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Spatial Distribution of Adults and Nymphs of *Euschistus heros* (F.) (Hemiptera: Pentatomidae) on Bt and Non-Bt Soybean

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Abstract

The knowledge of the arrangements of dispersion of insect pests in soybean cultivars is needed to improve the monitoring and control. The objective of this study was to evaluate the spatial distribution of adults and nymphs of *Euschistus heros* in Bt and non-Bt soybean into two regions, under field conditions. The experimental area located in Dourados and Douradina, Mato Grosso do Sul. For the evaluations we used the method of cloth-to- beat, where we collected one sample per plot randomly from each experimental area. Evaluations were performed at 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98 days after emergence (DAE). For data analysis, the rates of dispersion (variance/average Morisita index and exponent k Negative Binomial Distribution) and theoretical frequency distributions (Poisson, Binomial and Negative Binomial Positive) were estimated at 1-5 % probability. Adults of *E. heros* are arranged differently for Bt and non-Bt soybean and between regions, since in both cultivars and locations, individuals of the populations evaluated in probabilistic set arrangements negative binomial distribution (aggregate), Poisson (random) and positive binomial distribution (uniform), as the days after soybean emergence. The nymphs of *E. heros* showed aggregated spatial arrangement, being distributed similarly for Bt and non-Bt soybean and regions, setting a negative binomial distribution model.

Keywords: *Glycine max* L, damage, sampling, horizontal dispersion, Bt crop

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is the crop with the largest acreage in the world with high yield potential (Fonseca et al., 2013b; Yokomizo et al., 2013). The crop is attacked by different insect-pests from sowing till harvest, among which, *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae) being the most abundant and prevalent in agricultural areas in Brazil (Corrêa-Ferreira & Panizzi, 1999; Panizzi et al., 2012; Krinski et al., 2013). Bedbugs occur in soybean at all stages and are harmful from the beginning of pod formation until grain maturity (Zambiazzi et al., 2012). They reach the seeds through the insertion of the mouthparts in vegetables, making them shriveled and wrinkled, affecting consequently the yield and quality of grain (Panizzi & Slansky Junior, 1985).

The damage caused by *E. heros* when uncontrolled can get up to 30% on soybean (Vivan & Degrande, 2011). To reduce losses, control of this insect is accomplished through chemical insecticide applications, from the vegetative phase of the plant, and are not always effective (Corrêa-Ferreira, 2005; Sosa-Gómez & Silva, 2010).

Considering the efficiency of the conventional system, it can be considered that, the Bt-plants is a tool that assists in the productive systems (Homrich et al., 2008). The main objective in the generation of Genetically Modified Organisms (GMO) is to increase the plant resistance to insects (Yuan & Knauf, 1997; Yu et al., 2011; Dutra et al., 2012a), with this; despite the great advances in Bt technology poses to world agriculture, their adoption in some regions may be affected positively or negatively depending on the characteristics of each locality (Barros & Degrande, 2012).

Therefore, soybean yield is defined by the interaction between the plant and the environment and the management of the insect pests (Pereira et al., 2011), thus high yields are only obtained when such conditions are favorable, at all stages of growth culture.

The management of *E. heros* in soybean should include the adoption of control measures based on population levels of species monitored by periodic sampling. The first step in designing a sampling plan is the knowledge of the spatial distribution of the species of interest, to establish appropriate criteria on population (Barbosa, 2003). Thus, knowledge of a fast and efficient way of pest sampling, especially on large areas of cultivation, is essential that the Integrated Pest Management (IPM) is satisfactorily used (Fernandes et al., 2003).

Despite the benefits obtained from the Bt cultivar, it is not known exactly how transgenic plants affect the populations of organisms in an agroecosystem (Rodrigues et al., 2010). Understanding the behavior of distribution of non-target pests in transgenic plants makes it necessary to know the shape of the spatial arrangement of this insect in this new technology to determine whether or not to change some characteristics of the sample such as the sample size and sampling units.

There is demand for research with the goal of evaluating the spatial arrangement of *E. heros* in Bt and non-Bt soybean; moreover, there is still lack of scientific information about the behaviour of this pest in different localities. In this context, this study aimed to evaluate the spatial distribution of adults and nymphs of *E. heros* in Bt and non-Bt soybean in two regions under field conditions.

2. Material and Methods

The experiment was conducted under field conditions in two experimental areas located at Rincão Porã Farm, the geographic coordinates 22°14'25" S, 54º42'60.7" W and altitude of 403 m above mean sea level in the town of Dourados and Boa Sorte Farm in the geographical coordinates 22º01'07" S, 54º32'15" W and altitude of 310 m above mean sea level in the municipality of Douradina during harvest season, 2011/12. The soil of the area is classified as Typic Distroferric (RH) of loamy soil. The climate, according to Köppen's humid mesothermal, Cwa type, with annual average temperatures and precipitation ranging from 20 ºC to 24 ºC and 1.250 mm to 1.500 mm, respectively (Fietz & Fisch, 2006). For management of two experimental areas with soybean, we used the no-tillage system, and corn (*Zea mays* L.) as preceding crop. The area of this experiment was dried with glyphosate combined with mineral oil (2.0 $l.ha^{-1}$ + 0.5 $l.ha^{-1}$, respectively).

The seeds used were Bt soybean AL 6910 Intact RR2 PRO ™ and non-Bt RR BMX Potencia®, constituting the two treatments in two different locations. The crop was sown on October 22, 2011 and October 29, 2011 at Farm Rincão Porã and Farm Boa Sorte, respectively, with a density of 15 seeds per meter, thus maintaining a plant population of approximately 300.000 plants ha^{-1}. The row spacing was 0.50 meters. For fertilizer application in both cultivars, 300 kg ha^{-1} of NPK formulation (2:18:18) were used. We carried out the weed and disease control with application of herbicides and fungicides. No insecticides were carried out in the areas studied.

The spatial distribution of adults and nymphs of the stink *E. heros* was evaluated in two regions (Dourados and Douradina) in two fields each containing of 100 plots in each area, each plot consisted of 11 rows with 5 m long, totaling (27.5 m^2) with Bt and non-Bt soybean constituting two treatments. Sampling methodology used was the method of cloth-to-beat, consisting of two sticks of wood connected with a white cloth, with a length of 1 m and width of 1.4 m. For the samples, one end of cloth was placed between the rows of soybeans, being adjusted to the base of a row of plants and other plants extended over the adjacent row. The plants of a row (0.50 m^2) were shaken vigorously in order to bring down the pest insects on the cloth (Sturmer et al., 2012). Samples were taken at 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98 days after emergence (DAE) in Bt and non-Bt soybean.

For data analysis, the mean and variance of the number of adults and nymphs of *E. heros* per plot were obtained at each sampling date, using the relationship between those values as an indicator of the spatial distribution (Elliott, 1979). The dispersion indexes, as described below, were calculated using the Excel program.

Variance/mean ratio: ratio between the variance and mean ($I = s^2/m$), used to measure the deviation of a random arrangement of conditions, where values equal to unity indicates random spatial distribution, values smaller than unity uniform distribution and values greater than the aggregate distribution unit (Rabinovich, 1980). The departure from randomness can be tested using the chi-square test with n-1 degrees of freedom, $\chi2 = (n-1)\ s^2/m$ (Elliott, 1979).

Morisita index: the index of Morisita (I_δ) is relatively independent of the medium and the number of samples. So when $I_\delta = 1$ the distribution is random, when $I_\delta>1$ the distribution is contagious type of I_δ and when<1 indicates a regular distribution (Morisita, 1962).

Exponent k of the Negative Binomial distribution: the exponent k is a suitable dispersion index when the size and numbers of sample units are the same in each sample, as often this is influenced by the size of the sampling units. This parameter is an inverse measure of the degree of aggregation in this case negative values indicate a regular or uniform distribution, positive, values close to zero indicate aggregate provision and higher values indicate a willingness to eight randomly (Pielou, 1977; Southwood, 1978; Elliot, 1979). On this point, Poole (1974) uses another interpretation for it when $0<k<8$, the index indicates a clustered distribution, and when $0>k>8$ points to random distribution.

Theoretical frequency distribution: used to evaluate the spatial distribution of the observed species. As presented below, according to L. J. Young and J. H. Young (1998). Poisson distribution: also known as random distribution, characterized by having variance equal to the mean ($m = s^2$).

Positive Binomial Distribution: describes the uniform distribution and has less than average ($s^2<m$) variance.

Negative binomial distribution: has higher than average variance, thus indicating clumped distribution, and has two parameters: the mean (m) and the parameter k ($k> 0$).

Chi-square test of grip: for checking the fit of the data collected in the field to the theoretical frequency distribution test, we used the chi-square test that compares the total grip of the observed frequencies in the sampled area, with frequencies expected according to L. J. Young and J. H. Young (1998); those frequencies being defined by the product of the probabilities of each class and the total number of sampling units used. To conduct those tests, we chose to fix a minimum expected frequency equal to unity. Statistical analysis was performed using chi-square test at 1 and 5% probability test.

3. Results and Discussion

The presence of adults of *E. heros* on soybean plants in Dourados were detected at 35 days after emergence (DAE) in soybean, Bt and non-Bt in Dourados and Douradina (Figure 1A and 1B). The presence of the stink bug in the vegetative phase is due to the fact that often these bugs initiate colonization of crops at the end of the growing season and early flowering. At that time, the bugs come out of diapause or alternative hosts and migrate to soybean, progressively increasing their populations during the breeding phase (Corrêa-Ferreira, 2005).

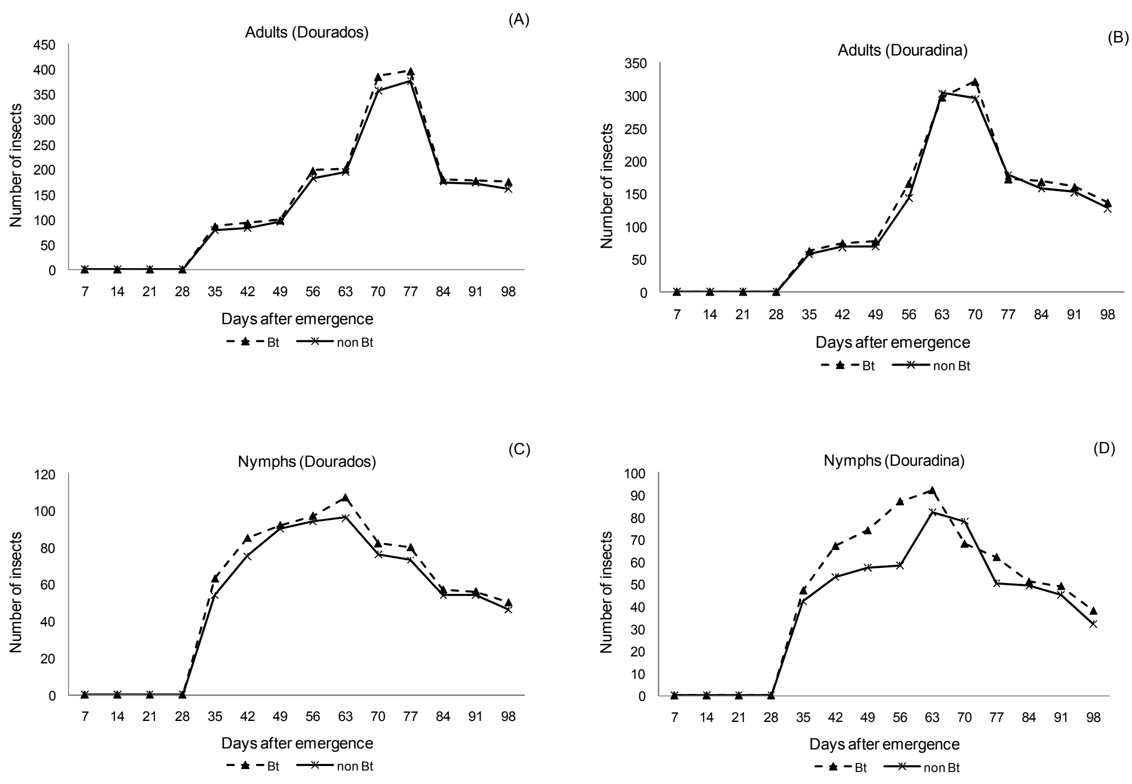

Figure 1. Percentage of the number of adults and nymphs of *Euschistus heros* occurring in Bt and non-Bt soybean [*Glycine max* (L)] as a function of days after emergence in Dourados and Douradina, Mato Grosso do Sul, Brazil, 2012

In 14 samples, a total of 3856 insects were found, with 51.47% to 48.52% cultivating Bt and non-Bt in Dourados for a total of 3181 insects, being 51.30% for the Bt cultivar and 48.69 % in the non-Bt in Douradina (Figure 1A and 1B). The peak population of adults was observed at 77 DAE (January) in Bt and non-Bt soybean, in Dourados and Douradina, respectively (Figure 1A and 1B).

The presence of nymphs of *E. heros* was observed in Bt and non-Bt soybean from the fifth sample, 35 DAE in both regions (Figure 1C and 1D). The largest numbers of nymphs of *E. heros* were found in Bt soybeans, which represented 51.92% of total plots sampled in Dourados, and 53.76% in Douradina (Figure 1C and 1D). The results observed in the present study corroborate studies by Thomazoni et al. (2010), Dutra et al. (2012b) which showed that Bt technology had greater abundance of this insect.

The highest population density of adults and nymphs of *E. heros* found in Bt soybean was due probably to the reduction of inter-specific competition between the various populations that made up the community of arthropods culture, it was caused probably due to reduced caterpillar population as the major defoliating caterpillars culture as velvetbean caterpillar *Anticarsia gemmatalis* (Hübner, 1818), false-medideira caterpillar *Chrysodeixis includens* (Walker, 1857), *Rachiplusia nu* (Guenée, 1852) and drill underarm *Crocidosema aporema* (Walsingham, 1914), those species targeted by toxin Cry1Ac and therefore were not found in this cultivation because of the resistance offered by such transgenic plants to those Lepidoptera insects (Bernardi et al., 2012).

No preference of adults and nymphs of *E. heros* was observed in relation cultivars versus regions for Bt and non-Bt soybean. The similar behavior in the two regions is due to the fact of using the same system of agricultural cultivation in recent years. According to Smaniotto and Panizzi (2013) adopted different cultivation practices, especially tillage and sowing in the second season, which resulted in significant changes in the dynamics of agricultural pests, thus the similarities of the study areas resulted in the same behaviour of *E. heros* in both regions.

Compared to the aggregation of adults of *E. heros*, the variance/mean (I) calculated in cultivating Bt presented nine surveys in Dourados and four in Douradina, with statistically higher values indicating that the unit aggregation, and had two samplings in the Bt soybean, with values ranging unit indicating uniformity (Table 1).

In Dourados for adults of *E. heros* soy non-Bt index I had seven samples with statistically higher values than unity (aggregate score). In Douradina index I had four samples with statistically higher values than unity (aggregate score) and two values equal to unity indicating uniform (Table 1) theoretical distribution. Pereira et al. (2004) studied the population of pests in legumes and found regular or uniform distribution of individuals in the population sampled.

In summary, the results of Morisita index (I_δ) for the treatment Bt, *E. heros* adults, showed that the largest unit of a total of ten values, eight samples in Dourados and five in Douradina representing an aggregate score. Analyzing the test Morista (I_δ) index to the non-Bt soybean in Dourados was observed ten samples, seven values were greater than unity (aggregate score); soybeans in Dourados not Bt, and observed in Douradina ten samples, four values were greater than unity and equal to the sampling unit, ie indicated uniformity (Table 1).

Analyzing the exponent Ķ for Bt soybeans in Dourados, found the ten samples, eight showed aggregate provision for adults of *E. heros*, because the values were variable from 1.961 to 5.091, and two samples indicated random arrangement with values 10.822 and 33.000. In Douradina was found for K, soy Bt four samples which showed aggregated arrangement of four uniform random and two (Table 1).

It was found for adults of *E. heros* in non-Bt soybeans in Dourados with ten samples in the field, eight of them indicated aggregate provision for adults, and two showed random distribution. In Douradina we observed five available samples showed aggregated two random three uniform dispersion of the index K (Table 1).

Based on the dispersion index K, it can be stated with certainty that the spatial distribution of *E. heros* is aggregated, corroborating the results obtained by Bueno et al. (2008) and Souza et al. (2011) in soybean with studies of spatial distribution of the small green stink (Fonseca et al., 2013a) in cotton with studies of spatial distribution of bedbug *Piezodorus guildine*.

Table 1. Dispersion indices for adults of *Euschistus heros* in Bt and non-Bt soybean [*Glicine max* (L)] function of days after emergence in Dourados and Douradina, Mato Grosso do Sul, Brazil, 2012

Samples			Dourados					Douradina				
	Numbers	(DAE)	Média	S^2	I	I_δ	K	Média	S^2	I	I_δ	K
Soybean (Bt)	1rd	7	0.000	-	-	-	-	0.000	-	-	-	-
	2rd	14	0.000	-	-	-	-	0.000	-	-	-	-
	3rd	21	0.000	-	-	-	-	0.000	-	-	-	-
	4rd	28	0.000	-	-	-	-	0.000	-	-	-	-
	5rd	35	0.860	1.051	1.222 *	1.259^{NS}	3.874^{AG}	0.620	0.521	0.905^{NS}	0.846^{NS}	-6.539^{UN}
	6rd	42	0.920	1.226	1.332 *	1.362 *	2.767^{AG}	0.740	0.800	1.082^{NS}	1.111^{NS}	9.069^{AL}
	7rd	49	0.980	1.474	1.504 *	1.515 *	1.943^{AG}	0.770	0.846	1.098^{NS}	1.128 *	7.847^{AG}
	8rd	56	1.970	3.949	2.004 *	1.507 *	1.961^{AG}	1.650	3.260	1.976 *	1.589 *	1.691^{AG}
	9rd	63	2.000	2.121	1.061^{NS}	1.030^{NS}	33.000^{AL}	2.970	3.080	1.037^{NS}	1.012^{NS}	80.264^{AL}
	10rd	70	3.850	5.220	1.356 *	1.092 *	10.822^{AL}	3.210	4.087	1.273 *	1.085 *	11.752^{AL}
	11rd	77	4.000	7.143	1.786 *	1.207 *	5.091^{AG}	1.720	1.335	0.776^{NS}	0.870^{NS}	-7.683^{UN}
	12rd	84	1.790	2.895	1.617 *	1.343 *	2.900^{AG}	1.680	2.624	1.562 *	1.333 *	2.990^{AG}
	13rd	91	1.770	2947	1.665 *	1.374 *	2.663^{AG}	1600	2646	1.654 *	1.407 *	2.446^{AG}
	14rd	98	1.750	2.856	1.632 *	1.360 *	2.769^{AG}	1.360	1.465	1.077^{NS}	1.057^{NS}	17.607^{AL}
Soybean (non-Bt)	1rd	7	0.000	-	-	-	-	0.000	-	-	-	-
	2rd	14	0.000	-	-	-	-	0.000	-	-	-	-
	3rd	21	0.000	-	-	-	-	0.000	-	-	-	-
	4rd	28	0.000	-	-	-	-	0.000	-	-	-	-
	5rd	35	0.790	0.875	1.107^{NS}	1.136^{NS}	7.373^{AG}	0.570	0.551	0.966^{NS}	0.940^{NS}	-16.753^{UN}
	6rd	42	0.830	1.052	1.267 *	1.322 *	3.109^{AG}	0.680	0.705	1.036^{NS}	1.054^{NS}	18.761^{AL}
	7rd	49	0.940	1.390	1.479 *	1.510 *	1.962^{AG}	0.690	0.822	1.191^{NS}	1.279 *	3.604^{AG}
	8rd	56	1.820	3.038	1.669 *	1.366 *	2.720^{AG}	1.430	1.763	1.233 *	1.162^{NS}	6.146^{AG}
	9rd	63	1.950	2.008	1.030^{NS}	1.015^{NS}	66.043^{AL}	3.030	5.423	1.790 *	1.259 *	3.836^{AG}
	10rd	70	3.560	4.229	1.188^{NS}	1.052^{NS}	18.953^{AL}	2.940	3.128	1.064^{NS}	1.022^{NS}	46.056^{AL}
	11rd	77	3.760	5.679	1.510 *	1.135 *	7.366^{AG}	1.780	1.567	0.880 *	0.933^{NS}	-14.894^{UN}
	12rd	84	1.740	2.720	1.563 *	1.322 *	3.091^{AG}	1.580	2.347	1.485 *	1.306 *	3.254^{AG}
	13rd	91	1.720	2.749	1.598 *	1.346 *	2.875^{AG}	1.520	2.454	1.615 *	1.403 *	2.473^{AG}
	14rd	98	1.600	2.040	1.275 *	1.171 *	5.813^{AG}	1.270	1.250	0.984 *	0.987 *	-79.048^{UN}

*Not significant at 5% probability. DAE= days after (plant) emergence.

Not significant at 5% probability.

^{Ag}gregate; ^{UN}uniform; ^{AL}random.

S^2 Variance, *I* Mean-variance ratio, I_δ Morisita index, *K* Exponent of the negative binominal.

Compared to aggregation nymphs of *E. heros* variance/mean (*I*) ratio calculated in cultivating Bt presented eight samples in Dourados, seven in Douradina, with statistically higher values indicating the unit aggregation (Table 2) .

In non-Bt soybeans in the index *I* in Dourados nymphs of *E. heros* had seven samples with statistically higher values than unity (aggregate score). In Douradina index *I* had six samples with statistically higher values than unity (aggregate score) (Table 2).

As most phytophagous Pentatomidae, associated with soybean species tend to be polyphagous (Slansky Jr, & Panizzi, 1987), which develop in over 32 plant species, although survival and cycle time are influenced by food (Medeiros & Megier, 2009). Although the dietary patterns of phytophagous bugs, notes the existence of food preferences in localized populations in which insects can act as a oligophagous even monophagous, depending on the availability of hosts (Panizzi, 1997; Cividanes & Parra., 1994) .

In summary, the results of Morisita index (I_{δ}) for the treatment Bt nymphs of *E. heros* was arranged in aggregate in nine samples in Dourados and Douradina in seven samples, a total of ten. Analyzing the test Morista (I_{δ}) index for the non-Bt crop in Dourados, observed ten samples, nine of which had values greater than unity (aggregation); Douradina in, there was the ten samples, seven, was mixed added (Table 2). In this context according to Ricklefs (2003) the spatial arrangement of populations of insects such a standard that can be random uniform and aggregated basis.

Analyzing the exponent K for nymphs on soybean Bt found in both regions of the ten samples, all indicated aggregate provision. It was found in soybean fields in both non-Bt ten samples in the field, most of them showed aggregated to the nymphs, and a sample arrangement shown in Douradina randomized, (49 DAE) the dispersion index K (Table 2). These results agree with Maruyama et al. (2002), mentioning the fact that the provision of insects in general tends to aggregate in the field normally.

Table 2. Dispersion indexes nymphs of *Euschistus heros* in Bt and non-Bt soybean [*Glicine max* (L)] function of days after emergence in Dourados and Douradina, Mato Grosso do Sul, Brazil, 2012

Samples			Dourados					Douradina				
	Numbers	(DAE)	Média	S^2	*I*	I_{δ}	*K*	Média	S^2	*I*	I_{δ}	*K*
Soybean (Bt)	1^{rd}	7	0.000	-	-	-	-	0.000	-	-	-	-
	2^{rd}	14	0.000	-	-	-	-	0.000	-	-	-	-
	3^{rd}	21	0.000	-	-	-	-	0.000	-	-	-	-
	4^{rd}	28	0.000	-	-	-	-	0.000	-	-	-	-
	5^{rd}	35	0.630	1.064	1.688 *	2.099 *	0.915 ^{AG}	0.470	0.555	1.180 ^{NS}	1.388 ^{NS}	2.610 ^{AG}
	6^{rd}	42	0.850	2.169	2.552 *	2.829 *	0.548 ^{AG}	0.670	0.991	1.479 *	1.719 *	1.398 ^{AG}
	7^{rd}	49	0.920	1.953	2.123 *	2.222 *	0.819 ^{AG}	0.740	0.821	1.109 ^{NS}	1.148 ^{NS}	6.794 ^{AG}
	8^{rd}	56	0.970	1.686	1.738 *	1.761 *	1.314 ^{AG}	0.870	1.266	1.455 *	1.524 *	1.913 ^{AG}
	9^{rd}	63	1.070	2.288	2.138 *	2.063 *	0.940 ^{AG}	0.920	1.893	2.057 *	2.150 *	0.870 ^{AG}
	10^{rd}	70	0.820	1.866	2.276 *	2.559 *	0.643 ^{AG}	0.680	1.068	1.571 *	1.844 *	1.191 ^{AG}
	11^{rd}	77	0.800	1.697	2.121 *	2.405 *	0.714 ^{AG}	0.620	0.844	1.361 *	1.586 *	1.716 ^{AG}
	12^{rd}	84	0.570	0.773	1.356 *	1.629 *	1.602 ^{AG}	0.510	0.717	1.406 *	1.804 *	1.256 ^{AG}
	13^{rd}	91	0.560	0.714	1.274 ^{NS}	1.494 *	2.043 ^{AG}	0.490	0.677	1.381 *	1.786 *	1.286 ^{AG}
	14^{rd}	98	0.500	0.576	1.152 ^{NS}	1.306 ^{NS}	3.300 ^{AG}	0.380	0.460	1.211 ^{NS}	1.565 ^{NS}	1.800 ^{AG}
Soybean (non-Bt)	1^{rd}	7	0.000	-	-	-	-	0.000	-	-	-	-
	2^{rd}	14	0.000	-	-	-	-	0.000	-	-	-	-
	3^{rd}	21	0.000	-	-	-	-	0.000	-	-	-	-
	4^{rd}	28	0.000	-	-	-	-	0.000	-	-	-	-
	5^{rd}	35	0.540	0.695	1.288 ^{NS}	1.537 *	1.877 ^{AG}	0.420	0.509	1.211 ^{NS}	1.510 ^{NS}	1.989 ^{AG}
	6^{rd}	42	0.750	1.260	1.680 *	1.910 *	1.103 ^{AG}	0.530	0.656	1.237 ^{NS}	1.451 ^{NS}	2.235 ^{AG}
	7^{rd}	49	0.900	1.970	2.189 *	2.322 *	0.757 ^{AG}	0.570	0.571	1.001 ^{NS}	1.003 ^{NS}	402.064 ^{AL}
	8^{rd}	56	0.940	1.411	1.501 *	1.533 *	1.878 ^{AG}	0.580	0.771	1.330 *	1.573 *	1.758 ^{AG}
	9^{rd}	63	0.960	1.958	2.040 *	2.083 *	0.923 ^{AG}	0.820	1.684	2.054 *	2.288 *	0.778 ^{AG}
	10^{rd}	70	0.760	1.457	1.917 *	2.211 *	0.829 ^{AG}	0.780	1.668	2.139 *	2.464 *	0.685 ^{AG}
	11^{rd}	77	0.730	1.290	1.767 *	2.055 *	0.952 ^{AG}	0.500	0.677	1.354 *	1.714 *	1.414 ^{AG}
	12^{rd}	84	0.540	0.736	1.363 *	1.677 *	1.490 ^{AG}	0.490	0.656	1.340 *	1.701 *	1.442 ^{AG}
	13^{rd}	91	0.540	0.695	1.288 ^{NS}	1.537 *	1.877 ^{AG}	0.450	0.593	1.319 *	1.717 *	1.412 ^{AG}
	14^{rd}	98	0.460	0.514	1.116 ^{NS}	1.256 ^{NS}	3.953 ^{AG}	0.320	0.402	1.255 ^{NS}	1.815 *	1.255 ^{AG}

*Not significant at 5% probability. DAE= days after (plant) emergence.

Not significant at 5% probability.

^{Ag}gregate; ^{UN}uniform; ^{AL}random.

S^2 Variance, *I* Mean-variance ratio, I_{δ} Morisita index, *K* Exponent of the negative binominal.

Tests of theoretical frequency distributions of adults of *E. heros*, with ten samples showed, sufficient numbers of classes to perform the adjustment test for Bt soybean in Douradina and Dourados (Table 3). Melo et al. (2006) and

Fonseca et al. (2013c) studied the distribution of pests and conducted theoretical frequency distributions for insect pests in eight to 12 samples in field.

The values of adults of *E. heros* to Bt and non-Bt soybean in Dourados and Douradina indicate that the data fitted to theoretical models of the Poisson distribution (random arrangement) negative binomial (aggregate score) and positive binomial (uniform set) in the samples (Table 3).

According to Degrande (1998), Fonseca et al. (2013a) insect begin the dispersion later in the areas of soybeans and over time the distribution becomes widespread throughout the field.

Table 3. Chi-square test of adherence by the expected Poisson distributions, negative binomial (Bn) positive binomial (Bp), spatial arrangement for *Euschistus heros* in Bt and non-Bt soybean [*Glicine max* (L)] frequencies in the days after emergence in Dourados and Douradina, Mato Grosso do Sul, Brazil , 2012

	Samples		Dourados			Arrangement	Douradina			Arrangement
	Numbers	(DAE)	Poisson	Bn	Bp		Poisson	Bn	Bp	
Soybean (Bt)	1rd	7	i	i	i	NP	i	i	i	NP
	2rd	14	i	i	i		i	i	i	
	3rd	21	i	i	i		i	i	i	
	4rd	28	i	i	i		i	i	i	
	5rd	35	7.959 *	8.753 NS	4.021 *	aggregate	8.292 *	10.626 NS	9.249 **	aggregate
	6rd	42	10.391 *	6.380 NS	9.078 *		11.121 *	8.941 NS	12.805 **	
	7rd	49	17.702 *	5.578 NS	14.304 *		11.702 **	8.733 NS	13.485 **	
	8rd	56	4.927 NS	20.303 *	8.215 *	random	1.402 NS	20.034 **	3.090 *	random
	9rd	63	4.324 NS	36.911 *	9.288 *		2.804 NS	45.906 **	5.501 *	
	10rd	70	7.018 NS	27.337 *	21.566 *		21.679 NS	42.482 **	63.059 **	
	11rd	77	46.555 NS	18.219 *	243.730 *		10.639 *	48.747 **	10.532 NS	uniform
	12rd	84	9.098 *	12.246 *	7.468 NS	uniform	10.556 *	10.460 NS	10.786 **	aggregate
	13rd	91	10.149 *	9.418 *	9.581 NS		10.596 NS	6.708 NS	13.365 **	
	14rd	98	8.383 *	10.450 *	8.560 NS		7.595 NS	18.872 **	11.015 **	random
Soybean (non-Bt)	1rd	7	i	i	i	NP	i	i	i	NP
	2rd	14	i	i	i		i	i	i	
	3rd	21	i	i	i		i	i	i	
	4rd	28	i	i	i		i	i	i	
	5rd	35	5.811 *	8.528 NS	6.184 *	aggregate	9.101 *	9.752 NS	9.983 **	aggregate
	6rd	42	10.020 *	5.303 NS	12.336 *		17.585 **	16.021 NS	19.459 **	
	7rd	49	16.095 *	4.674 NS	15.244 *		15.968 **	9.915 NS	18.639 **	
	8rd	56	0.817 NS	26.547 *	1.861 *	random	7.267 *	30.933 NS	7.109 **	
	9rd	63	4.974 NS	38.150 *	8.991 *		123.014 **	16.990 NS	440.705 **	
	10rd	70	4.918 NS	30.681 *	18.860 *		8.342 NS	32.821 **	24.886 **	random
	11rd	77	41.289 *	26.630 NS	195.420 *	aggregate	6.656 NS	38.551 **	8.726 **	
	12rd	84	8.169 NS	13.794 NS	7.542 *	random	6.530 *	13.297 **	6.135 NS	uniform
	13rd	91	8.804 NS	11.430 *	8.218 *		7.976 NS	8.929 *	9.578 **	random
	14rd	98	5.350 NS	15.461 *	8.316 *		9.383 NS	21.096 **	10.720 **	

**Not significant at 1% and * significant at 5%probability. DAE= days after (plant) emergence.

ns Not significant at 1% and 5% probability.

i Class insufficiently.

NP not present.

Tests of theoretical frequency distributions for nymphs of *E. heros* presented in ten samples, sufficient numbers of classes to perform the adjustment test for Bt soybean in Dourados and Douradina. The values of the nymphs of *E. heros* for Bt cultivar in Dourados and Douradina indicate that the data fitted to theoretical models of the negative binomial distribution (aggregate score) in samples taken (Table 4). The dispersion is probably induced by changes in food availability around the nymphs and competition for food (Panizzi, 1991; Panizi & Oliveira, 1998).

Brown stink bug nymphs disperse the site of oviposition, however can cover only relatively small distances. For example, small nymphs of the green stink bug, *Piezodorus guildinii* move up to 12 m from the starting point during its development in soybean fields. Importantly, the nymphs move more in the longitudinal direction than in the transverse direction of the rows of soybean (Panizzi et al., 1980; Cividanes & Parra, 1994).

The use of multiple indices of dispersion to calculate the spatial distribution of a pest is an important recommendation (Barbosa, 2003), as a single index does not provide all the attributes considered ideal from statistical point of view, while the use of more than one index can provide greater certainty to conclusions (Martins et al., 2012).

Table 4. Chi-square test of adherence by the expected Poisson distributions, negative binomial (Bn) positive binomial (Bp), spatial arrangement nymphs of *Euschistus heros* in Bt and non-Bt soybean [*Glicine max* (L.)] frequencies in the days after emergence inDourados and Douradina, Mato Grosso do Sul, Brazil, 2012

Samples			Dourados			Arrangement	Douradina			Arrangement
	Numbers	(DAE)	Poisson	Bn	Bp		Poisson	Bn	Bp	
Soybean (Bt)	1rd	7	i	i	i	NP	i	i	i	NP
	2rd	14	i	i	i		i	i	i	
	3rd	21	i	i	i		i	i	i	
	4rd	28	i	i	i		i	i	i	
	5rd	35	20.461 **	7.458 NS	21.847 **	aggregate	4.502 *	2.518 NS	5.254 *	aggregate
	6rd	42	30.310 **	12.056 NS	29.451 **		11.556 **	4.811 NS	15.652 **	
	7rd	49	11.718 **	7.326 NS	10.364 **		5.807 *	7.688 NS	6.018 *	
	8rd	56	9.443 *	4.814 NS	11.316 **		10.248 *	7.230 NS	11.515 **	
	9rd	63	21.138 **	6.070 NS	25.156 **		25.884 **	8.065 NS	29.092 **	
	10rd	70	30.310 **	6.738 NS	29.451 **		16.840 **	5.841 NS	21.933 **	
	11rd	77	18.256 **	3.6704 NS	21.525 **		14.142 **	5.214 NS	15.324 **	
	12rd	84	12.679 **	4.201 NS	14.136 **		13.020 **	3.780 NS	14.752 **	
	13rd	91	7.365 *	2.704 NS	8.514 **		10.815 *	3.041 NS	12.480 **	
	14rd	98	7.219 *	5.722 NS	8.038 **		4.133 *	1.815 NS	4.938 *	
Soybean (non-Bt)	1rd	7	i	i	i	NP	i	i	i	NP
	2rd	14	i	i	i		i	i	i	
	3rd	21	i	i	i		i	i	i	
	4rd	28	6.219 *	4.434 NS	6.928 **	aggregate	i	i	i	
	5rd	35	15.124 **	7.969 NS	16.720 **		7.659 *	4.210 NS	8.577 **	aggregate
	6rd	42	21.950 **	9.480 NS	29.451 **		11.581 **	6.547 NS	12.628 **	
	7rd	49	16.440 **	8.466 NS	14.333 **		3.486 *	6.124 NS	3.847 *	
	8rd	56	8.419 *	2.931 NS	9.641 **		12.370 **	4.538 NS	13.613 **	
	9rd	63	22.972 **	5.801 NS	27.462 **		25.789 **	8.908 NS	28.352 **	
	10rd	70	32.222 **	6.743 NS	31.628 **		30.113 **	9.880 NS	33.317 **	
	11rd	77	22.212 **	3.790 NS	25.758 **		15.695 **	6.241 NS	17.356 **	
	12rd	84	11.372 **	3.283 NS	12.975 **		9.339 *	2.600 NS	10.730 **	
	13rd	91	7.554 *	2.387 NS	8.745 **		7.442 *	1.929 NS	8.743 **	
	14rd	98	3.112 *	2.917 NS	3.666 NS		3.987 *	1.006 NS	4.900 *	

**Not significant at 1% and * significant at 5%probability. DAE= days after (plant) emergence.

ns Not significant at 1% and 5% probability.

i Class insufficiently.

NP not present.

Adults of *E. heros* are arranged differently between soybean cultivars and between regions, since in both cultivars and locations, individuals of the populations studied showed different patterns of spatial distribution as a function of days after emergence. However, with respect to the spatial arrangement of the nymphs of *E. heros*, it was found that the location of Bt soybean cultivation and did not influence the distribution, because when analyzing the results obtained from the theoretical frequency distributions, represented by aggregate in both cultivars and in both regions disposal. This result corroborates with the study of Souza et al. (2013) corroborates this work, in which they reported that nymphs of *E. heros* of the first to third instars clumped spatial distribution.

The absence of caterpillars target of Bt soybean seems to have favored the growth of populations of adults and nymphs of *E. heros* in cultivating Bt. Thus, it is suggested that future studies seek to develop sequential samples of this pest in soybean in both the Bt crop cultivation and non-Bt, aiming to define the exact number of sample units to be used.

The precise number of sample units for the sampling process of these insects is extremely important since those species are the direct cause considerable crop losses. Thus the application of insecticides should be performed only in locations where the presence of insect pests and taking into account the principles of integrated pest management and arrangement of spatial distribution. This will result in a more efficient management, with less environmental impact.

4. Conclusion

Adults of *E. heros* are arranged differently for Bt and non-Bt soybean and between regions, since in both cultivars and locations, individuals of the populations evaluated in probabilistic set arrangements negative binomial distribution (aggregate), Poisson (random), and positive binomial distribution (uniform), as the days after soybean emergence.

The nymphs of *E. heros* showed aggregated spatial arrangement, being distributed similarly for Bt and non-Bt soybean and regions, setting a negative binomial distribution model.

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