Crop Protection 74 (2015) 9–17

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Optimizing a fixed-precision sequential sampling plan for adult *Acrotomopus atropunctellus* (Boheman) (Coleoptera: Curculionidae), new pest on sugarcane

María L. del P. Pérez [a, *], Marcos G. Isas [a], Analía R. Salvatore [b], Gerardo Gastaminza [b], Eduardo V. Trumper [c]

[a] Instituto de Tecnología Agroindustrial del Noroeste Argentino (ITANOA), Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. William Cross 3150, C.P. T4101XAC Las Talitas, Tucumán, Argentina
[b] Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Argentina
[c] Instituto Nacional de Tecnología Agropecuaria (INTA), Ruta Nac. Nro. 9 km 636, C.P. 5988 Manfredi, Córdoba, Argentina

ARTICLE INFO

Article history:
Received 14 January 2015
Received in revised form
25 March 2015
Accepted 26 March 2015
Available online

Keywords:
Sequential sampling
Relative net precision
Taylor's power law
Green's model

ABSTRACT

Sugarcane weevil borer, *Acrotomopus atropunctellus* (Boheman) (Coleoptera: Curculionidae) has been detected across all sugarcane planting areas in the Argentinian Northwest with increasing population densities. The monitoring for its occurrence and the population density usually is made by visual inspection and consequently demands much effort and time. The objectives of this study were 1) to describe the sampling distribution pattern of *A. atropunctellus* adults 2) to develop and validate a fixed-precision sequential sampling plan for density estimation, and 3) to find the optimum inspection time for each sampling unit. On-farm data collection was performed at sugarcane fields located in Ranchillos (Tucumán, Argentina) during 2011–2012 to 2013–2014 sugarcane growing seasons. Thirty sampling units consisting on one meter of sugarcane furrow were randomly selected at 1-wk intervals. Within each sampling unit, weevils were counted and recorded independently for five increasing examination time per sampling unit (ETSU) (2, 4, 6, 8 and 10 min). For each ETSU, the sampling distribution pattern was assessed by Taylor's power law (TPL). The average sample number (ASN) and sampling stop lines were calculated according to Green's sequential sampling model, based on TPL estimated parameters, for fixed precision levels, C = 0.1 and C = 0.25. The resampling for validation of sample plans (RVSP) program was used to evaluate the performance of the different sampling plans. Parameters *a* and *b* from TPL regressions did not vary significantly between different ETSUs. All estimates of *b* coefficients were significantly >1 which can indicate an aggregated sampling distribution pattern. For each precision level, Green's sequential plans predicted very similar ASN between ETSU. This was confirmed through the validation process, with the five sampling protocols providing very similar mean sample sizes and mean precision levels. Variability of these parameters from validation results did not vary significantly among the different ETSUs. The relative net precision was the only performance parameter that varied with the ETSU, with the shortest ETSU resulting in the most efficient sampling plan. We conclude that *A. atropunctellus* has an aggregated sampling distribution and that the fixed precision sequential sampling plan developed using Green's model and based on a two-minute inspection of the sampling unit is the most convenient choice for estimating its population density in sugarcane. Our analysis of the ETSU effect on the performance of sugarcane weevil sampling protocols could contribute to develop more efficient monitoring plans for other arthropods.

1. Introduction

Sugarcane (*Saccharum* spp) is the main raw material for sugar production in the world (Romero et al., 2009). In addition, this crop is used to produce bio-ethanol for renewable energy, fiber for

* Corresponding author.
E-mail addresses: mlperez@eeaoc.org.ar (M.L.P. Pérez), marcosisas@hotmail.com (M.G. Isas), asalvatore@eeaoc.org.ar (A.R. Salvatore), ggastaminza@eeaoc.com.ar (G. Gastaminza), eduardo.trumper@gmail.com (E.V. Trumper).

http://dx.doi.org/10.1016/j.cropro.2015.03.024
0261-2194/

paper, fertilizer, alcoholic beverages and forage (Bastos Andrade and Cardoso, 2010; Dos Anjos et al., 2010; Ripoli and Ripoli, 2010; Rivera de Castillo, 1980). Sugarcane production is affected by many arthropod pests which cause significant crop and industrial yield losses (Dinardo-Miranda, 2010; Goebel and Sallam, 2011; Long and Hensley, 1972). Within the order Coleoptera, the Curculionidae family includes many pest species of sugarcane around the world (Lanteri et al., 2002; Terán and Precetti, 1982). One of them is the sugarcane weevil borer, *Acrotomopus atropunctellus* (Boheman) (Coleoptera: Curculionidae). When infestations occur in early plant phenological stages, this insect can cause the death of sugarcane's shoots due to perforations caused by adults and tunnels bored by the larvae. Furthermore, the larva decreases sugarcane stool longevity. *A. atropunctellus* has been detected across all sugarcane planting areas in the Argentinean Northwest with increasing population densities, raising concerns about potential economic impact on sugarcane production (Salvatore et al., 2006).

The design of a sound Integrated Pest Management (IPM) strategy should be based, among other principles, on knowledge about the relationship between pest infestation levels and crop damage (Higley and Pedigo, 1997) and on key aspects of the pests population ecology (Binns et al., 2000; Castle and Naranjo, 2008; Dhawan and Peshin, 2009). Sampling is performed to quantify pest densities, treatment thresholds, and pest forecasting (Parajulee et al., 2006). The development of sampling plans for estimating population density, including the size of the sampling unit, the number of sampling units to take, and the allocation of sampling units within the sample universe, depends on an understanding of the underlying spatial distribution of an insect (Naranjo and Flint, 1994). Sequential sampling schemes for estimating the population density of *A. atropunctellus* with fixed statistical precision would be valuable for ecological and pest management research in sugarcane to facilitate efficient use of time. The efficiency of a sampling plan can be assessed in terms of the precision attained relative to the cost of sampling, which in turn is determined by the cost of field scout manpower (man hours). Apart from the influence of sample size (number of sampling units), the cost of sampling depends on the size of the sampling unit or the time devoted to its inspection. Morris (1955) established that the sampling unit should be of such a size as to provide a reasonable balance between the variance and the cost. Although too few sampling units can reduce the reliability of the estimate, taking too many samples increases the cost of the sampling program (Dent, 2000).

The sampling methods used for most sugarcane pests are traps (Coleoptera and Lepidoptera) and visual observation (Dinardo-Miranda, 2010). Currently, as no traps have been developed for *A. atropunctellus*, monitoring is made by visual inspection of stalks, leaves and soil. Consequently, sampling demands much effort and time. Sampling protocols generally are developed after a sampling technique and procedure have already been established upon a given sampling unit size (Dogramaci et al., 2006; Soltani Ghasemloo and Aleosfoor, 2013). However, the size of the sampling unit can have a strong influence on the efficiency of the protocol (Jian et al., 2011; Rossi and Nuutinen, 2004; Serra and Trumper, 2006; Southwood, 1978; Underwood and Chapman, 1996) because it depends on the distribution pattern.

An additional aspect of the sampling procedure that could affect efficiency, in a similar way to the size of the sampling unit, is the time assigned to the visual inspection of the sampling unit. Sampling units for arthropod monitoring are usually defined in terms of units of space (for example crop row meter, crop squared meter, soil or water volume in cubic meter) or in terms of ecological units (leaf or any other plant organ, plant, etc.). When arthropods are difficult to find within the sampling unit because of their size, behavior and/or color similarity with the substrate, it is not always clear when to end sampling efforts. Furthermore, in some cases the only way scouting personnel can be sure when to end inspection of the sampling unit, is implementing a procedure to get an absolute measure. However, this would be extremely time consuming. Counting small insects that are often concealed in big plants usually requires some sort of stopping rule, for example a fixed number of minutes (Kelsey, 1968). However, if visual examination of each sampling unit is ended too soon, insect counts can underestimate the true density, which will impede robust description of the sampling distribution.

When the time devoted to each sampling unit is reduced, the population density can be underestimated, which affects the statistical characterization of the sampling distribution. Accordingly, the distribution pattern is more difficult to detect since a low density can mask the real spatial distribution. When population density is low, the sequential sampling plans requires more time, which makes it difficult to implement. The objectives of this study were 1) to describe the distribution pattern of *A. atropunctellus*, 2) to find the appropriate inspection time for each sampling unit, and 3) to develop and validate a fixed precision sequential sampling plan for the adult weevils.

2. Materials and methods

2.1. Field sampling

The monitoring was carried out in commercial ratoon sugarcane fields (cultivar LCP85-384) located in Ranchillos (26°59′ 5″ S, 65°00′ 36″ W), Tucumán, Argentina. The data collection was conducted from November to April during growing seasons 2011–2012, 2012–2013 and 2013–2014. In each growing season, one sugarcane plot of 1.6 ha was sampled repeatedly at 1-wk intervals. Thirty samples were randomly selected at each date. Each sampling unit consisted of one meter of sugarcane furrow and five examination times per sampling unit (ETSU) (2, 4, 6, 8 and 10 min). For each ETSU, the monitoring was conducted by visual inspection of the soil surface, then the stalks and leaves of the plants. The same sequence was applied with every ETSU. The time allotted to inspection of the soil surface was a small proportion of the ETSU because according to prior experience most of the weevils are found above ground. Consequently, greater ETSUs had smaller proportions of time allotted to inspection of the soil surface. Based on a prior monitoring experience the maximum ETSU was set at 10 min in order to maximize the probability of finding insects, particularly on bigger plants with more microhabitats for hiding. In each sampling unit, adult weevils were sequentially counted and recorded for each ETSU.

2.2. Sampling distribution

Taylor's power law (TPL) establishes a relationship between the variance (S^2) and the mean density (m), $S^2 = am^b$, that is considered to be constant and characteristic for each species (Taylor, 1961). Most of the specific literature consider the parameter a to be a sampling factor depending on the size of the sampling unit and characteristics of the habitat from which the sample is taken (Taylor, 1961), while coefficient b is frequently interpreted as an index of aggregation (Taylor et al., 1978; Young and Young, 1998). However, it has been shown that both a and b relate to the variance/mean ratio (Wilson, 1994), and to k, the negative binomial dispersion parameter (Binns et al., 2000). Consequently, both TPL parameters describe the spatial pattern of a population, given a specific sampling technique and sampling unit size.

A log–log transform was used to linearize the TPL equation ($\log S^2 = \log a + b \log m$), and linear regression was used to estimate a

and b, where a is the antilog of the intercept and b, the slope of the regression. Homogeneity of both TPL regression parameters for growing seasons 2011–2012, 2012–2013 and 2013–2014 were tested incorporating the growing season as dummy variables in the regression analysis (Draper and Smith, 1998) before pooling the data. Then, with the data of the three growing seasons combined, linearized TPL was fitted for each ETSU. The intercepts and slopes of the fitted regressions were compared with values of zero and one, respectively, through one sample t-tests (Draper and Smith, 1998). The goodness-of-fit for each linear regression model was evaluated by coefficients of determination (R^2).

2.3. Sample size analysis

The average sample number (ASN) is the minimum expected number of sampling units (n) required to estimate the mean population with a fixed precision level, and can it be calculated according to Green's (1970) formula: $n = (am^{b-2})/C^2$, where m is the mean density and C, the precision level, is expressed as the relative variation of the mean (VR_m), and measured by SEM/m. The precision levels used were $C = 0.10$ and $C = 0.25$ which are usually considered as appropriate values for ecological research purposes and for pest management decision making, respectively (Southwood, 1978), and can be considered as two distinct scenarios to assess the robustness of the sampling protocols. ASN curves were calculated for each ETSU, by replacing a and b with the corresponding estimated values (see Section 2.2).

2.4. Sequential sampling protocol construction and validation

Fixed-precision sequential sampling stop lines were generated using the procedure detailed by Green (1970). The following equation was used: $T_n = (C^2/a)^{(1/1-b)}/n^{(b-1/b-2)}$, where T_n is the number of insects accumulated throughout n sampling units; C is the precision level as defined before, a and b are coefficients from TPL regression.

The resampling for validation of sample plans (RVSP) program was used to validate a fixed-precision sampling plan based on Green's model (Naranjo and Hutchison, 1997). Twelve data sets were chosen at random from the three growing seasons with replacement. Data sets used for validation of the sampling plan were not included in development of the plan. With the fixed-precision sequential sampling plan, the simulation procedure randomly selected successive sampling units from each of the 12 data sets with replacement until the cumulative total (T'_n) was $\geq T_n$. Resampling simulations were repeated 1000 times for each data set. Mean actual density, mean sample number, and mean actual precision values were obtained for each data set of 1000 simulations.

2.5. Relative net precision

The assessment of sampling protocols with different ETSU was complemented through their relative net precision (RNP) (Buntin, 1994). RNP gives equal consideration to precision and time as variables, and incorporates the cost of a sampling method as a component of the level of precision, according to the following equation: $RNP = 1/(RV_m.SC)$, where RV_m is the relative variation of the mean and represents the precision level, and SC is the sampling cost. In this work, SC was considered as equivalent to sampling time, which in turn is composed of time for walking from one sampling unit to the next, and time for sampling unit processing. Because the first component was taken as constant, SC was considered directly proportional to time devoted to each sampling unit. Thus, RNP was calculated for each ETSU. Higher RNP values indicate higher efficiency levels. RNP was calculated according to two approaches. A conventional method, as originally proposed by Pedigo and Buntin, 1994 and Beschinski and Pedigo (1981) consisted of calculating the RNP with the sample mean and SEM from the 44 sampling data sets using a fixed simple size of 30 sampling units. As an alternative method (Burkness and Hutchison, 1998), RNP calculations were also done using actual mean estimates of sample size and precision from Green's plan, through resampling simulations using specific software as explained in Section 2.4. Then, within each approach, the effect of the ETSU on RNP was assessed through analysis of covariance (ANCOVA), with weevil density as the covariate, and Tukey's *post hoc* tests. All statistical analyses were carried out with Infostat Software package (Di Rienzo et al., 2008).

3. Results and discussion

3.1. Sampling distribution pattern

With the longest ETSU (10 min) as a starting point, mean densities ranged considerably among growing seasons (Table 1). The TPL parameters were estimated from 56 data sets comprising the three growing seasons. TPL regressions yielded good fits, particularly in the last two growing seasons, with TPL intercept ($\log a$) and b values ranging from 0.14 to 0.18, and from 1.05 to 1.26, respectively (Table 1). The dummy-variable regression analysis indicated that neither the slope nor the intercept of TPL regressions differed significantly among the three growing seasons (Table 1).

Taking the pooled data set of the three growing seasons (2011/12–2013/14), TPL linear regression intercept ($\log a$) was significantly greater than 0 ($t = 5.12$; $df = 55$; $P = 0.0001$) indicating that regression through the origin was not appropriate. Following Wilson's (1994) approach for analyzing population dispersion patterns based on TPL equation ($S^2 = am^b$), the combination of a and b values estimated for the pooled data (Table 1), yield variance-to-mean ratios greater than 1 for most of the mean density range. From this standpoint the dispersion pattern of *A. atropunctellus* populations in sugarcane appears to be mostly aggregated. Several species of weevils in different crops (Faleiro et al., 2002; Monterrey et al., 1994; Salama and Abd-Elgawad, 2010) including the sugarcane pests *Rhabdoscelus obscurus* (Boisduval) (Coleoptera: Curculionidae) in Australia and *Sphenophorus levis* Vaurie in Brazil (Sabongi Izeppi et al., 2014; Sallam and Garrad, 2001), have been categorically reported to show aggregated distributions.

In order to assess the potential effects of limiting the inspection time within each sampling unit on the characterization of the sampling distribution, the TPL was fitted for each ETSU taking the pooled data set of the three growing seasons. TPL parameters were estimated from 44 data sets, while the remaining 12 data sets were used for resampling validation analysis. The intercept ($\log a$) was significantly greater than 0 in all five regressions, again making regression through the origin unnecessary. The same analysis based on TPL equation as described above using the estimated a and b parameters from Table 2, shows that the dispersion pattern of *A. atropunctellus* in sugarcane is mostly aggregated, regardless of the ETSU.

TPL regressions provided a good fit to the sampling distribution data. Although the density range of *A. atropunctellus* recorded in our work was not as wide as often found for other pests and crops, it should be representative of its incidence on sugarcane in Northeastern Argentina considering it resulted from three years of monitoring with little variation among growing seasons. In similar studies carried out with rather narrow insect density ranges, TPL yielded very good fits to the sampling distributions (see for example Butler and Trumble, 2012; Prager et al., 2013).

Table 1
Taylor's power law regression statistics and parameter estimates (+/−SE) for each growing season and for all three seasons of pooled data, with 10 min examination time per sampling unit.

Growing season	n	b (SE)	a (SE)	MSE	F	p	R^2	Mean density range
2011/12	17	1.25 (0.187)	1.53 (1.21)	0.84	45.03	0.0001	0.73	0.13–0.60
2012/13	20	1.22 (0.095)	1.51 (1.11)	3.86	164.59	0.0001	0.90	0.03–1.60
2013/14	19	1.05 (0.061)	1.13 (1.07)	1.75	296.83	0.0001	0.95	0.06–1.13
Pooled data	56	1.18 (0.06)	1.38 (1.07)	6.74	415.85	0.0001	0.88	0.03–1.60

Table 2
Taylor's power law regression statistics and parameter estimates (+/−SE) for different examination time per sampling unit (ETSU). Regressions were fit with the pooled data set from growing seasons 2011/12–2013/14.

(ETSU)	n	b (SE)	a (SE)	MSE	F	p	R^2	Mean density range
2	44	1.124 (0.042)	1.39 (1.09)	11.21	732.7	0.0001	0.94	0.03–1.06
4	44	1.164 (0.055)	1.48 (1.08)	6.85	442.25	0.0001	0.91	0.03–1.2
6	44	1.167 (0.053)	1.47 (1.07)	6.04	483.98	0.0001	0.92	0.03–1.4
8	44	1.177 (0.068)	1.41 (1.08)	6.03	301.1	0.0001	0.87	0.03–1.6
10	44	1.167 (0.06)	1.40 (1.07)	5.88	327.01	0.0001	0.88	0.03–1.6

Consequently, we believe our TPL parameter estimates are reliable for characterizing the sampling distribution of *A. atropunctellus* on sugarcane.

Green's sequential sampling model depends on the values of the TPL parameters estimated through regression analysis. Even small changes in these values can give rise to important changes in the sampling effort (number of sampling units) required to achieve a given precision level. Hence considering the factors that can cause these coefficients to change is relevant. In this work, physical sampling units were established as 1 m of furrow. However the time devoted to the visual inspection of plants within each meter of furrow determined different effective sizes of sampling units. It has been argued that TPL's parameters are affected by the size of the sampling unit (Southwood, 1978). Consequently, estimates of *a* and *b* were expected to change with different ETSUs. If the weevils density obtained with the longest ETSU (10 min) is taken as the reference level, shorter ETSUs can be considered as sub-samples and then the shorter the ETSU, the higher the sampling error in terms of estimation of the real density per sampling unit. Also, shorter ETSUs determine narrower ranges of weevil numbers with lower means (Table 2), which could have a statistical influence *per se* on the estimation of the TPL parameters. On the other hand, it can be considered that taking shorter ETSUs truncates the actual frequency distribution of weevils numbers per sampling unit, leading to a more uniform distribution because the less time available for visual inspection of the plants, the less opportunity to detect differences among sampling units. If this was the case, shorter ETSUs should have yielded lower values of the TPL *a* and/or *b* parameters, consistent with a less aggregated sampling distributions (Wilson, 1994; Binns et al., 2000). Despite these predicted effects, TPL regression parameters did not differ significantly among ETSUs (Table 2). Arguably the increase in plant size along the crop growing season could have a similar effect to that expected from increasing the ETSU. However, considering that the range of *A. atropunctellus* densities was narrow, plant size did not seem to influence the counts. The spatial resolution of the sampling unit (1 m of furrow) seems to provide a robust representation of the real spatial distribution of weevils, and consequently of its sampling distribution.

3.2. Sample size analysis

The average sample number (ASN) expected with Green's method specific for different ETSU and precision levels $C = 0.1$ and $C = 0.25$ are represented in Fig. 1. Within each precision level, ASN curves for different ETSUs were close to each other, indicating that very similar sample sizes are required regardless of the ETSU. For example, in the 2012/13 growing season the average *A. atropunctellus* density was nearly 1 insect m^{-1}. The estimation of this population density with a precision level $C = 0.10$, using 2, 4, 6, 8 and 10 ETSU sampling plans, would require sample sizes of 148, 155, 155, 148 and 148 sampling units, respectively. With a desired precision $C = 0.25$, sample sizes of 24, 25, 25, 24 and 24 units would be required, respectively. Thus, sampling protocols based on visually inspecting 1 m of sugarcane furrow are not sensitive to the ETSU in terms of predicted sample size. Smaller sampling units or shorter ETSUs are expected to generate estimates of arthropod densities with greater variances and hence require more sampling units to achieve the same reliability of estimations. However, the sampling effort required with the different ETSUs does not differ, as shown above. This remarkable similarity in the sample sizes required by protocols with different inspection times assigned to the same spatial unit is a direct consequence of the similarity of the TPL parameters discussed above. A similar result was reported by Serra and Trumper (2006) in large larvae of *Spodoptera frugiperda* (Smith), where the sequence of sampling unit sizes did not result in a sequence of ASN curves in the same order.

3.3. Validation of the sequential sampling protocol

To implement a sequential sampling program, the monitoring is carried out until the cumulative number of insects reaches or surpasses the stop line for a given required precision level. The mean population density can be estimated as the slope of this curve at the sample size n, i.e. as T_n/n (Naranjo and Castle, 2010; Pedigo and Buntin, 1994). For example, if the stop line is reached at the 17th sampling unit with a cumulative number of insects of 24, the density of weevils is 1.4 weevils m^{-1} (24 weevils/17 m). Because critical or stop lines of a sequential sampling protocol are completely consistent with the ASN curves as they have the same origin (Green, 1970) and both types of curves represent the predicted sampling effort required to achieve a predefined precision level, the performance of the sampling protocol was assessed through the following validation results.

The twelve independent data sets used for the resampling simulations covered a density range from 0.17 to 1.13 insects m^{-1}, which was very similar to the density range of the whole data sets used for estimating TPL parameters (Table 2). Bootstrap resampling

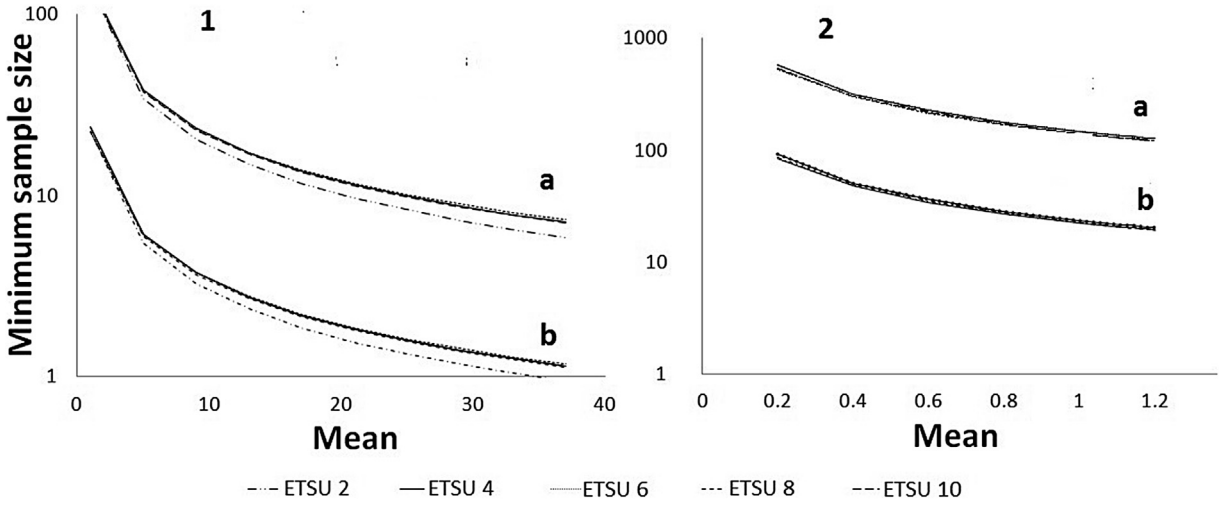

Fig. 1. Average sample size (ASN) required to estimate density of *A. atropunctellus* with fixed precision levels C = 0.10 (a) and C = 0.25 (b) for 2, 4, 6, 8 and 10 min of examination time (ETSU). For a clear visual discrimination of the curves, axis was transformed to logarithmic scale and the same figure is presented in two scales 1 and 2.

simulation analysis for Green's sampling plans using different ETSU and desired precision of $C = 0.25$ demonstrated that the average sample number obtained was slightly higher (2–5%) than that predicted by Green's model (Fig. 2). The actual precision values obtained in the resampling simulation ranged from 0.24 to 0.22 depending on the ETSU. These slight improvements in precision levels (4–12%) attained by the sampling protocol with respect to the nominal precision (Fig. 3), could be explained as follows. Green's critical equation, under the assumption that sampling stops exactly on the boundary, can yield no integer T_n values, whereas, in fact, sampling can only stop once the sampling unit has been completely examined. Indeed, the implementation of sequential sampling plans rarely stops right on the critical line, but instead sampling units are collected until the number of accumulated individuals is greater than T_n, i.e. above the stop line required to achieve the predefined precision level. In other words our predictions had higher average precision than desired and, consequently, on average more sampling units were taken than necessary. Another issue to consider is the variability of sample size and precision. Although the real ASN and precision levels obtained through resampling simulation ranged widely around the mean values, their coefficients of variation across the range of weevils densities evaluated were not higher than 4.5% and 2.9%, respectively, and did not vary with the ETSU of the sampling plan. Consequently, the sequential sampling protocol presented here appears as a reliable and robust tool for estimating *A. atropunctellus* density on sugarcane.

Similar results were obtained when desired precision was fixed at $C = 0.1$, with around 10% higher actual precision (=lower C) achieved by the sampling protocols. Hutchison et al. (1988) and Naranjo and Flint (1995) found that the fixed precision level can be relaxed (increasing C slightly above the nominal value) to achieve the desired actual average precision. However this calibration to get the exact precision level is beyond the aim of this work and the purpose of the sampling protocol performance assessment was primarily to allow for an informed selection of an ETSU. In this regard, the resampling simulations using sampling protocols with different ETSUs did not reveal any clear advantage of any one in particular.

The validation results suggest that, regardless of the ETSU, the performance of the fixed-precision sequential sampling plan using Green's model for estimating *A. atropunctellus* density on sugarcane is acceptable. Precision achieved using the sampling plan described in our work departed from expected precision by around 10% while, for example, Elliott et al. (2003) report 20% differences between expected and observed precision using their sampling plans for *Schizaphis graminum* (Rondani) and *Rhopalosiphum padi* (Linnaeus) in winter wheat (*Triticum aestivum* Linnaeus).

3.4. Optimum sampling protocol

Choosing the best ETSU can be based on the balance between the precision level, C (=RV), and the sampling cost, SC, which in turn is determined by the ASN and the ETSU. As explained before, these parameters are condensed in the RNP. Given a predetermined, fixed precision level C, the lower the SC (the higher RNP), the better, and since increasing ETSU implies increasing SC, the only way for higher ETSU to counterbalance this effect is by a reduction of ASN. This assessment can be done in terms of predicted and real performance of the sampling protocols.

Considering the first criterion, the different ETSUs did not result in relevant predicted differences of ASN, as shown in Section 3.2 (Fig. 1). However, the total cost in time did vary dramatically increasing almost as a power of two with each 2-min increase in ETSU. For example, specific Green's equations predict that with a density of 1 weevil m^{-1}, sample sizes of 23, 24, 24, 23 and 23 sampling units would be required to achieve a precision level of $C = 0.25$ while the total examination time would be 46, 96, 144, 188 and 230 min for 2, 4, 6, 8 and 10 ETSU, respectively. On the other hand, precision is supposed to be equal among the sampling protocols with different ETSU because for the sake of comparison these protocols were set with the same fixed precision parameter ($C = 0.25$ in this example). Thus, the only relevant effect of increasing the ETSU, predicted by Green's equations, is an increase in sampling cost and consequently a decrease in the RNP.

When the same analysis was carried out with the results of the resampling simulations, two issues were considered. First, while predictions from sequential sampling protocols are deterministic and hence only one result can be obtained for each population density and any given precision level plugged into the model equation, resampling simulations provided variability both in terms of sample size and real precision. Because we calculated RNP for each one of the 1000 simulation runs from each data set, for nominal precisions $C = 0.1$ and $C = 0.25$, such variability could be assessed. Second, as SC is partially determined by sample size and this, in turn, is strongly affected by population density of the target

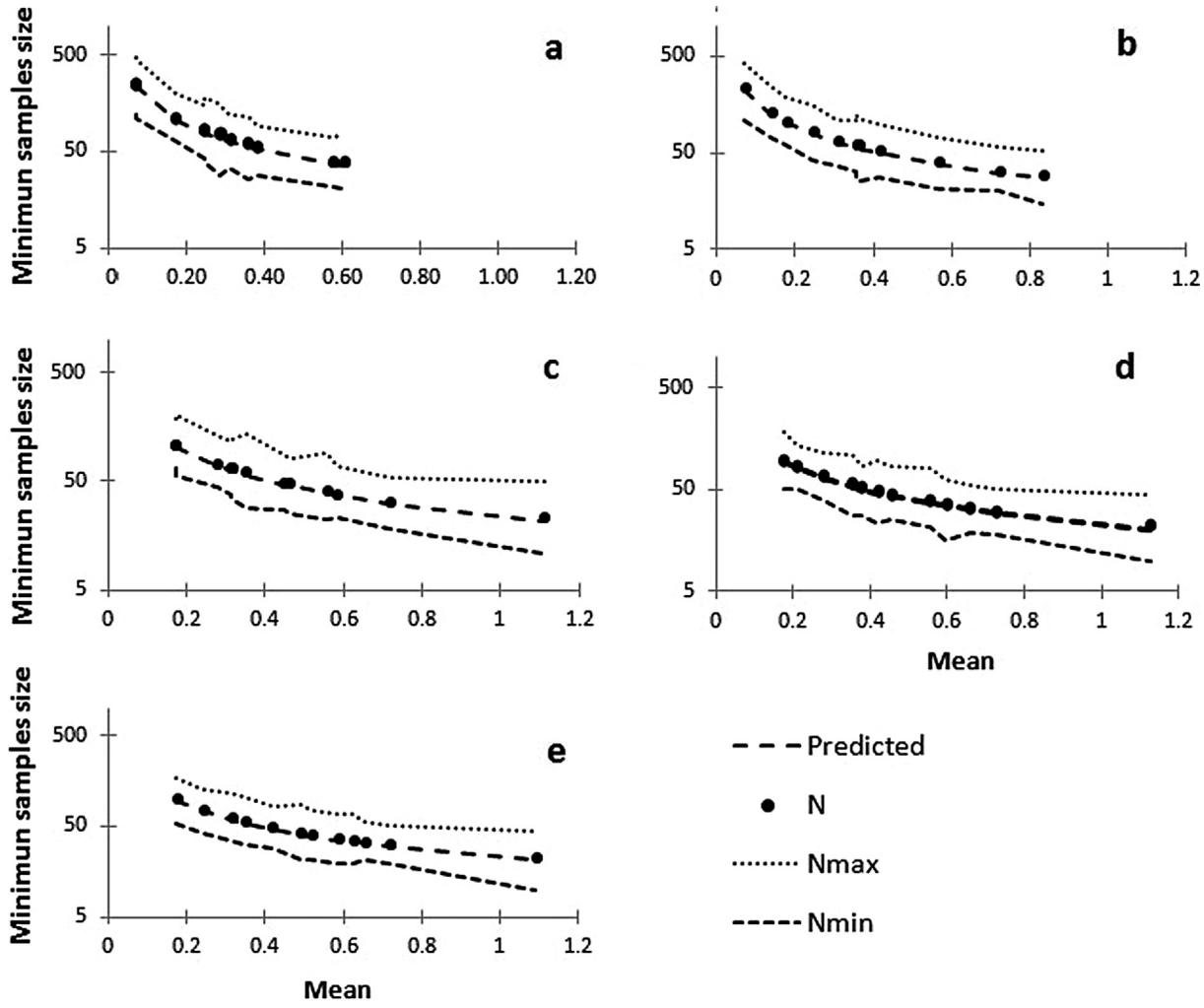

Fig. 2. Validation of fixed-precision sequential sampling plans based on Green's (1970) method for estimating the density of *A. Atropunctellus* adults on sugarcane based on resampling of twelve independent data sets. Average sample number obtained with fixed precision level C = 0.25 for 2 (a), 4 (b), 6 (c), 8 (d) and 10 (e) minutes of examination time per sampling unit, compared with that predicted by Green's model.

species, the comparison of RNP among ETSUs had to filter the potential effect of density on RNP using density as a covariate in the ANCOVA.

The analysis of resampling simulation results showed that both sample size (Fig. 2) and precision (Fig. 3) varied around the predicted value for any given population density. The extent of this variation is represented by coefficients of variations ranging 16.2–22.3% and 3.8–15%, respectively. A slight trend of higher precision variability with longer ETSU was found, with ETSU = 2 showing the lowest level of precision coefficient of variation. Although a narrow range of real precision achieved by a sampling protocol should be preferred, we believe the difference between ETSU is not big enough to have any practical consequences. The same kind of analysis showed that RNP also varied, with coefficients of variations ranging 21.7–29.6%. However, the ETSU did not affect this variability significantly. On the other hand, taking weevils density as a covariate, the ANCOVA of the RNP values generated through resampling simulations allowed the identification of significant effects of the ETSU both with C = 0.1 ($F = 40.4$; df:5.57; $P < 0.0001$) and C = 0.25 ($F = 30.9$; df:5.59; $P < 0.0001$) precision levels. The post hoc analyses yielded the same ranking of ETSUs with both precision levels showing a clear trend of higher RNP values with smaller ETSU (Table 3).

With both methods for calculating RNP and both precision values, the results were similar and the sampling protocol based on ETSU = 2 was significantly more efficient. Considering this conclusion, the corresponding stop lines for the sequential sampling plan aiming at two precision levels were calculated (Fig. 4). Sequential sampling stop lines increased with greater levels of precision and increasing weevils per sampling unit. Constant precision sequential sampling stop lines were calculated using Green's model (See Section 2.4). Tables for required sample sizes to achieve any desired level of precision can be obtained by substituting the estimates of *a* and *b* from Table 2 into Green's equation and calculating *Tn* for a range of mean density values. Assuming that these sampling plans yield the average specified level of precision *C*, they will provide a sampling method for interested readers to obtain time-efficient estimates of population density. As previously shown (Section 3.3), the average precision achieved by the sequential sampling plan was slightly higher (smaller C value) than expected. The lack of any trend in Fig. 3 indicates that the sequential sampling plans achieved unbiased estimates of C.

Although our work did not strictly assess the influence of different spatial units on the characterization of sampling spatial distributions, we intended to explore whether the ETSUs assigned to each sampling unit have similar effects and potential

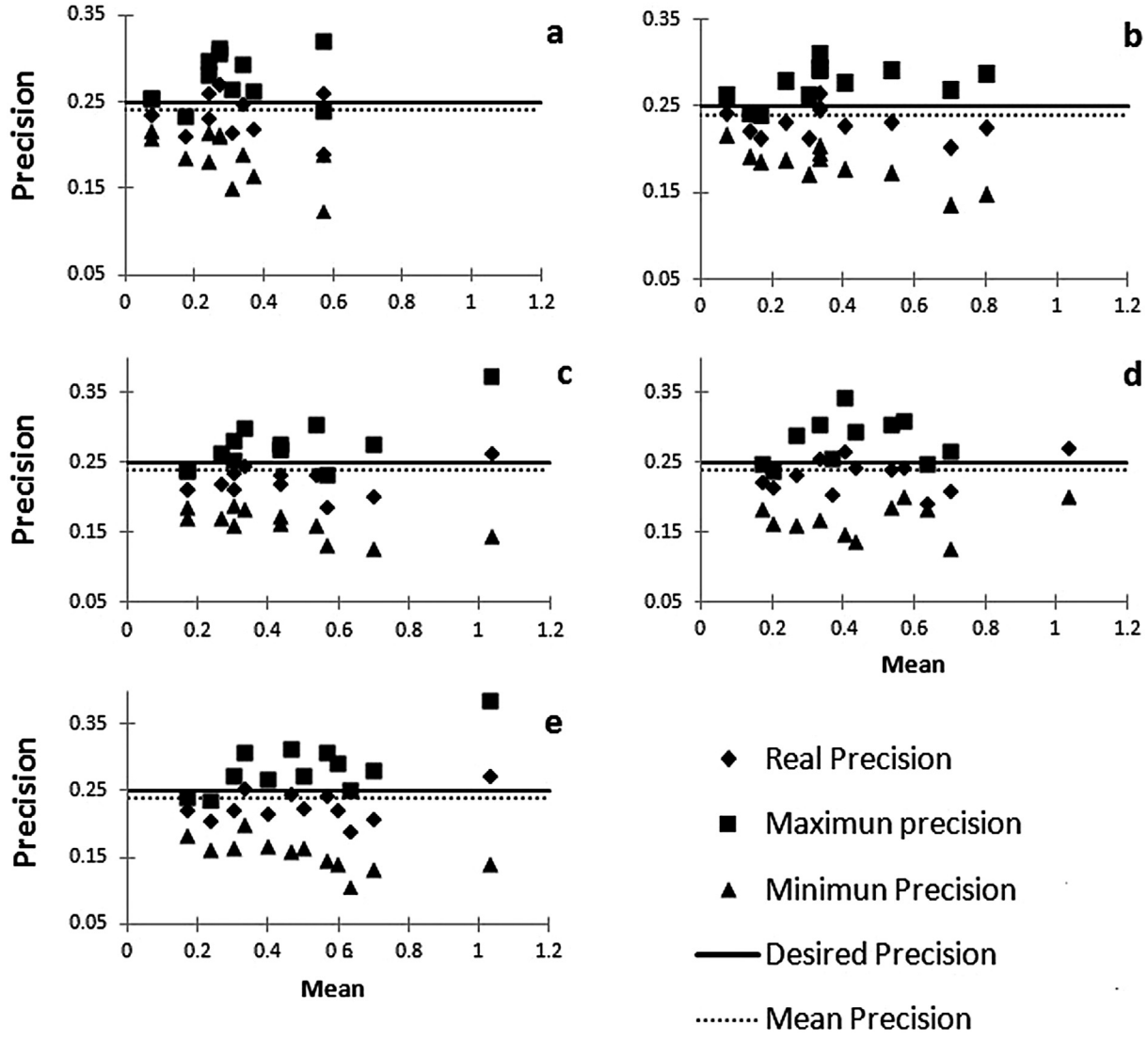

Fig. 3. Precision (mean, maximum and minimum) obtained at different means by using bootstrap simulations for 2 (a), 4 (b), 6 (c), 8 (d) and 10 (e) minutes of examination time per sampling unit with a fixed precision C = 0.25.

Table 3
Relative net precision (RNP) values for each examination time per sampling unit (ETSU), with observed and validation data sets.

ETSU (minutes)	Observed		Validation			
	Mean RNP	S.E.	Precision 0.10		Precision 0.25	
			Mean RNP	S.E.	Mean RNP	S.E.
10	0.01008[a]	0.00096	0.003323[a]	0.000672	0.007451[a]	0.001826
8	0.01260[a]	0.00096	0.004374[a]	0.000668	0.010213[a]	0.001812
6	0.01704[b]	0.00095	0.005822[ab]	0.000665	0.013947[ab]	0.001801
4	0.02527[c]	0.00095	0.008357[b]	0.000670	0.020229[b]	0.001808
2	0.04392[d]	0.00097	0.015211[c]	0.000739	0.034163[c]	0.001857

Different letters indicate significant differences between means ($P < 0.05$).

consequences on the efficiency of sampling protocols. Our results showed that the time devoted to counting weevils within a meter of furrow does not have a relevant influence on the estimation of Taylor's power law parameters. This result suggested that subsequent Green's sequential sampling protocols for different ETSU would yield similar ASN predictions for any given precision level. The validation of the five sampling plans showed very similar performances in terms of sample sizes required, mean real precision achieved, and variability of these parameters. Given these similarities, the only significant difference among the five sampling protocols was the RNP, with the shortest ETSU emerging as the best performance. We conclude that *A. atropunctellus* has an aggregated sampling distribution and that the fixed-precision sequential sampling plan based on a two minute inspection of the sampling unit is acceptable for estimating its population density on sugarcane. Our results about the influence of the time assigned to each

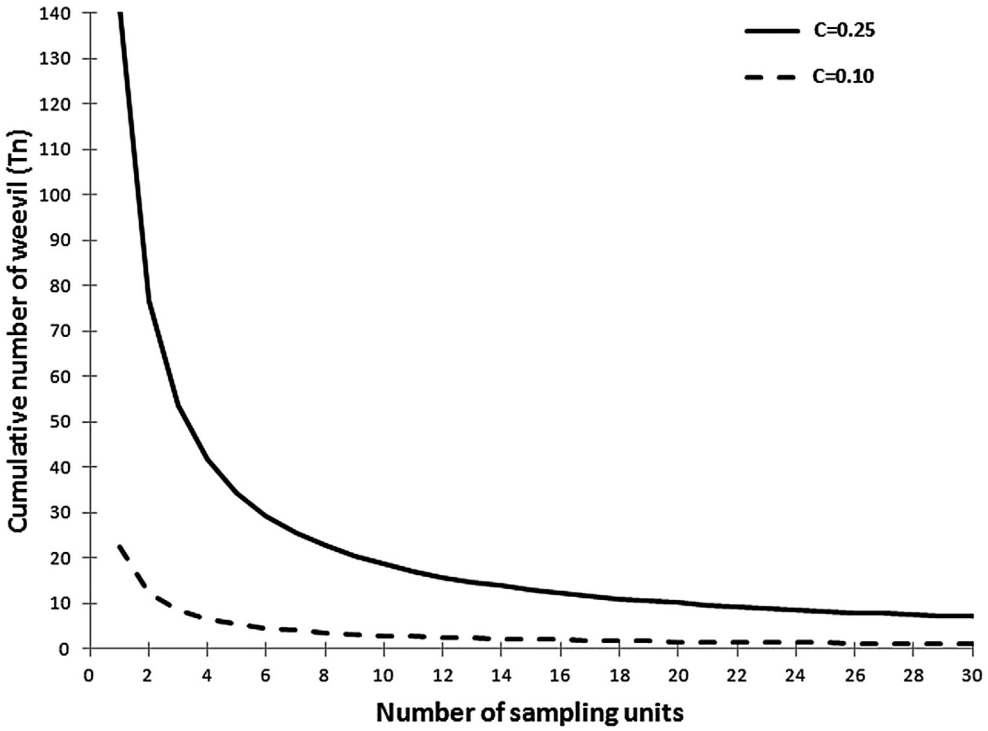

Fig. 4. Sequential sampling stop lines for precision levels C = 0.25 and C = 0.1 for estimating density of *A. atropunctellus* on sugarcane.

sampling unit on the performance of sugarcane weevil sampling protocols could contribute to develop more efficient monitoring schemes for other arthropods.

Acknowledgments

This study was supported by Estación Experimental Agroindustrial Obispo Colombres (EEAOC) and Instituto de Tecnología Agroindustrial del Noroeste Argentino (ITANOA) of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). We are grateful to the Editor and three anonymous reviewers for helpful comments on an earlier version of this manuscript.

References

Bastos Andrade, L.A., Cardoso, M.D.G., 2010. Cana de açúcar como produçao de cachaça. In: Dinardo-Miranda, L.L., Vasconcelos, A.C.M., Andrade Landell, M.G. (Eds.), Cana de açúcar. Instituto Agronômico, Campinas, pp. 747–762.

Beschinski, E.J., Pedigo, L.P., 1981. Ecology of predaceous arthropods in iowa soybean agroecosystems. Environ. Entomol. 10, 980–985.

Binns, M.R., Nyrop, J.P., Van der Werf, W., 2000. Sampling and Monitoring in Crop Protection. The Theoretical Basis for Developing Practical Decision Guides. CAB International, Wallingford, Oxon, p. 284.

Burkness, E.C., Hutchison, W.D., 1998. Action thresholds for striped cucumber beetle (Coleoptera: Chrysomelidae) on 'Carolina' cucumber. Crop Protec. 17, 331–336.

Buntin, G.D., 1994. Developing a primary sampling program. In: Pedigo, L.P., Buntin, G.D. (Eds.), Handbook of Sampling Methods for Arthropods in Agriculture. CRC, Boca Raton, FL, pp. 99–118.

Butler, C.D., Trumble, J.T., 2012. Spatial dispersion and binomial sequential sampling for the potato psyllid (Hemiptera: Triozidae) on potato. Pest. Manag. Sci. 68, 865–869.

Castle, S.J., Naranjo, S.E., 2008. Comparison of sampling methods for determining relative densities of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) on citrus. J. Econ. Entomol. 101, 226–235.

Dhawan, A.K., Peshin, R., 2009. Integrated pest management: concept, opportunities and challenges. In: Peshin, R., Dhawan, A.K. (Eds.), Integrated Pest Management: Innovation-development Process. Springer, pp. 51–81.

Dent, D., 2000. In: Insect Pest Management, Second ed. CABI, Wallingford, U.K., p. 410.

Dinardo-Miranda, L.L., 2010. Pragas. In: Dinardo-Miranda, L.L., Vasconcelos, A.C.M., Andrade Landell, M.G. (Eds.), Cana de açúcar. Instituto Agronômico, Campinas, pp. 349–403.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2008. InfoStat, Versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Dogramaci, M., Shefler, J.W., Giles, K., Edelson, J.V., 2006. Spatial pattern and sequential sampling of squash bug (Heteroptera: Coreidae) adults in watermelon. J. Econ. Entomol. 99, 559–567.

Dos Anjos, I.A., Da Silva, D.N., Campana, M.P., 2010. Cana de açúcar como forrageira. In: Dinardo-Miranda, L.L., Vasconcelos, A.C.M., Andrade Landell, M.G. (Eds.), Cana de açúcar. Instituto Agronômico, Campinas, pp. 725–746.

Draper, N.R., Smith, H., 1998. Applied Regression Analysis. Wiley Interscience, New York, p. 706.

Elliott, N.C., Giles, K.L., Royer, T.A., Kindler, S.D., Tao, F.L., Jones, D.B., Cuperus, G.W., 2003. Fixed precision sequential sampling plans for the greenbug and bird cherry-oat aphid (Homoptera: Aphdidae) in winter wheat in Oklahoma. J. Econ. Entomol. 96 (5), 1585–1593.

Faleiro, J.R., Ashok Kumar, J., Rangnekar, P.A., 2002. Spatial distribution of red palm weevil *Rhynchophorus ferrugineus* oliv. (Coleoptera: Curculionidae) in coconut plantations. Crop Protec. 21, 171–176.

Goebel, F.R., Salam, N., 2011. New pest threats for sugarcane in the new bioeconomy and how to manage them. Curr. Opin. Environ. Sust. 3 (1), 81–89.

Green, R.H., 1970. On fixed precision level sequential sampling. Res. Popul. Ecol. 12, 249–251.

Higley, L.G., Pedigo, L.P., 1997. Economic Thresholds for Integrated Pest Management. Univ. Nebraska Press, Lincoln, NE, p. 327.

Hutchison, W.D., Hogg, D.B., Poswal, M.A., Berberet, R.C., Cuperus, G.W., 1988. Implications of the stochastic nature of Kuno's and Green's fixed-precision stop lines: sampling plans for the pea aphid (Homoptera: Aphididae) in alfalfa as an example. J. Econ. Entomol. 81, 749–758.

Jian, F., Larson, R., Jayas, D.S., White, N.D.G., 2011. Evaluation of sampling units and sampling plans for adults of *Cryptolestes ferrugineus* (Coleoptea: Laemophloeidae) in stored wheat under different temperatures, moisture contents, and adult densities. J. Stored Prod. Res. 47, 334–340.

Kelsey, J.M., 1968. Sampling pasture insects. N. Z. Entomol. 4, 12–19.

Lanteri, A.A., Marvaldi, A.E., Suárez, S.M., 2002. Gorgojos de la Argentina y sus plantas huéspedes. Tomo I: Apionidae y Curculionidae. Publicación Especial de la Soc. Entomol. Argent. Nº 1, p. 98.

Long, W.H., Hensley, S.D., 1972. Insect pests of sugar cane. Ann. Rev. Entomol. 17, 149–176.

Monterrey, J., Cordova, M., Fernandez, S.Y., Guaharay, F., 1994. Comportamiento y manejo del picudo negro *Cosmopolites sordidus* en musáceas del pacífico seco de Nicaragua. Manejo integrado de plagas. Avance técnico. Tomo V.

Morris, R.F., 1955. The development of sampling techniques for forest defoliators with particular reference to the spruce budworm. Can. J. Zool. 33, 225–294.

Naranjo, S.E., Castle, S.J., 2010. Sequential sampling plans for estimating density of glassy-winged sharpshooter, *Homolodisca vitripennis*, on citrus. Crop Protec. 29,

1363–1370.
Naranjo, S.E., Flint, H.M., 1994. Spatial distribution of preimaginal *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development of fixed-precision sequential sampling plans. Environ. Entomol. 23, 254–266.
Naranjo, S.E., Flint, H.M., 1995. Spatial distribution of adult *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development and validation of fixed-precision sampling plans for estimating population density. Environ. Entomol. 24, 261–270.
Naranjo, S.E., Hutchison, W.D., 1997. Validation of arthropod sampling plans using a resampling approach: software and analysis. Am. Entomol. 43, 48–57.
Parajulee, M.N., Shrestha, R.B., Leser, J.F., 2006. Sampling methods, dispersion patterns, and fixed precision sequential sampling plans for western flower thrips (Thysanoptera: Thripidae) and cotton fleahoppers (Hemiptera: Miridae) in cotton. J. Econ. Entomol. 99 (2), 568–577.
Pedigo, L.P., Buntin, G.D., 1994. Handbook of Sampling Methods for Arthropods in Agriculture. CRC Press, Boca Raton, FL.
Prager, S.M., Butler, C.D., Trumble, J.T., 2013. A sequential binomial sampling plan for potato psyllid (Hemiptera: Triozidae) on bell pepper (*Capsicum annum*). Pest. Manag. Sci. 69, 1131–1135.
Ripoli, M.L.C., Ripoli, T.C.C., 2010. A palha como fonte de energia. In: Dinardo-Miranda, L.L., Vasconcelos, A.C.M., Andrade Landell, M.G. (Eds.), Cana de açúcar. Instituto Agronômico, Campinas, pp. 791–808.
Rivera de Castillo, A., 1980. Subproductos de la industria azucarera. Cienc. Soc. 5, 2.
Romero, E.R., Scandaliaris, J., Digonzelli, P.A., Leggio Neme, M.F., Giardina, J.A., de Ullivarri, J.F., Casen, S.D., Tonatto, M.J., Alonso, L.G.P., 2009. La caña de azúcar, características y ecofisiología. In: Romero, E.R., Digonzelli, P.A., Scandaliaris, J. (Eds.), Manual del Cañero. EEAOC, Las Talitas, pp. 15–22.
Rossi, J.P., Nuutinen, V., 2004. The effect of sampling unit size on the perception of the spatial pattern of earthworm (*Lumbricus terrestris* L.) middens. Appl. Soil Ecol. 27, 189–196.
Sabongi Izeppi, T., Dinardo-Miranda, L.L., Barbosa, J.C., 2014. Distribucao espacial de *Sphenophorus levis* em cana-de-acucar e sugestao de método de amostragem. VIII Workshop Agroenergia, 29 e 30 de maio de 2014. ISBN 978-85-85564-30-8. http://www.infobibos.com/agroenergia/cd/Resumos/ResumoAgroenergia_2014_024.pdf.
Salama, H.S., Abd-Elgawad, M.M.M., 2010. Spatial patterns of the red palm weevil and applied entomopathogenic nematode *Heterorhabditis bacteriophora*. Arch. Phytopathol. Plant Prot. 7, 689–699.
Sallam, M.N., Garrad, S.W., 2001. Distribution and sampling of adults of *Rhabdoscelus obscurus* (Boisduval) (Coleoptera: Curculionidae) and their damage in sugarcane. Aust. J. Entomol. 40, 281–285.
Salvatore, A.R., Acosta, M., Willink, E., 2006. El picudo perforador de la caña de azúcar *Acrotomopus atropunctellus* (Coleoptera: Curculionidae) en caña de azúcar Tucumán, Argentina. Resumen presentado en XII Jornadas Fitosanitarias Argentina, Catamarca, junio de.
Serra, G.V., Trumper, E.V., 2006. Sequential sampling protocols for *Spodoptera frugiperda* (Lepidoptera: Noctuidae), on *Zea mays* fields: influence of sampling unit size. Bull. Entomol. Res. 96, 471–477.
Soltani Ghasemloo, V., Aleosfoor, M., 2013. Dispersion pattern and fixed precision sequential sampling of *Sitobion avenae* (Fabricus) (Hemiptera: Aphididae) in wheat fields of Badjgah (Fars province) in Iran. J. Entomol. Acarol. Res. 45, 120–127.
Southwood, T.R.E., 1978. Ecological Methods, with Particular Reference to the Study of Insect Populations, second ed. Chapman and Hall, London, p. 524.
Taylor, L.R., 1961. Aggregation, variance and the mean. Nature 189, 732–735.
Taylor, L.R., Woiwod, I.P., Perry, J.N., 1978. The density-dependence of spatial behaviour and the variety of randomness. J. Anim. Ecol. 47, 383–406.
Terán, O.F., Precetti, A.A.C., 1982. *Sphenophurus levis* e *Metamasius hemipterus*, como pragas na cana-de-açucar. Bol. Téc. Copersucar 1, 24–26.
Underwood, A.J., Chapman, M.G., 1996. Scales of spatial patterns of distribution of intertidal invertebrates. Oecologia 107, 212–224.
Wilson, L.T., 1994. Estimating abundance, impact, and interactions among arthropods in cotton agroecosystems. In: Pedigo, L.P., Buntin, G.D. (Eds.), Handbook of Sampling Methods for Arthropods in Agriculture. CRC, Boca Raton, FL, pp. 475–514.
Young, L.J., Young, J.H., 1998. Statistical Ecology, a Population Perspective. Kluwer Academic Publishers, Boston.