hat{n}=\left(\frac{s}{\bar{x}C}\right)^2$, where $\hat{n}$ is the optimal sample size, s is the standard deviation, $\bar{x}$ is the mean, and C is the accuracy (Karandinos 1976; Southwood and Henderson, 2000).

To determine the spatial disposition, the variance/mean ratio (Ledo *et al.*, 2012), the Morisita index (Ledo *et al.*, 2012), and Lloyd's agglomeration mean index (Lloyd, 1967; Ledo *et al.*, 2012) were used. The variance/mean ratio $=\frac{s^2}{\bar{x}}$; with variance (s^2) and mean ($\bar{x}$), indicates that when the calculated value is >1, the population is aggregated; if $=1$, it is random; if <1, it is ordered. The Morisita index, $I_\delta=n\left[\frac{\sum x_i^2-\sum x_i}{\left(\sum x_i\right)^2-\sum x_i}\right]=\frac{n\bar{x}I_A}{n\bar{x}-1}$, with $\sum x_i$, the sum of the sampling observations, $\sum x_i^2$ sum of the square of the sampling observations, and $\left(\sum x_i\right)^2$ the square of the sum of the sampling observations. If $I_\delta > 0$ indicates aggregation, if $= 0$ it indicates randomness, and if < 0 it presents ordered dispersion. Lloyd's mean agglomeration index (I_A) was calculated through: $I_A=\frac{\dot{x}}{\bar{x}}$ y $\dot{x}=\bar{x}+\left(\frac{s^2}{\bar{x}}-1\right)$, where $\bar{x}$ is the mean, s^2 is the variance, and $\dot{x}$ is the average of individuals in relation to other individuals. For the relationship between the mean agglomeration index (I_A) and the mean ($\bar{x}$) to be easier to compare, the aggregation percentage was estimated, as the quotient between the index I_A and the mean: $\frac{I_A}{\bar{x}}*100\%$. To compare the efficiency of both sampling frames, the accuracy was estimated through the mean standard error ($S_{\bar{x}}$) and the efficiency with the sampling effort (E_M), defined as: $E_M=S_{\bar{x}}(t)$, where t, is the time invested and $S_{\bar{x}}=\frac{s}{\sqrt{n}}$.

A factorial analysis was carried out through PROC GLM from SAS, to identify the effect of the factors: plot (1, 2, 3 and 4), sampling frame (Frame30 and Frame15), and their

interaction. The effect of the sampling dates (July-23, August-11, and August-30, 2012) was analyzed as means repeated in time (PROC MIXED, SAS).

RESULTS AND DISCUSSION

Soil fertility

The soil pH of the plots was alkaline, and differences were found in the clay content, which allows classifying the sampled soil as loamy clay or clay. The content of organic matter (OM) in the soil was rich and it did not present salinity. The macro and micronutrients (N, P, K, Ca, Mg, Fe, Cu, Zn and Mn) were found in adequate levels (>1.0 mg kg^{-1}) (Table 1), except for the zinc content (Zn) of 0.90 mg kg^{-1} in plot 1 MEX 69-290, considered as marginal. The soil from the four plots presented good fertility (Salgado-García *et al.*, 2013).

Sample of the optimal sample

The means and variances are lower in Frame15 than in Frame30 (Table 2). Approximately half the number of eggs was collected with Frame15 than with Frame30, but the variance was significantly lower. The samples obtained with Frame15 were more homogeneous. In addition, the sample sizes estimated with a high level of accuracy (0.1) (Villanueva-Jiménez *et al.*, 1993; Southwood and Henderson, 2000) with Frame15 were lower than those calculated with Frame30.

Spatial disposition of spotted spittlebug eggs

The variance/mean ratio and the Morisita index (I_δ) indicate that all the plots present aggregated distribution, except for plot 2, Frame15, variety MEX 69-290, where the variance/mean ratio ($0.938<1$) indicates random distribution. With the Morisita index ($0.989<1$) all the plots presented aggregated distribution. In every case, higher aggregation percentages (I_A) were obtained with Frame15 than those estimated with Frame30, which can indicate that the aggregation of eggs is higher in the 15 cm closer to the stems of the variant than the 30 cm closer to the variant (Table 3).

Effectiveness of the sampling unit in different plots

To compare the accuracy between Frame15 and Frame 30, the standard error ($S_{\bar{x}}$) was estimated, and the unit of effort (E_M) was calculated for efficiency (Table 4).

Table 1. Chemical and physical characteristics of the sol in the sugarcane plots sampled in the trial.

Plot	Variety	pH	Organic matter (%)	Electric conductivity (dS m^{-1})	Total Nitrogen (%)	Phosphorus (mg kg^{-1})	Potassium	Calcium	Magnesium	Iron	Copper	Zinc	Manganese	Texture
							cmol (+) kg^{-1}			mg kg^{-1}				
1	MEX 69-290	8.1	3.9	0.12	0.20	12	0.4	35.2	4.2	16	0.9	0.9	20	Clay loam
2	MEX 69-290	7.6	4.3	0.12	0.22	36	0.5	28.3	5.2	27	2.2	2.2	32	Clay
3	MEX 91-662	8.0	4.5	0.14	0.22	28	0.5	36.2	7.2	32	1.1	1.1	26	Clay
4	MEX 91-662	7.4	3.1	0.11	0.15	46	0.5	22.6	5.6	27	4.8	4.8	32	Clay loam

Table 2. Mean ($\bar{x}$), standard deviation (s) and optimal sample sizes ($\hat{n}$) of *Aeneolamia albofasciata* eggs in sugarcane plots obtained with Frame15 (M_{15}) and Frame30 (M_{30}).

Plot	Variety	$\bar{x}_{F15}$	s_{F15}	$\hat{n}_{F15}$	$\bar{x}_{F30}$	s_{F30}	$\hat{n}_{F30}$
1	MEX 69-290	2.7121	1.7167	40	4.4274	3.5881	65
2	MEX 69-290	3.4994	1.8124	26	6.6557	3.9214	34
3	MEX 91-662	2.7484	2.0836	57	4.4063	3.4517	61
4	MEX 91-662	4.4485	2.2297	25	7.8406	4.5478	33

Table 3. Variance/mean ratio, Mosirita index (I_δ) and Lloyd's mean agglomeration index (I_A) to determine the degree of aggregation of *Aeneolamia albofasciata* eggs in sugarcane plots obtained with Frame15 (M_{15}) and Frame30 (M_{30}).

Plot	Variety	$\frac{s^2}{\bar{x}_{F15}}$	$I_{\delta_{F15}}$	$I_{A_{F15}}$	$\frac{I_{A_{F15}}}{\bar{x}}$ (%)	$\frac{s^2}{\bar{x}_{F30}}$	$I_{\delta_{F30}}$	$I_{A_{F30}}$	$\frac{I_{A_{F30}}}{\bar{x}}$ (%)
1	MEX 69-290	1.086	1.041	1.031	38	2.907	1.439	1.430	32
2	MEX 69-290	0.938	0.989	0.982	28	2.310	1.201	1.196	17
3	MEX 91-662	1.579	1.222	1.210	44	2.703	1.394	1.386	31
4	MEX 91-662	1.117	1.032	1.026	23	2.637	1.212	1.208	15

s^2=variance; $\bar{x}$ =mean; I_A=aggregation index.

The time to obtain 40 samples with Frame30 was 5.89 h, while to obtain the samples with Frame15 it was 2.44 h; in addition, the sampling effort (E_M) obtained with Frame15 was lower than with Frame30 (Table 4). Therefore, the use of Frame15 was more efficient in function of the standard error and the sampling effort. This agrees with what was reported by King (1975), who demonstrated that the use of small samples is as efficient to estimate spittlebug eggs as the samples of larger size.

The plot and the sampling frame

Table 5 presents the separation of Tukey's means for the number of spotted spittlebug eggs, the plots used and the sampling frames. The factorial analysis (PROC GLM, SAS) showed significant differences between the frames ($P<0.0001$) and between the plots ($P<0.0001$), but not between the interaction frames-plot ($P=0.1509$). The differences between plots can be due to local and ecological effects, as well as to differences in

Table 4. Comparison of the effectiveness between Frame15 (M_{15}) and Frame30 (M_{30}) to obtain samples of *Aeneolamia albofasciata* eggs in sugarcane plots, based on the standard error ($S_{\bar{x}}$) and the sampling efficiency (E_M).

Plot	Variety	$S_{\bar{x}_{F15}}$	$E_{M_{F15}}$	$S_{\bar{x}_{F30}}$	$E_{M_{F30}}$
1	MEX 69-290	0.307	0.751	1.877	11.058
2	MEX 69-290	0.361	0.881	2.273	13.389
3	MEX 91-662	0.528	1.289	1.725	10.164
4	MEX 91-662	0.627	1.532	3.112	18.33

management. In the four plots of this study, oviposition happened in slightly alkaline soils and with clay contents higher than 36%, but the differences in the soil are so small in texture, humidity, or pH, that they could hardly be attributed to these factors.

Sampling dates and egg density

The analysis of measurements repeated in time (PROC MIXED, SAS) of the total eggs extracted did not show significant evidence of the effect of the sampling date ($P=0.4568$) or of the interaction sampling date-sampling frame ($P=0.2659$), although it reiterates the differences found between sampling frames ($P<0.0001$) (Table 6).

According to García-García *et al.* (2006), one month after the rainy season begins, a large amount of eggs can be found from the mixture of different generations; that is, those recently oviposited, as well as eggs with different degrees of diapause (Morales and Gallardo, 1996). The samplings were carried out at the end of the months of July and August, during the rainy season, time when the spotted spittlebug population was already established in the entire crop.

These biological characteristics explain the average number of eggs found in 250 g of soil analyzed with both frames (Table 6). Therefore, a greater agglomeration was observed, with lower variance and standard error in the number of spotted spittlebug eggs found in the area defined by the Frame15, as indicated by the aggregated disposition found both by the variance/mean ratio, the Morisita index, and Lloyd's mean conglomeration index.

Table 5. Means separation and Tukey grouping ($p\leq0.05$) of the variables variety and plot in sampling of *Aeneolamia albofasciata* eggs in sugarcane, with sampling frames Frame15 and Frame30.

Sampling Frame	$\bar{x}$	Plot	$\bar{x}$
Frame15 (A)*	3.3521	4 (MEX 91-662) (A)*	4.4486
		2 (MEX 69-290) (A)	3.4995
		3 (MEX 91-662) (B)	2.7485
		1 (MEX 69-290) (B)	2.7122
Frame30 (B)	5.8325	4 (MEX 91-662) (A)	7.8407
		2 (MEX 69-290) (A)	6.6557
		1 (MEX 69-290) (A)	4.4275
		3 (MEX 91-662) (A)	4.4064

* Different letters in the same column denote statistical significant differences.

Table 6. Mean, standard deviation and Tukey grouping ($p\leq0.05$) of total *Aeneolamia albofasciata* eggs by sampling frame (Frame15 and Frame30), through time, year 2012.

Sampling Frame	$\bar{x} \pm s$	Date	$\bar{x} \pm s$
Frame15 (A)*	3.3521 ± 2.0774	1 (jul/23) (A)	3.4994 ± 1.8124
		2 (aug/11) (A)	3.3590 ± 1.9255
		3 (aug/30) (A)	3.9327 ± 2.1469
Frame30 (B)	5.8325 ± 4.1374	1 (jul/23) (A)	6.6557 ± 3.9214
		2 (aug/11) (A)	5.5513 ± 4.3801
		3 (aug/30) (A)	5.4653 ± 3.7266

* Different letters in the same column denote statistical significant differences.

CONCLUSIONS

The use of a sampling frame for spotted spittlebug eggs of 15×15×5 cm (Frame15) reduces the volume of soil extracted and decreases the sampling effort. Its use allows obtaining more accurate samples and with lower variation than those obtained with the traditional sampling frame of 30×30×5 cm (Frame30). The estimation of the sampling size was also lower with Frame15. Therefore, the use of Frame30 can be substituted by Frame15 in sugarcane agriculture in Veracruz, Mexico.

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REFERENCES

García-García, C., López-Collado, J., Nava-Tablada, M.E., Villanueva-Jiménez, J.A., y Vera-Graziano, J. (2006). Modelo de predicción de riesgo de daño de la mosca pinta *Aeneolamia postica* (Walker) Fennah (Hemiptera: Cercopidae). *Neotropical Entomology 35*(5):677-688. DOI: 10.1590/S1519-566X2006000500017.

Parada-Domínguez, O., Alatorre-Rosas, R., Guzmán-Franco, A.W., Hernández-Rosas, F., Rojas-Avelizapa, L.I. y Ruíz-Vera, V.M. (2019). Efecto de nematodos entomopatógenos en ninfas de *Aenolamia albofasciata* y su persistencia en suelos cañeros de Veracruz. *Revista Mexicana de Ciencias Agrícolas 22*(1):115-127. DOI: 10.29312/remexca.v0i22.1862.

Alatorre-Rosas, R., y Hernández-Rosas, F. (2015). Mosca pinta, *Aeneolamia* spp. y *Prosapia* spp. (Hemiptera: Cercopidae). In: Arredondo-Bernal, H. y Rodríguez del Bosque, L.A. Casos de Control Biológico en México. Colegio de Postgraduados, México. pp.141-164. ISBN: 978-607-715-258-3.

Obando, J., Bustillo, A., Castro, U., y Mesa, N. (2013). Selección de cepas de *Metarhizium anisopliae* para el control de *Aeneolamia varia* (Hemiptera: Cercopidae). *Revista Colombiana de Entomología 39*(1):26-33. URL: http://www.scielo.org.co/pdf/rcen/v39n1/v39n1a05.pdf.

Hernández-Rosas, F. y Figueroa-Rodríguez, K.A. (2011). Barrenador (*Diatraea saccharalis*) y mosca pinta (*Aenolamia* spp. *Prosapia* spp.) en caña de azúcar y sus enemigos naturales. *Agroproductividad 4*(3):3-9. URL: https://revista-agroproductividad.org/index.php/agroproductividad/article/view/578/447.

Badilla, F. (2002). Un programa exitoso de control biológico de insectos-plagas en la caña de azúcar en Costa Rica. *Manejo Integrado de Plagas y Agroecología* 64:77-87. URL: https://repositorio.catie.ac.cr/handle/11554/6868.

Thompson, V., y León, G.R. (2005). La identificación y distribución de los salivazos de la caña de azúcar y los pastos (Homoptera: Cercopidae) en Costa Rica. *Manejo Integrado de Plagas y Agroecología* 75:43-51. URL: http://www.bio-nica.info/biblioteca/Thompson2005Cercopidae.pdf.

León-Hernández, M., González-Cárdenas, J.C., González-Acosta, A., González-Castro, A. y Alanís-Méndez, J.L. (2014). Control de la mosca pinta de pastos *Aenolamia* spp. con tres cepas del hongo *Metarhizium anisopliae*. *Revista Biológico Agropecuaria 2*(1):116-121. DOI: 10.47808/revistabioagro.v2i1.270.

Ramos-Hernández, F., Guzmán-Martínez, J.C., Cruz-Tobon, M. y Hernández-Rosas, F. (2018). Dinámica espacio-temporal de mosca pinta de la caña de azúcar en la zona centro del estado de Veracruz, XL Convención EXPOATAM 2018, Boca del Río, Veracruz. 1-10 pp.

Auad, A.M., Simoes, A.D., Leite, M.V., B. da Silva, S.E., dos Santos, D.R., y Monteiro, P.H. (2011). Seasonal dynamics of egg diapause in *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) on elephant grass. *Arquivos do Instituto Biológico 78*(2):325-330. DOI: 10.1590/1808-1657v78p3252011.

Bautista-Galvéz, A. y González-Cortés, A. (2005). Tres dosis de *Metarhizium anisopliae* sobre mosca pinta (*Aenolamia* spp.) en caña de azúcar en la región de Los Ríos, Estado de Tabasco. *Ciencia y Universidad 21*(41):37-40. URL: https://www.redalyc.org/articulo.oa?id=15404105.

Ortiz-Laurel, H., Rosas-Calleja, D., Rossel-Kipping, D., Herrera-Corredor, J.A. (2014). Mecánica para la contención de eclosión de huevos de mosca pinta (*Aeneolamia* spp.) en caña de azúcar (*Saccharum officinarum*). *Agroproductividad 7*(2):27-34. URL: https://revista-agroproductividad.org/index.php/agroproductividad/article/view/510/391.

NOM-021-RECNAT. Norma Oficial Mexicana (2001). Especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreo y análisis. Secretaría del Medio Ambiente y Recursos Naturales. Diario Oficial de la Federación. 7 diciembre 2001. URL: http://www.ordenjuridico.gob.mx/Documentos/Federal/wo69255.pdf.

Villanueva-Jimenez, J.A., De Yta, C.E.J., Alatorre-Rosas, R. y Ortega, A.L.D. (1993). La mosca pinta (*Aenolamia* spp.) en caña de azúcar de la región de Cardel, Veracruz: Avances en su diagnóstico, combate y propuesta de estimación de daño. Memoria. Simposio Nacional. La Capacitación y Desarrollo Tecnológico en el Campo Cañero Mexicano. Tomo II. GEPLACEA. Xalapa, Veracruz. 367-801 pp.

Southwood, T.R.E. and Henderson, P.A. (2000). Ecological Methods. Blackwell Science. 3.rd Ed. Oxford, UK. 594 pp. URL: https://www.researchgate.net/profile/Peter-Henderson-8/publication/260051655_Ecological_Methods_3rd_edition/links/556dd4ad08aeccd7773f64ca/Ecological-Methods-3rd-edition.pdf.

Karandinos, M.G. (1976). Optimum sample size and comments on some published formulae. *Bulletin of Entomological Society of America 22*(4):417-421. DOI: 10.1093/BESA/22.4.417.

Ledo, A., Condés, S. y Montes, F. (2012). Revisión de índices de distribución espacial usados en inventarios forestales y su aplicación en bosques tropicales. *Revista Peruana de Biología*. 19:113-124. URL: http://www.scielo.org.pe/pdf/rpb/v19n1/a17v19n1.pdf.

Lloyd, M. (1967). Mean Crowding. *Journal of Animal Ecology 36*(1):1-30. DOI: 10.2307/3012.

SAS Institute Inc. (2014). SAS/STAT® 14.1 user's guide. SAS Institute Inc., Cary, North Carolina, 10393 pp.

Salgado-García, S., Palma-López, D.J., Castelán-Estrada, M., Lagunés-Espinoza, L.C., y Ortiz-Laurel, H. (2013). Manual para el muestreo de suelos, plantas y aguas e interpretación de análisis para la producción sostenible de alimentos. Grupo MASCAÑA-LPI-2: AESS. Colegio de Postgraduados-Campus Tabasco. H. Cárdenas, Tabasco, México. 101 p.

King, A.B.S. (1975). The extraction, distribution and sampling of the eggs of the sugar-cane Froghopper, *Aeneolamia varia saccharina* (Dist.) (Homoptera, Cercopidae). *Bulletin of Entomological Research* 65:157-164. DOI: 10.1017/S000748530000585X.

Morales, S.J., y Gallardo, J.S. (1996). Diapausa y resistencia a la sequía de los huevos de *Aeneolamia varia* (Homoptera: Cercopidae) en Venezuela. *Bioagro 8*(1):3-13. URL: http://www.ucla.edu.ve/bioagro/Rev8(1)/1.%20Diapausa.pdf.