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Sampling grids for monitoring Euschistus heros infestation in soybean

Sampling grids for monitoring *Euschistus heros* infestation in soybean

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ABSTRACT: *Euschistus heros* is one the most important pest insect in soybean fields in Brazil. Therefore, site-specific management applied for controlling *E. heros* can result in economic and environmental benefits. However, sampling protocols for assessing spatial-temporal variability of this soybean pest and, then, for guiding a more sustainable pest management, were still unknown. Thus, the objective of this study was to compare the efficiency of sampling grid sizes for monitoring the spatial variability of *E. heros* infestation along the soybean reproductive stages. Nine sampling campaigns were carried out using the beating sheet method, from R_2 to R_7 soybean phenological stages. It was used a sampling grid of 10×10 m, totaling 338 points in an soybean field of 3.4 ha, located in Londrina, PR, Brazil, during the 2011/2012 growing season. Increased sampling grid sizes (i.e., 10×10 m, 10×20 m, 20×20 m, 20×30 m, 30×30 m, 30×40 m, and 40×40 m) were simulated by deleting points from the initial (reference) sampling grid. The data were analysed through statistical and geostatistical procedures. Thematic maps were built and compared using Pearson's correlation and relative deviation coefficient (RDC). Spatial variability distribution pattern of *E. heros* infestation was dependent on sampling grid size and infestation pressure, in which smaller sampling grids and higher *E. heros* infestations enabled a more accurate monitoring. Based on study, an accurate protocol ($r > 0.70$ and RDC < 20%) for monitoring high infestation (i.e., > 2 individual per meter) of *E. heros* in soybean reproductive stages should include sampling grids smaller or equal to 20×20 m.

Key words: neotropical brown stink bug, pest sampling protocol, spatio-temporal variability, precision agriculture.

INTRODUCTION

The indiscriminate use of insecticides has raised concerns due to frequent emergence of resistant populations of pests to insecticides and outbreaks of secondary pests in soybean agroecosystems in Brazil (Sosa-Gómez and Silva 2010; Bueno et al. 2015). The risks to human health, environmental contamination, and potential harmful effects on bees and other beneficial insects have driven to the use restriction of some soybean insecticides (Lundin et al. 2015). These restrictions have mostly affected the insecticides used for controlling bedbugs, which are one of the most important groups of pests in the soybean production.

From the complex of bedbugs that occur in soybean, it stands out in Brazil the *Euschistus heros* (Fabricius 1794) (Heteroptera: Pentatomidae), due to its difficulty to control, higher prevalence compared to other species of stink bugs, high population densities, and potential for damaging (Sosa-Gómez and Silva 2010; Bueno et al. 2015). With the reduced number of chemical molecules available for controlling this pest, resistance management has been a major concern of entomologists in order to maintain the efficiency of these products (Sosa-Gómez and Silva 2010). Thus, the rational use of insecticides has been considered one of the main strategies to be implemented for handling the insecticide-induced resistance of bedbugs.

Pest sampling has been historically performed using traditional protocols that considering the *E. heros* distribution within the field as a random pattern. Consequently, these protocols indicate to make 10 to 15 pest sampling (subsamples) within the entire area, and the average infestation from all sampling is used for guiding whether a pest controlling strategy need to be implemented (Bueno et al. 2013, 2015). However, as several studies have reported the population of insect pests can also presents a structured spatial dependence (Burgio et al. 2012; Riffel et al. 2012; Pazini et al. 2015; Pavlu and Molin 2016). Consequently, average population of insects does not represent the actual condition of most part of the sampled fields. To overcome this challenge, precision agriculture tools can be useful for performing georeferenced pest sampling campaigns, which takes into account the spatial variability of insects, and, consequently, a site-specific pest management (Riffel et al. 2012; Aita et al. 2015).

Studies have shown that site-specific application of pesticides can reduce the amount of product applied, leading to lower insecticide-induced resistance of pest insects, as well as it may contribute to the conservation of natural enemies (Reay-Jones et al. 2010; Aita et al. 2015; Pavlu and Molin 2016). On the other hand, the cost of pest sampling for identifying the spatio-temporal variability patterns and elaborating the management maps stands out as one of the major constraints for the economical sustainability of site-specific control of agricultural pests (Burgio et al. 2012; Carleton et al. 2013; Pazini et al. 2015). Therefore, a suitable dimensioning of the sampling grid is crucial to achieve both technical and economic feasibility of the specific management of pests (Pazini et al. 2015). For instance, using larger pest sampling grids (i.e., few evaluation points per area) makes the field work easier, less time-consuming, and cheaper; however, it can compromise the quality of information obtained in the field (Carleton et al. 2013), leading to improper management decisions.

A suitable sampling protocol should be capable to detecting efficiently the spatial variability of insect population in the field, allowing to estimate values for unsampled locations though interpolation techniques and to obtain results without bias and with minimum variance (Vieira 2000). The hypothesis tested in this study was that *E. heros* infestation presents spatial dependence and, thus, increased sampling grid size affects negatively the accuracy of thematic maps. To test our hypothesis, we conducted a field experiment aiming to compare the spatial variability of *E. heros* infestation along the reproductive stage of soybean using increasing size of sampling grids.

MATERIAL AND METHODS

The data were collected in an experimental soybean field of the Embrapa Soja (lat 23°28′44′S; long 50°59′03′W), in Londrina, PR, Brazil, during the 2011/2012 growing season. Daily rainfall occurred during the period that *E. heros* sampling were performed is shown in Figure 1. The experimental area of 3.4 ha was sown in November 2011, with the soybean cultivar BRS 316 RR in rows spaced at 0.45 m apart, and a population of 378,000 plants·ha^{-1}. The soybean management practices
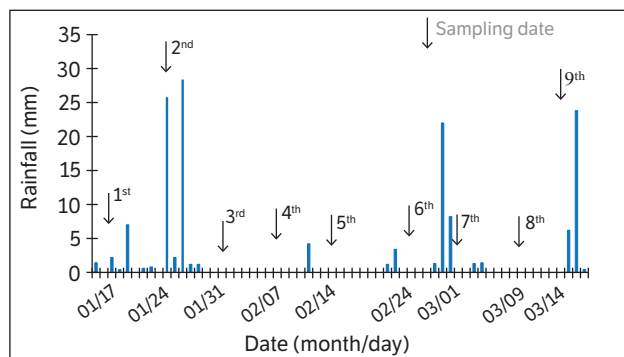
were carried out following technical recommendations for the crop, except for the control of bedbugs, which was not performed in order to carry out this study.

The perimeter of the area was georeferenced and divided by a regular squared sampling grid of 10 × 10 m, totalling 338 points of evaluation. The sampling campaigns were performed between R_2 and R_7 phenological stage [Ritchie et al. (1985) adapted by Yorinori (1996)], as follows: R_2 stage — full bloom [1st evaluation: Jan 17th, 2012; 2nd evaluation: Jan 24th, 2012)]; R_3 stage — beginning pod [3rd evaluation: Jan 31th, 2012]; R_4 stage — full pod [4th evaluation: Feb 07th, 2012]; $R_{5.1}$ stage — beginning seed (10%) [5th evaluation: Feb 14th, 2012]; $R_{5.4}$ stage — beginning seed (50 — 75%) [6th evaluation: Feb 24th, 2012]; R_6 stage — full seed [7th evaluation: Mar 01st, 2012; 8th evaluation: Mar 09th, 2012]; R_7 stage — beginning maturity [9h evaluation: Mar 14th, 2012]. *Euschistus heros* sampling were performed using the beating sheet method. At each sampling point, we evaluated four replication of 1 m plant row within a radius of two meters from the center point. All adults and nymphs from the 3rd to 5th instar of *E. heros* were quantified.

Based on the reference sampling grid (i.e., 10 × 10 m) with 338 points, 6 new sampling grid sizes were obtained using the technique of systematic points elimination (Oliveira et al. 2015): 10 × 20 m, 20 × 20 m, 20 × 30 m, 30 × 30 m, 30 × 40 m, and 40 × 40 m, containing, respectively, 166, 81, 56, 35, 28, and 19 points (Figure 2).

Initially the raw datasets, from each sampling grids and dates of evaluation, were subjected to outlier analysis. Any values that fell outside of the range of 3 standard deviation from the mean were considered outliers. Posteriorly, an exploratory statistical analysis was performed in order to obtain the position measurements (minimum, mean, and maximum) and dispersion

(coefficient of variation, CV%). Coefficient of variation values were classified as low variability (< 10%), medium (10 – 20%), high (20 – 30%) and very high (> 30%) as reported by Pimentel-Gomes and Garcia (2002). The hypothesis of normality was tested by the Shapiro Wilk's test (p > 0.05). The statistical analyses were performed using Statistical Analysis System (SAS) 9.3 software (SAS Institute, Cary, NC, USA).

The data were also analysed using geostatistical techniques through the Geoest software package (Vieira et al. 2002). The semivariogram were adjusted through theoretical models (i.e., spherical, exponential, and Gaussian), which were selected based on the highest coefficient of determination (r^2) and the smallest residual sum of squares (RSS) obtained by the technique of cross-validation. Based on model chosen was obtained the following parameters: range (a), nugget effect (C_0), →
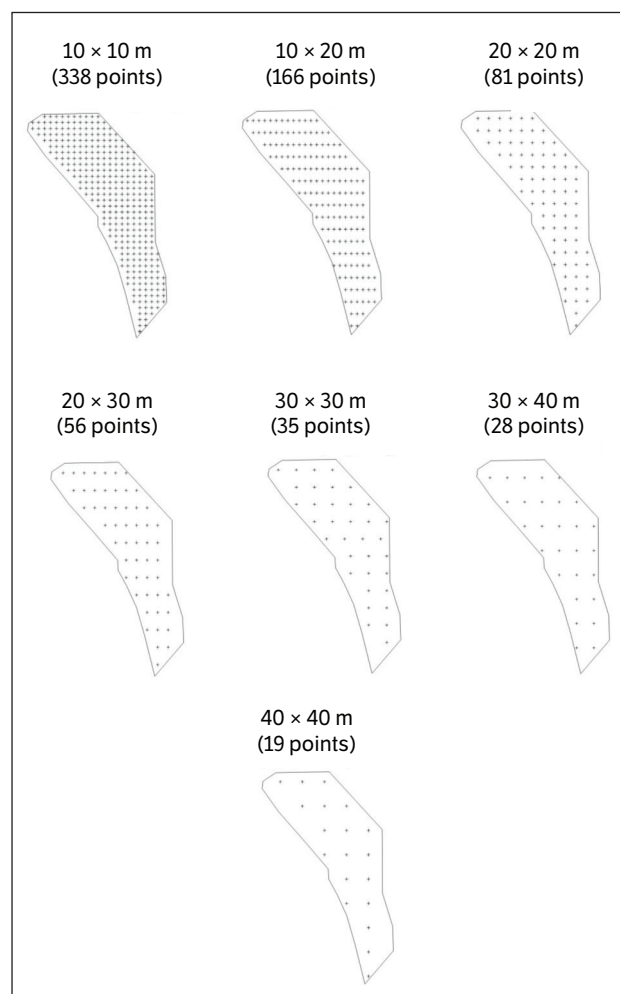
Figure 2. Sampling grid sizes tested for assessing the spatio-temporal variability of *Euschistus heros* infestation in a soybean field, Londrina, Paraná.

Figure 1. Daily rainfall occurred during the experiment conduction, highlighting the *Euschistus heros* sampling campaigns, in Londrina, Paraná.

contribution (C_1) and sill ($C_1 + C_0$). Spatial dependence of the data was verified from the equation $[(C_0 / (C_0 + C_1) \times 100]$ and classified according to Cambardella et al. (1994) as strong ($\leq 25\%$), moderate ($25 – 75\%$) and weak ($\geq 75\%$).

Thematic maps were elaborated using kriging as stochastic interpolation for data with defined spatial structure (Vieira 2000), and inverse square of distance as deterministic interpolation for data that did not obtain satisfactory adjustment to neither of the tested theoretical models (i.e., pure nugget effect, PNE). The Surfer 9 software (Golden Software, Golden, CO, USA) was used to elaborate the maps.

The sampling size grid effects on the accuracy of thematic maps were analysed though the Pearson's correlation ($p < 0.05$) and the relative deviation coefficient (RDC, in %). In order to use these methods, it was identified in each grid the interpolated values correspondent to 338 points sampled by reference grid (i.e., 10×10 m) for obtaining the same number of observation (n) in all maps. The RDC expresses the dissimilarity between two maps in module. The calculation was performed using an adaptation of the equation used by Cherubin et al. (2015):

$$RDC = \Sigma \ [(DENij – DENiref) / DENiref] \times (100/n)$$

where: n is the number of interpolated points (338); DENiref. Means the density of *E. heros* at the point "i" (value determined in the reference sampling grid, 10×10 m); DENij represents the density of *E. heros* at point "i" determined using increased sampling grid sizes j (j1 = 10×20 m; j2 = 20×20 m; j3 = 20×30 m; j4 = 30×30 m; j5 = 30×40 m; j6 = 40×40 m).

RESULTS AND DISCUSSION

Regardless of the phenological stage of soybean, the mean value of the *E. heros* found in the field was similar among sampling grid sizes (Table 1). However, using larger sampling grid sizes it was verified a decreased data amplitude (i.e., range between minimum and maximum values), resulting in lower capacity to detect the spatial variability of *E. heros* distribution in the field. The dispersion of data was high for all sampling grids, especially until the R_4 stage, period that lower number of insects was found in the field. From the $R_{5.4}$ stage, the *E. heros* infestation was considerably increased, decreasing CVs to below 45%; whereas from the R_6 stage, *E. heros* infestation was higher, exceeding eight individuals

per meter, resulting in lower CVs (i.e., ranging between 15 and 31%). Pazini et al. (2015) also found high CVs (i.e., > 100%) in infestation data of stalk stink bug (*Tibraca limbativentris*) in rice fields. It shows that regardless of species, bedbugs have similar spatial pattern of distribution in the field.

In general, CV values are inversely proportional to average values, and higher infestation of insects tend to provide lower CV, as also reported by Riffel et al. (2012) and Pazini et al. (2015), studying the spatial variability of soybean defoliating caterpillars (*Anticarsia gemmatalis* and *Pseudoplusia includes*) and rice stalk stink bug (*Tibraca limbativentris*), respectively. High CV values are commonly associated with counting data, and reflects the variability of the number found point-to-point (Chiba et al. 2010). Therefore, high CV values observed in this study suggest high data variability of the *E. heros* infestation in the area (Oliveira et al. 2015), requiring the use of sampling plans with higher number of samples to efficiently reproduce this spatial variability in unsampled locals.

Until the $R_{5.1}$ stage, regardless of the sampling grid size, none datasets showed normal distributions. In contrast, from the R_6 stage, with increased *E. heros* infestation, all sampling grids larger than 10×20 m presented normal distribution of data (Table 1). Several studies of pest population dynamics have reported that, under low infestation, the data do not follow a normal distribution curve (Reay-Jones et al. 2010; Riffel et al. 2012; Carleton et al. 2013). In this case, using conventional sampling protocols, which takes into account the arithmetic mean value, non-normal data do not represent satisfactorily the variability of the insect population in the field (Chiba et al. 2010). On the other hand, data normality is not a requirement of geostatistical analysis; however, asymmetric distributions with high number of outliers deserve attention (Webster and Oliver 2007).

The results obtained from geostatistical analysis (Table 2) showed that until $R_{5.1}$ stage (5th evaluation), all semivariograms of the *E. heros* infestation presented pure nugget effect (PNE) and, consequently, none theoretical model could be efficiently adjusted to dataset. Therefore, the infestation had a randomized pattern of spatial variability in the field (Pavlu and Molin 2016). The absence of spatial dependence of data may be associated to lower number of insect (< 1 individual per meter) found in these initial reproductive stage of soybean crop (i.e., R_1 to R_5) (Reay-Jones et al. 2010) as well as due to high data dispersion, commonly observed in studies with counting dataset (Chiba et al. 2010).

$\rightarrow$

Table 1. Descriptive statistical analysis of spatial distribution of the *Euschistus heros* sampled through increasing size of sampling grids during different reproductive phenological stages of the soybean crop, in Londrina, Paraná.

Statistical parameters	Sampling grids (m)						
	10 × 10	10 × 20	20 × 20	20 × 30	30 × 30	30 × 40	40 × 40
1st evaluation (phenological stage R_2)							
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	2.00	1.66	1.50	1.50	1.25	0.75	1.00
Mean	0.42	0.40	0.42	0.48	0.43	0.31	0.36
CV[1] (%)	87.68	83.12	78.78	86.88	81.97	75.61	82.32
W-test[2]	0.89*	0.90*	0.90*	0.89*	0.91*	0.88*	0.85*
2nd evaluation (R_2 phenological stage)							
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	1.50	1.25	1.25	1.25	1.25	1.00	0.75
Mean	0.38	0.36	0.35	0.37	0.36	0.37	0.36
CV (%)	86.36	88.06	90.98	87.19	98.65	82.42	82.24
W-test	0.90*	0.89*	0.89*	0.90*	0.87*	0.90*	0.85*
3rd evaluation (R_3 phenological stage)							
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	1.00	1.00	1.00	1.00	1.00	0.75	1.00
Mean	0.20	0.21	0.21	0.22	0.25	0.28	0.17
CV (%)	120.64	115.03	122.08	110.78	97.01	86.35	138.25
W-test	0.78*	0.80*	0.78*	0.78*	0.84*	0.86*	0.63*
4th evaluation (R_4 phenological stage)							
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	1.25	1.25	0.75	1.00	1.00	0.75	0.75
Mean	0.24	0.22	0.21	0.26	0.28	0.19	0.20
CV (%)	111.86	115.76	115.63	101.20	96.03	123.73	123.67
W-test	0.81**	0.80*	0.78*	0.85*	0.86*	0.77*	0.78*
5th evaluation ($R_{5.1}$ phenological stage)							
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	1.75	1.50	1.50	1.25	1.25	1.25	1.25
Mean	0.52	0.50	0.55	0.47	0.51	0.43	0.54
CV (%)	71.12	70.30	65.87	68.46	65.52	83.26	64.18
W-test	0.93*	0.93*	0.94*	0.91*	0.92*	0.88*	0.93[ns]
6th evaluation ($R_{5.4}$ phenological stage)							
Minimum	0.75	0.75	0.75	0.75	1.00	1.75	0.75
Maximum	10.50	7.50	7.33	8.50	7.33	7.00	6.25
Mean	3.77	3.52	3.46	3.69	3.52	3.69	3.58
CV (%)	42.83	44.53	43.29	43.87	40.66	37.38	45.02
W-test	0.96*	0.95*	0.97[ns]	0.95*	0.91*	0.91*	0.95[ns]
7th evaluation (R_6 phenological stage)							
Minimum	3.00	3.50	3.50	5.00	3.75	3.50	3.50
Maximum	17.00	17.00	17.00	14.50	14.50	12.75	13.50
Mean	8.81	8.78	8.79	9.00	8.71	8.71	8.43

Table 1. Continuation

7th evaluation (R$_6$ phenological stage)						
CV (%)	26.77	28.75	28.47	27.19	29.13	28.38	30.46
W-test	0.99*	0.98*	0.98^{ns}	0.97^{ns}	0.98^{ns}	0.97^{ns}	0.99^{ns}
8th evaluation (R$_6$ phenological stage)						
Minimum	5.25	5.25	6.00	6.50	7.50	7.75	7.00
Maximum	24.00	19.50	19.50	16.50	16.50	18.66	13.00
Mean	11.80	11.16	11.10	11.01	11.11	11.38	10.78
CV (%)	24.11	22.83	22.65	22.30	18.84	21.86	15.15
W-test	0.97*	0.98*	0.98^{ns}	0.98^{ns}	0.94^{ns}	0.94^{ns}	0.95^{ns}
9th evaluation (R$_7$ phenological stage)						
Minimum	6.00	6.25	7.00	6.50	6.50	7.50	8.25
Maximum	22.33	19.00	17.75	17.00	15.00	15.00	14.50
Mean	11.94	11.88	11.94	11.71	11.37	11.51	11.98
CV (%)	21.80	22.27	22.14	19.49	16.88	18.73	17.35
W-test	0.99*	0.98*	0.98^{ns}	0.98^{ns}	0.97^{ns}	0.95^{ns}	0.91^{ns}

[1]Coefficient of variation; [2]W-test: Shapiro-Wilk test for normal distribution; *Significant at levels of p < 0.05; ^{ns}Non-significant. When significant, it indicates that the normal distribution hypothesis is rejected.

In the following sampling campaigns (6th to 9th evaluations), a structured spatial dependence among sampling points was verified for data collected using 10 × 10 m, 10 × 20 m, and 20 × 20 m sampling grids. The spatial dependence of the *E. heros* data was classified as moderate to weak (Cambardella et al. 1994), with index of spatial dependence ranging from 33 to 78%. It is worth highlighting that stronger spatial dependence among sampling points makes the data interpolation more accurate (Vieira et al. 2002; Pazini et al. 2015) and, then, better predictions of *E. heros* infestation for non-evaluated locals can be performed. Based on that, our finding revealed that mapping of brown stink bug infestation throughout a crop season is a complex and challenge task. Reay-Jones et al. (2010) and Pazini et al. (2015) also reported it for other bedbug species. The seasonality and mobility of pest insects into the soybean fields result in a high spatio-temporal variability of the data and consequently, there are no models able to describe accurately this distribution pattern.

The Gaussian theoretical model showed better adjustment for 69% of the data that presented a structured spatial dependence (Table 2). The ability of models to describe the measured data pattern decreased (i.e., lower coefficients of determination, r²) when increased sampling grid sizes were used. The r² values ranged from 0.89 to 0.71 for data collected using the 10 × 10 m

sampling grid, from 0.80 to 0.52 for the 10 × 20 m sampling grid and from 0.58 to 0.34 for the 20 × 20 m sampling grid. In addition, r² = 0.32 was obtained from data collected using 20 × 30 m sampling grid during the 6th evaluation. For all sampling grids, data collected during the 6th evaluation, when soybean was beginning the seed growth, presented a more stable pattern of distribution of the *E. heros*.

For all data that presented spatial dependence, the range values obtained from the adjusted semivariograms ranged from 89 to 150 m (Table 2). There was not a clear trend to increase or decrease the range value as a function of sampling grid size. The range value expresses the maximum distance that existing dependence spatial between two points (Vieira 2000; Webster and Oliver 2007), and, therefore, it is a key parameter for interpreting the spatial variability of *E. heros* infestation in the soybean field.

Datasets collected using sampling grid sizes larger than 20 × 30 m at 6th evaluation and larger than 20 × 20 m from 7th evaluation to 9th evaluation were not possible to adjust efficiently a theoretical model (i.e., semivariograms presented PNE). According to reported by Pavlu and Molin (2016), PNE does not mean necessarily that dataset presents a randomized pattern, but it may be a result of the large sampling grid sizes that prevented the detection of variability existing in the area. In addition,

Table 2. Geostatistical parameters of spatial distribution of the *Euschistus heros* sampled through increasing size of sampling grids during different reproductive phenological stages of the soybean crop, in Londrina, Paraná,

Geostatistical parameters	Sampling grids (m)						
	10 × 10	10 × 20	20 × 20	20 × 30	30 × 30	30 × 40	40 × 40
1st evaluation (R$_2$ phenological stage[1])							
Model	PNE	PNE	PNE	PNE	PNE	PNE	PNE
2nd evaluation (R$_2$ phenological stage)							
Model	PNE	PNE	PNE	PNE	PNE	PNE	PNE
3rd evaluation (R$_3$ phenological stage)							
Model	PNE	PNE	PNE	PNE	PNE	PNE	PNE
4th evaluation (R$_4$ phenological stage)							
Model	PNE	PNE	PNE	PNE	PNE	PNE	PNE
5th evaluation (R$_{5.1}$ phenological stage)							
Model	PNE	PNE	PNE	PNE	PNE	PNE	PNE
6th evaluation (R$_{5.4}$ phenological stage)							
Model	Gau	Gau	Gau	Gau	PNE	PNE	PNE
Nugget effect (C$_0$)	1.24	0.86	0.71	0.93	_	_	_
Range (m)	133.55	141.8	138.9	122.9	_	_	_
Sill (C$_0$ + C$_1$)	2.34	2.16	2.13	2.09	_	_	_
r^2	0.89	0.80	0.58	0.32	_	_	_
SDI	53.07	39.64	33.43	44.30	_	_	_
7th evaluation (R$_6$ phenological stage)							
Model	Gau	Gau	Sph	PNE	PNE	PNE	PNE
Nugget effect (C$_0$)	3.72	4.20	3.71	_	_	_	_
Range (m)	88.99	90.92	150.53	_	_	_	_
Sill (C$_0$ + C$_1$)	5.87	6.55	7.03	_	_	_	_
r^2	0.81	0.58	0.34	_	_	_	_
SDI	63.34	64.06	52.72	_	_	_	_
8th evaluation (R$_6$ phenological stage)							
Model	Gau	Sph	Gau	PNE	PNE	PNE	PNE
Nugget effect (C$_0$)	6.07	4.43	3.62	_	_	_	_
Range (m)	123.50	116.70	137.30	_	_	_	_
Sill (C$_0$ + C$_1$)	7.81	6.18	6.69	_	_	_	_
r^2	0.71	0.52	0.51	_	_	_	_
SDI	77.62	71.79	54.00	_	_	_	_
9th evaluation (R$_7$ phenological stage)							
Model	Sph	Exp	Gau	PNE	PNE	PNE	PNE
Nugget effect (C$_0$)	4.73	3.87	4.98	_	_	_	_
Range (m)	99.66	123.58	90.00	_	_	_	_
Sill (C$_0$ + C$_1$)	7.22	7.66	7.41	_	_	_	_
r^2	0.84	0.77	0.49	_	_	_	_
SDI	65.51	50.57	67.16	_	_	_	_

[1]Soybean phenological scale as described by Ritchie et al. (1985) and adapted by Yorinori (1996). r^2 = Coefficient of determination; SDI = Spatial dependence index; Gau = Gaussian; Sph = Spherical; Exp = Exponential; PNE = Pure nugget effect.

larger sampling grid sizes enable obtaining a reduced number of sampling points, which can be not enough for an accurate modelling (Cherubin et al. 2014). In order to increase the changes to have accurate estimates and high reliability model parameters, Webster and Oliver (2007) suggested that at least 100 observations (sampling points) are necessary for using geostatistical analysis. Based on this criterion, only the 2 smallest sampling grid sizes tested in this study allowed collecting the minimum dataset suggested in the literature (i.e., 10 × 10 m = 338 points and 10 × 20 m = 166 points).

The thematic maps of *E. heros* showed a loss of spatial information according to increase the sampling grid size, and consequently, decrease the number of observation (Figure 3). These results are consistent with those observed by Riffel et al. (2012) for defoliating caterpillars in soybean fields. A paired comparison between the map generated by the reference-sampling grid (10 × 10 m) and map generated based on increased sampling grids was provided in Table 3. In general, coefficients of correlation (r) decreased according to increasing size of sampling grids, suggesting that maps from larger sampling grids lost similarity with reference map. Cherubin et al. (2015) also reported this same pattern in study involving soil chemical properties.

Moreover, an increased correlation among maps was observed from the 6th evaluation, when higher *E. heros* infestations were found. Coefficients of correlation above 0.70 were verified only for maps obtained by 10 × 20 m and 20 × 20 m from $R_{5.4}$ to R_7 stage. In these soybean phonological stages the infestation of *E. heros* was > 3.5

individual per meter, being considered above of the economic threshold level established by pest integrated management in soybean fields in Brazil (Bueno et al. 2013). These results were confirmed by the RDC data (Figure 4), in which smaller sampling grid sizes decreased the data deviation among maps, as well as high insect infestation (i.e., from $R_{5.4}$ to R_7) also led to greater similarity among maps (i.e., RDC < 0.20). Negative linear correlations were observed for both RDC *versus* average the *E. heros* infestation (–0.83; p< 0.05), and RDC *versus* CV values (–0.85; p < 0.05).

The *E. heros* infestation reached the economic threshold level (i.e., 2 individuals per meter) in R_6 phenological stage, when more than 90% of area had reached the critical control level. If this field would be dedicated to seed production the *E. heros* infestation reached the economic threshold level (i.e., 1 individuals per meter) since R_2 phenological stage. However, this damaging infestation occurred only in small regions within the soybean field, which represent about 5% of area.

In this sense, future studies focusing to define the sampling frequency. In this study, it was verified that sampling frequency once a week could be too much long, especially in the more aggressive infestation phases. For instance, the *E. heros* infestation drastically increased from 0.52 individuals per meter in the 5th evaluation ($R_{5.1}$ stage) to 3.77 individuals per meter in the following week (6th evaluation; $R_{5.4}$ stage). The hypothesis for this sudden increase in the *E. heros* infestation is mainly based on insect migration from adjacent soybean fields. Reay-Jones et al. (2010) verified a significant influence of adjacent
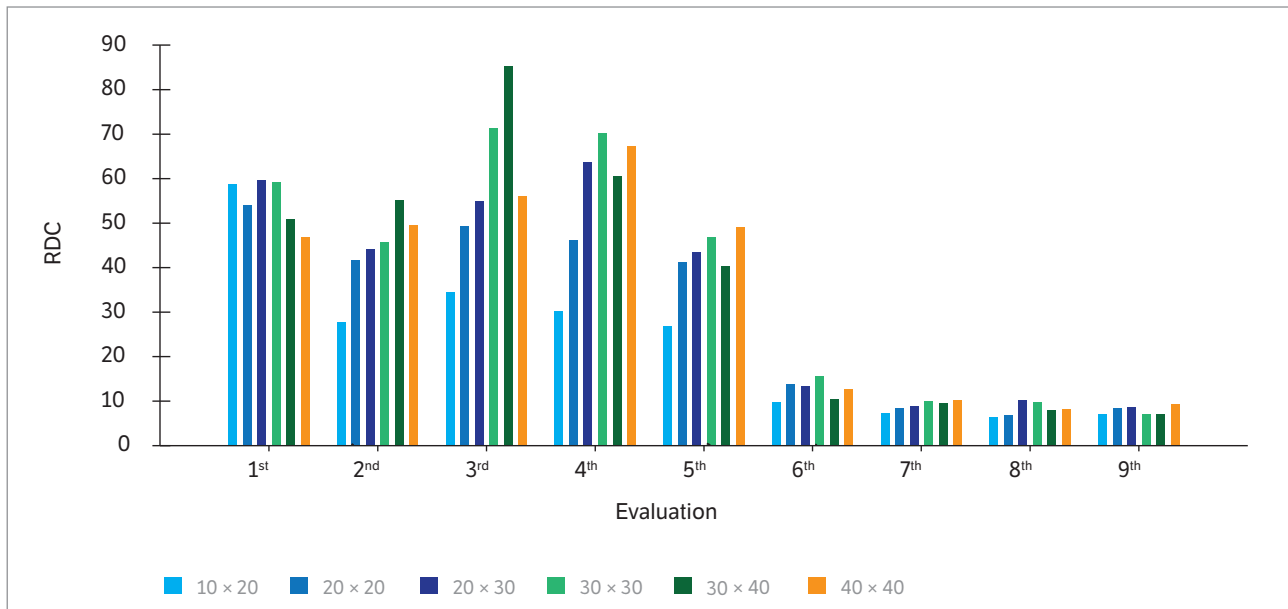
Table 3. Pearson's correlation coefficient among maps of the *Euschistus heros* population obtained by the reference sampling grid (10 × 10 m) and other sizes of sampling grids during different reproductive phenological stages of the soybean crop, in Londrina, Paraná.

Evaluation phenological stage[1]	Sampling grids (m)					
	10 × 20	20 × 20	20 × 30	30 × 30	30 × 40	40 × 40
1st - R_2	0.34*	0.42*	0.43*	0.32*	0.16*	0.45*
2nd - R_2	0.74*	0.55*	0.52*	0.48*	0.30*	0.40*
3rd - R_3	0.75*	0.57*	0.47*	0.31*	0.26*	0.11*
4th - R_4	0.68*	0.38*	0.21*	0.18*	0.03ns	−0.09ns
5th - $R_{5.1}$	0.63*	0.41*	0.25*	0.21*	0.27*	0.18*
6th - $R_{5.4}$	0.95*	0.94*	0.87*	0.73*	0.90*	0.93*
7th - R_6	0.82*	0.77*	0.66*	0.65*	0.69*	0.67*
8th - R_6	0.77*	0.88*	0.36*	0.38*	0.72*	0.61*
9th - R_7	0.72*	0.70*	0.44*	0.52*	0.60*	0.34*

*Significant Pearson's linear correlation (p < 0.05); nsNon-significant correlation; [1]Soybean phenological scale as described by Ritchie et al. (1985) and adapted by Yorinori (1996). N = 338 points.
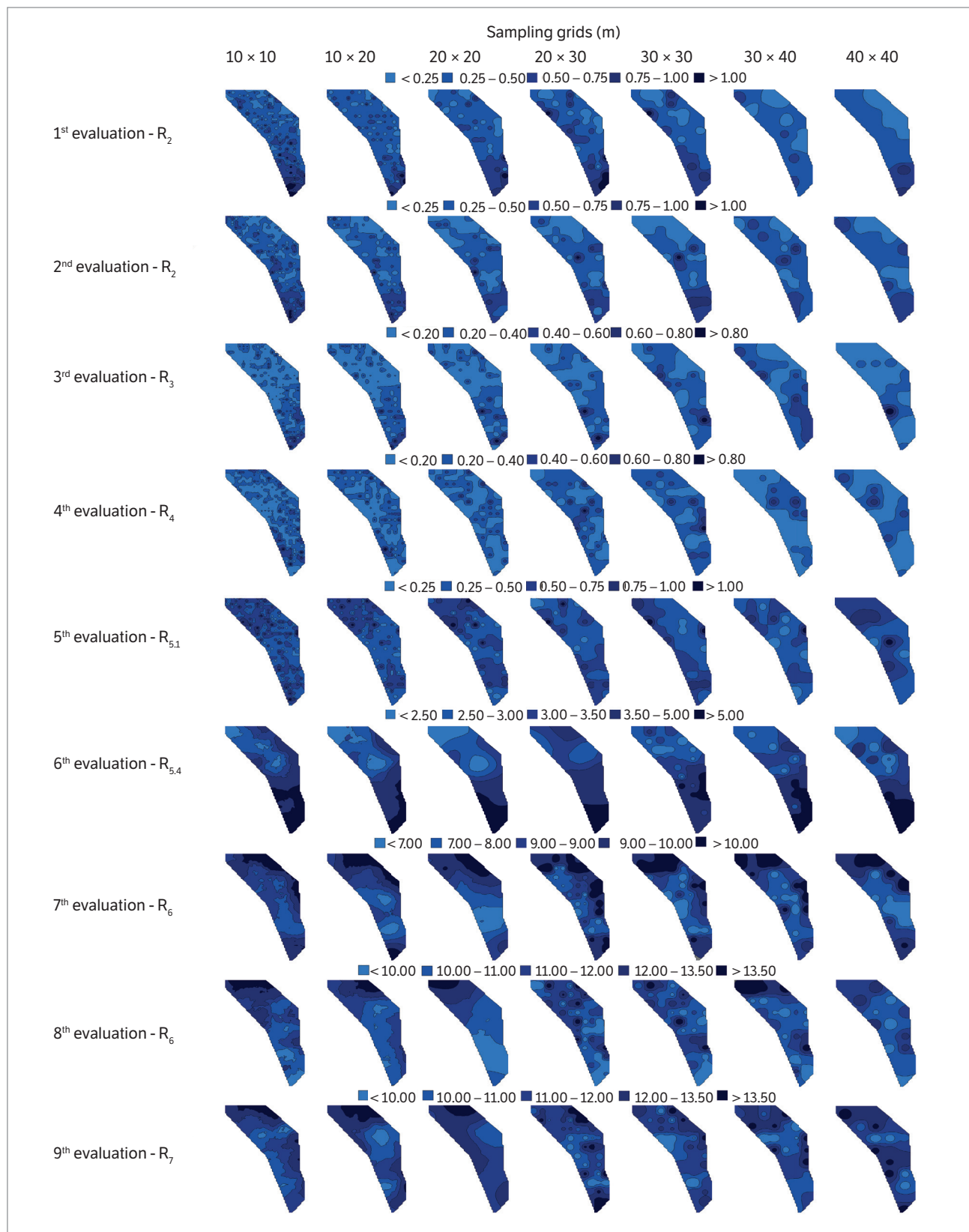
Figure 3. Thematic maps of spatial-temporal distribution of the *Euschistus heros* infestation obtained through increasing size of sampling grids during different reproductive phenological stages of the soybean crop, in Londrina, Paraná.

Figure 4. Relative deviation coefficient (RDC, in %) among maps of the *Euschistus heros* infestation obtained by the reference sampling grid (10 × 10 m) and others sizes of sampling grids during different reproductive phenological stages of the soybean crop, in Londrina, Paraná.

fields on spatial distribution of bedbugs in cotton fields, especially within small fields, as we assessed in this study. In addition, shorter sampling frequency could help to identify early the presence of insects younger than fourth-instar nymphs, since at this stage the pest is already damaging to soybean (Fonseca et al. 2014).

Agricultural pest mapping across large fields also need to be better understood (Reay-Jones et al. 2010). Only few studies has expanded them evaluation to large areas. An example is the study performed by Burgio et al. (2012), who monitored the spatial variability of *Agriotes sordidus* (Coleoptera Elateridae) infestation within an area of 500 ha cropped with several crops in northern Italy. Large-scale studies are essential for better understanding of the population dynamic of bedbugs and other pest among soybeans fields in different phenological stages. Furthermore, it enables to verified potential effects of soybean cultivars, management practices and microclimate on pest spatial distribution. Consequently, this information could be used to monitor more intensively areas with higher pest infestation risks, increasing the efficiency of pest control management and reducing crop yield losses.

Although this study showed promising technical findings for improving the integrated pest management in soybean crop, it is worth highlighting that protocol to monitoring the spatio-temporal variability of pest

using beating sheet method requires dense sampling grids (i.e., numerous points of evaluation in a given area). Consequently, large number of sampling points associated to the intensive frequency of evaluation imply in a time-consuming and tedious fieldwork, becoming practicality unfeasible in large commercial scale (Burgio et al. 2012). Our findings are in the same direction of those reported by Pazini et al. (2015), who were not able to define an ideal sampling grid size to monitor *Tibraca limbativentris* infestation due to high spatial variability of this pest in the field.

The difficulty to determine a consistent pattern of pest spatial variability led Reay-Jones et al. (2010) and Pavlu and Molin (2016) to conclude that a more efficient strategy to recognize the spatial distribution pattern of the bedbugs in cotton and *Sphenophorus levis* in sugarcane fields is indirectly through the mapping of pest damages caused to crops, since crop damages are irreversible and statics. However, this alternative is not feasible for soybean crop, because damages caused by bedbugs to grains cannot be identified visually in field, but they need to be analyzed in laboratory. Therefore, future researchs should to be oriented towards new methodologies to make fieldwork easier and more efficient. For instance, important advances in entomology field have been achieved using autonomous pest control robots (see Chung et al. 2014) and remote sensing by satellite

imaging or unmanned aerial vehicles (see Nansen and Elliott 2016).

CONCLUSION

Below the established economic threshold level for an integrated pest management (i.e., 2 individual per meter) *E. heros* infestation has no a defined pattern of the spatial variability distribution in the field, whereas under more intensive infestation *E. heros* presents a weakly structured pattern of distribution.

Increased sampling grid sizes are not efficient for detecting the spatio-temporal variability of *E. heros* within soybean field, resulting in less accurate thematic maps.

The ideal sampling grid size for monitoring *E. heros* infestation in soybean crop dependent on the infestation level and desired accuracy level as well. Based on study, an accurate protocol (r > 0.70 and RDC < 20%) for monitoring high infestations of *E. heros* in reproductive soybean phenological stages should include sampling grids smaller or equal to 20 × 20 m.

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