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Temporal variation and spatial distribution of *Euschistus heros* (Hemiptera: Pentatomidae) during the soybean grain formation period

Variação temporal e distribuição espacial de *Euschistus heros* (Hemiptera: Pentatomidae) durante o período de formação de grãos da cultura da soja

Variación temporal y distribución espacial de *Euschistus heros* (Hemiptera: Pentatomidae) durante el período de formación de granos del cultivo de la soja

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Abstract
The neotropical brown stink bug *Euschistus heros* (Hemiptera: Pentatomidae) is one of the most important pests in soybean and knowledge of its distribution is necessary to determine the moment of control and enable the appropriate use of strategies to avoid production losses. This research aimed to study the temporal variation and the spatial distribution of *E. heros* in soybean. The study was carried out in an area of 6600 m^2 of soybean, with 60 plots of 100 m^2 each, with two sampling points/plot being evaluated weekly by the beat cloth method to count nymphs and adults. The evaluations occurred from fruiting (R3) to physiological maturation (R8). The data were subjected to dispersion indices (variance/mean ratio, Morisita index, and exponent *k*), theoretical frequency distributions (Poisson, negative binomial, and positive binomial), and spatial analysis by inverse distance weighting (IDW). This study demonstrated the infestation of *E. heros* throughout the evaluation period, and the dispersion indices showed the trend of nymph aggregation and the sum of nymphs and adults (total population). However, adults tended towards a moderate to uniform aggregation. Theoretical frequency distributions for nymphs and total population indicated randomness at the beginning of the infestation, tending to aggregation from R5 onwards. Aggregation is confirmed in adults, but the population tended towards uniformity at the end of the cycle. The use of the IDW method is an important tool for the rational management of the neotropical brown stink bug in the soybean crop.
Keywords: Neotropical brown stink bug; Agroecosystem; Infestation; Sampling; Mapping.

Resumo
O percevejo marrom *Euschistus heros* (Hemiptera: Pentatomidae) é uma das mais importantes pragas em soja e o conhecimento da forma de distribuição deste inseto é necessário para determinar o momento de controle e possibilitar o uso apropriado das estratégias para evitar perdas na produção. O presente trabalho, objetivou estudar a variação temporal e a distribuição espacial de *E. heros* na cultura da soja. O trabalho foi conduzido em uma área de 6600 m^2 de soja, com 60 parcelas de 100 m^2 cada, sendo avaliada dois pontos amostrais/parcela semanalmente pelo método de

pano de batida, contabilizando ninfas e adultos. As avaliações ocorreram a partir da frutificação (R3) até a maturação fisiológica (R8). Os dados foram submetidos aos índices de dispersão (razão variância/média, Morisita e Expoente k) e distribuições teóricas de frequências (Poisson, binomial negativa e binomial positiva), além da análise espacial por Inverse Distance Weight (IDW). Este estudo demonstrou a infestação de *E. heros* durante todo o período de avaliação, e os índices de dispersão demonstraram a tendência de agregação de ninfas e o somatório de ninfas e adultos (total da população), contudo, os adultos tenderam de uma moderada agregação à uniformidade. As distribuições teóricas de frequência para ninfas e total da população indicaram aleatoriedade no início da infestação tendendo a agregação a partir de R5. Em adultos, a agregação é confirmada, contudo, a população tendeu a uniformidade no final do ciclo. O uso do método IDW é uma ferramenta importante visando um manejo racional deste percevejo na cultura da soja.
Palavras-chave: Percevejo-marrom; Agroecossistema; Infestação; Amostragem; Mapeamento.

Resumen
La chinche marrón es una de las plagas más importantes de la soja, y el conocimiento de la forma de distribución de este insecto es necesario para determinar el momento del control y permitir el uso adecuado de las estrategias para evitar pérdidas en la producción. El presente trabajo tuvo como objetivo, estudiar la variación temporal y la distribución espacial de *Euschistus heros* (Hemiptera: Pentatomidae) en el cultivo de la soja. El trabajo se realizó en un área de 6000 m² de soja, con 60 parcelas de 100 m² cada una, siendo evaluados semanalmente dos puntos de muestreo/parcela por el método de paño de batida, contando ninfas y adultos. Las evaluaciones ocurrieron desde la fructificación (R3) hasta la maduración fisiológica (R8). Los datos fueron sometidos a los índices de dispersión (razón de varianza/media, Morisita y Exponente *k*) y distribuciones teóricas de frecuencias (Poisson, binomial negativa y binomial positiva), además del análisis espacial por Inverse Distance Weighting (IDW). Este estudio demostró la infestación de *E. heros* durante todo el período de evaluación, y los índices de dispersión demostraron la tendencia de agregación de ninfas y la suma de ninfas y adultos (población total), sin embargo, los adultos tendieron hacia una moderada agregación a la uniformidad. Las distribuciones teóricas de frecuencia para ninfas y la población total indicaron aleatoriedad al inicio de la infestación, con tendencia a la agregación a partir de R5. En los adultos se confirma la agregación, sin embargo, la población tendió a la uniformidad al final del ciclo. El uso del método IDW es una herramienta importante para un manejo racional de esta chinche en el cultivo de la soja.
Palabras clave: Chinche marrón; Agroecosistema; Infestación; Muestreo; Mapeo.

1. Introduction

Soybean *Glycine max* (L.) Merrill belongs to the family Fabaceae and is considered one of the crops of greatest economic interest in the world due to its wide adaptability to different soils and climate conditions, which allows its production in almost the entire Brazilian territory and a great potential for foreign markets (Rocha et al., 2018).

New agricultural frontiers ended up being established in Brazil over the years, with an increase in the cultivated area. In addition, the use of Bt technology in crops such as soybean, corn, and cotton aiming at the management of multiple species of insect pests of the orders Lepidoptera and Coleoptera led to an increase in the use of insecticides and a decrease in their spectrum of action, and pests that were not previously key and not susceptible to toxin expression started to use this food source without competition (Catarino et al., 2015; Barbosa et al., 2020). One of the main species favored with the insertion of this technology was the phytophagous stink bug complex in the soybean crop (Panizzi et al., 2022).

Phytophagous stink bugs can cause quantitative and qualitative losses, such as a decrease in grain weight, delay in maturation, and reduction in oil content and germination power of seeds (Panizzi et al., 2022).

The neotropical brown stink bug *Euschistus heros* (Hemiptera: Pentatomidae) is the most abundant within the soybean stink bug complex, feeding on Fabaceae, Solanaceae, Brassicaceae, and Compositae (Panizzi, 2015). More recently, it has been reported to feed on Malvaceae (cotton), becoming an important pest in the Brazilian Midwest (Weber et al., 2018).

This stink bug can cause direct damage to the soybean crop through suction performed by nymphs and adults on pods and stems, piercing the plant tissue with their mandibular and maxillary stylets, releasing digestive enzymes, and extracting the plant fluid (Scopel et al., 2016; Tessmer et al., 2021).

In addition, the occurrence of the yeast *Eremothecium coryli* and the phenomenon known as “crazy soybean,” which consists of delaying soybean maturation, making mechanized harvesting difficult due to the presence of green stems and the retention of leaves by the plants, are considered as indirect damages (Sosa-Gómez et al., 2019).

The main tool for the management of this stink bug is chemical control, and most of the used insecticides have a broad spectrum of action, such as organophosphates and pyrethroids, often used in the vegetative period, when they are not effective for population reduction, directly affecting natural enemies (Marques et al., 2019). Therefore, knowing the population dynamics of this insect in the soybean crop is necessary to establish sampling plans (Grigolli et al., 2012) and accurately describe its occurrence in the field, aiming at reducing damage and providing rational management (Fernandes et al., 2018).

Furthermore, the use of geostatistics can be a complementary tool to graphically visualize how these insects are distributed in the field, providing useful visual information about the shape and location of the spatial distribution of pests under field conditions (Sciarretta & Trematerra, 2014). Therefore, knowledge of spatial distribution can be an important tool when associated with the integrated pest management to be considered when choosing to carry out chemical control of this insect, aiming at higher efficiency and reduction of control costs and damage to the environment (Pereira et al., 2018, Silva et al., 2021). In addition, little information can be found in the literature on the spatial distribution of *E. heros* during the soybean grain formation period. In this context, this study aimed to know the temporal variation and spatial distribution of *E. heros* in soybean.

2. Methodology

The study was carried out in the field during the 2020/21 growing season at Fazenda Três Meninas located in the municipality of Dourados, MS, Brazil, at the latitude of 22°15′34″ S and longitude of 54°24′52″ W and altitude of 400 m.

Sowing was carried out on November 5, 2020, with the cultivar M5947 IPRO®. The inter-row spacing was 0.50 m, with 15 plants per linear meter and base fertilization with 300 kg/ha of the formulation 4–30–10. Weed and disease management was carried out according to recommendations for the crop.

A total area of 6,600 m^2 was divided into 60 sampling units, each unit consisting of 22 crop rows 10 meters long (110 m^2), numbered from 1 to 60 and distributed sequentially in the study area.

The characterization of the phenological crop development followed Fehr and Caviness (1977), consisting of dividing the crop into vegetative (V1 to Vn) and reproductive (R1 to R8) stages. The stink bug population survey was carried out weekly from pod development (R3) to full maturation (R8), using the beat cloth method (2 points/plot/week) and counting the number of *E. heros* nymphs and adults, visually and directly on the beat cloth.

The obtained data were used to characterize the temporal and spatial variation of *E. heros* nymphs and adults, determining the mean, variance, and aggregation indices. The aggregation indices were variance/mean ratio, Morisita index, and negative binomial K index (Fernandes et al., 2018), while the theoretical frequency distribution used to verify the spatial distribution consisted of the Poisson distribution, positive binomial distribution, and negative binomial distribution, according to the methodology described by Fonseca et al. (2017).

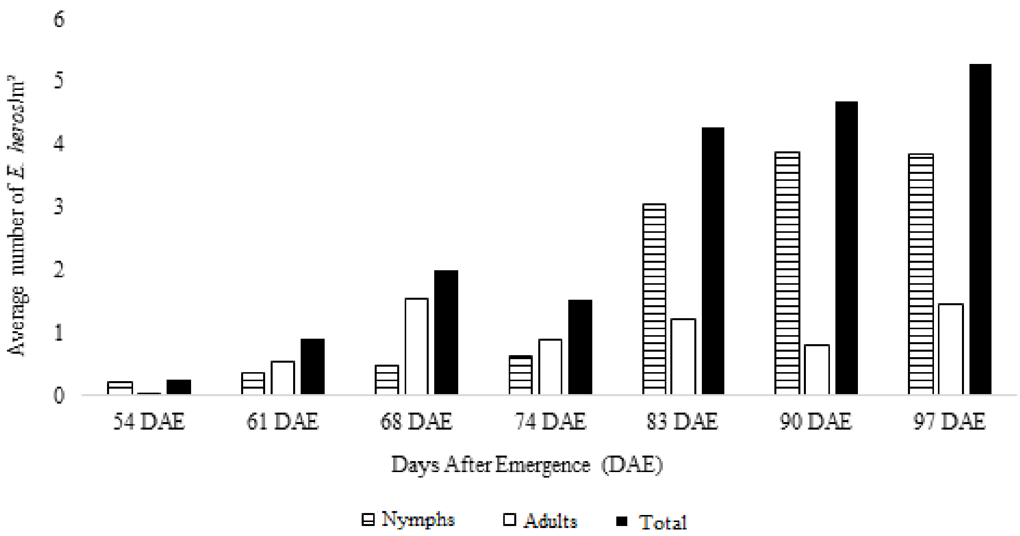
Maps of the number of individuals collected during the assessments were interpolated for the visual representation of the statistical models, using the inverse distance weighting (IDW) method through the ArcGIS® software. The generated maps describe the density of stink bugs (nymphs, adults, and nymphs + adults) in the experimental area.

3. Results and Discussion

This study reported the population dynamics and spatial distribution of *E. heros* during fruiting, grain filling, and physiological maturation of the soybean crop. Both the number of nymphs and adults increased throughout the cycle. Furthermore, based on frequency distributions and spatial analysis, *E. heros* showed a random distribution at the beginning of fruiting, tending to aggregate in subsequent evaluations.

Temporal variation of *E. heros*: Figure 1 shows the temporal variation of *E. heros* in soybean during the fruiting period under field conditions. A total of 749 nymphs and 388 adults of *E. heros* were counted, totaling 1137 stink bugs over seven evaluations. Nymphs accounted for 65.9% of the total population, while adults accounted for 34.1%.

Figure 1. Temporal variation of *E. heros* in soybean during the fruiting period, Dourados, MS, Brazil, 2021.



Source: Authors.

The nymph population grew throughout the evaluations, surpassing the number of adults when the soybean was at R6 (Figure 1), a moment characterized by full and green grains. This fact is corroborated by Silva et al. (2014), who observed an increase in the number of nymphs after the grain formation and filling period, which may be explained by the appearance of pods, making soybean more nutritionally suitable for reproduction, and increasing the number of nymphs (Fonseca et al., 2017).

The adult population increased until R5, with a stabilization in the growth of the recorded population (Figure 1). However, the total number of *E. heros* (nymphs + adults) increased until R8 (physiological maturation), demonstrating its ability to colonize the crop until the harvest time.

The temporal variation of adults was in line with Silva et al. (2014), who found the stabilization of adult growth when the plants were at the grain filling stage (R5 to R5.4).

The infestation of *E. heros* remained above the economic injury level in the most critical period of the crop to the attack of stink bugs (reproductive period), reaching 1 stink bug/m^2 for seed production and 2 stink bugs/m^2 for grain production (Panizzi et al., 2012). Therefore, frequent monitoring is essential in the period considered most sensitive to the stink bug attack.

Dispersion indices: The spatial distribution model of an insect pest is defined according to the degree of aggregation of each species through the relationship between the mean and the sample variance. The results of the index variance/mean ratio (I) for nymphs showed 85.7% of the values higher than 1 (R3, R4, R5, R5.4, R6, and R8), inferring an aggregate distribution (Table 1). The Morisita index (Iδ) showed that 85.7% of the values were higher than 1 (R3, R4, R5, R5.4, R6, and R8), indicating an aggregate distribution and confirming the aggregation presented by the variance/mean ratio. The results of the exponent k showed that 42.9% of the evaluation data indicated a highly aggregate distribution, confirming the results from the

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variance/mean ratio and Morisita index.

Table 1. Stage, mean infestation, and spatial distribution indices of *E. heros* during the fruiting period of soybean, Dourados, MS, Brazil, 2021.

Stage		$\dot{m}$	S^2	I	$I\delta$	K	$\chi 2$
	Nymph	0.233	0.317	1.360^{ns}	2.637*	0.040^{ag}	80.290
R3	Adult	0.033	0.033	0.983^{ns}	0.00^{ns}	-1.966^{un}	58.000
	Total	0.266	0.335	1.254^{ns}	2.000^{ns}	1.048^{ag}	74.000
	Nymph	0.366	0.540	1.476^{ns}	2.338*	0.770^{ag}	87.000
R4	Adult	0.533	0.800	1.491*	1.935*	1.085^{ag}	88.000
	Total	0.900	1.620	1.797*	1.887*	1.130^{ag}	106.000
	Nymph	0.483	0.490	1.016^{ns}	1.034^{ns}	29.535^{al}	59.970
R5	Adult	1.533	2.080	1.359^{ns}	1.233*	4.272^{ag}	80.170
	Total	2.016	2.360	1.168^{ns}	1.083^{ns}	11.997^{al}	68.920
	Nymph	0.633	1.080	1.711*	2.134*	0.891^{ag}	100.950
R5.4	Adult	0.883	0.820	0.924^{ns}	0.914^{ns}	-11.704^{un}	54.550
	Total	1.516	1.610	1.061^{ns}	1.040^{ns}	24.675^{al}	62.630
	Nymph	3.050	7.300	2.394*	1.452*	2.187^{ag}	141.260
R6	Adult	1.233	1.910	1.549*	1.444*	2.245^{ag}	91.410
	Total	4.266	7.460	1.741*	1.171*	5.774^{ag}	102.770
	Nymph	3.883	3.870	0.996^{ns}	0.999^{ns}	-953.289^{un}	58.760
R7	Adult	0.800	0.710	0.881^{ns}	0.851^{ns}	-6.743^{un}	52.000
	Total	4.683	4.690	1.002^{ns}	1.000^{ns}	1941.120^{al}	59.140
	Nymph	3.833	5.570	1.452*	1.116*	8.486^{al}	85.650
R8	Adult	1.450	1.740	1.202^{ns}	1.139^{ns}	7.170^{ag}	70.930
	Total	5.283	8.510	1.611*	1.114*	8.647^{al}	95.050

* = Significant at the 5% level by the chi-square test; ^{ag} = Aggregate; ^{un} = Uniform; ^{ns} = Not significant; $\dot{m}$ = Mean; S^2 = Variance; I = variance/mean ratio; Iδ = Morisita index; k = Exponent k; X^2 = Chi-square. Source: Authors.

The aggregate distribution of nymphs measured through the variance/mean ratio and Morisita index corroborate the results found by Souza et al. (2013), who observed values higher than 1 for these indices.

The variance/mean ratio (I) and Morisita index (Iδ) for adults presented 57.1% of values higher than 1 (R4, R5, R6, and R8) and 42.9% lower than 1 (R3, R5.4, and R7), which means a moderately aggregate to a uniform distribution. Likewise, the exponent k showed an oscillation from moderately aggregate to a uniform distribution, with corroboration of 42.9% of the evaluations in both. The conformity between the three dispersion indices attests with higher reliability to the form of dispersion of adults in this study.

The reported distribution of adults is confirmed by findings by Fonseca et al. (2014), in which the values of the variance/average ratio and exponent k vary from aggregate to uniform, while the values of the Morisita index show the aggregation of nymphs.

The sum of nymphs and adults presented values for the variance/mean ratio (I) and Morisita index (Iδ) indices higher than 1 at all phenological stages, except for R7 related to the Morisita index, reporting an aggregated distribution. According to the values of the exponent k for the total population, the distribution presents variations, being initially highly aggregate in the period before grain filling (R3 and R4), becoming random from then on, with 28.6 and 57.1% of the evaluations, respectively.

Frequency distribution: Some evaluations showed an insufficient number of classes for data interpretation. In this sense, insufficiency was observed for nymphs at R3, R4, R5, R5.4, and R6 for the positive binomial distribution and R5 for the negative binomial distribution. On the other hand, adults showed insufficiency at R3 for the three theoretical distribution models and R4 for the positive binomial distribution. The total population showed an absence in the number of classes at R3 for positive and negative binomial distributions and R4 for the negative binomial distribution.

Variations in the spatial distribution of *E. heros* nymphs were observed from R3 to R5, the period of grain formation and filling, ranging between randomness and aggregation. However, the nymphs show an aggregate distribution after grain filling until the harvest period (Table 2).

Table 2. Stage, chi-square (χ^2) test of adherence to the Poisson frequency, positive binomial, and negative binomial theoretical distributions for *E. heros* in the fruiting period of soybean, Dourados, MS, Brazil, 2021.

Stage		Poisson		Negative binomial		Positive binomial	
		X^2	DF(nc-2)	X^2	DF(nc-3)	X^2	DF(nc-3)
R3	Nymph	7.291^{**}	1	15.987^{**}	1	-	-
	Adult	0.01	*i*	0.0001	*i*	0.001	*i*
	Total	4.299^{*}	1	1.145	0	1.296	*i*
R4	Nymph	3.142^{ns}	1	0.423^{ns}	1	5.088	*i*
	Adult	5.651^{ns}	2	0.257^{ns}	1	6.943	0
	Total	6.439^{*}	2	7.203^{*}	2	46.182	0
R5	Nymph	0.003^{ns}	1	0.0014	0	0.001	0
	Adult	5.182^{ns}	4	3.22^{ns}	3	19.513^{**}	2
	Total	4.796^{ns}	5	4.774^{ns}	4	3.524^{ns}	3
R5.4	Nymph	2.683^{ns}	2	1.964^{ns}	2	19.423	0
	Adult	3.449^{*}	2	3.117^{ns}	1	4.032^{*}	1
	Total	10.529^{*}	3	10.796^{*}	3	10.377^{**}	2
R6	Nymph	40.525^{**}	6	7.643^{ns}	8	-	-
	Adult	6.229ns	3	0.514^{ns}	3	89.529^{**}	1
	Total	12.255ns	7	6.379^{ns}	9	102.002^{**}	2
R7	Nymph	12.792ns	8	12.835^{ns}	6	12.751^{ns}	7
	Adult	722.232^{**}	2	963.012^{**}	1	594.592^{**}	1
	Total	9.348ns	8	9.336^{ns}	7	7.378^{ns}	7
R8	Nymph	19.184^{*}	8	12.693^{ns}	8	56.819^{**}	4
	Adult	1.937^{ns}	3	0.744^{ns}	3	6.851^{*}	2
	Total	26.587^{**}	9	10.452^{ns}	9	67.130^{**}	6

* = Significant at the 5% level by the chi-square test; ** = significant at the 1% level by the chi-square test; ns = not significant at the 1% and 5% levels by the chi-square test; i = insufficient number of classes; DF = degrees of freedom; nc = number of classes. Source: Authors.

The results obtained for nymph distribution are in accordance with those found by Souza et al. (2013), who observed a strong trend toward nymph aggregation in different soybean cultivars.

Adults had a better fit to the negative binomial distribution, confirming the aggregation of their distribution. The results of nymphs and adults found in this study corroborate those obtained by Fonseca et al. (2014), who stated a better fit to the negative binomial distribution in all evaluated dates, indicating an aggregate distribution.

Moreover, Weber et al. (2018) reported a trend toward the aggregate distribution of *E. heros* in cotton, which agrees with the data found in the study in question.

The total population showed a variation in the distribution before the beginning of grain filling (R3 and R4), with a random distribution with a trend towards uniformity during the filling stage. However, aggregation becomes marked from R6 until the harvest period.

The movement and spatial distribution of insects can occur as a function of reproduction in response to climate change (temperature and humidity), aggregation pheromones, and availability of food sources (Tillman & Cottrell, 2016).

Therefore, the plants became more nutritionally suitable for feeding stink bugs with pod formation, resulting in an increase in the population during grain filling and maturation (Fonseca et al., 2017). Consequently, an aggregate pattern is observed, corroborating with Fonseca et al. (2017), who studied *Edessa meditabunda* (Hemiptera: Pentatomidae), and Silva et al. (2022), who studied *Piezodorus guildinii* (Hemiptera: Pentatomidae), both in soybean.

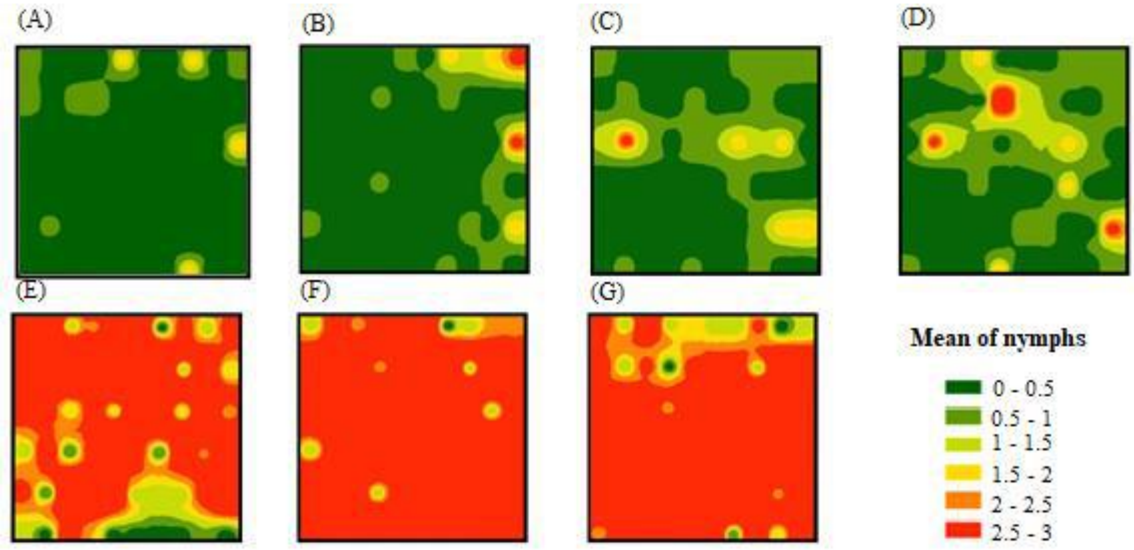
Spatial analysis: Studies aiming to determine the spatial distribution of insects in crops of economic interest are essential. However, only the distribution pattern of insects (random, aggregate, or uniform) is taken into account when only dispersion and frequency distribution indices are used, not considering the location of the insect in the field, which is obtained by spatial analysis (Silva et al., 2021).

The use of the IDW method allowed the visualization of the distribution of nymphs (Figure 2) and adults (Figure 3) separately and associated (Figure 4) over time in the soybean crop.

Nymph infestation (Figure 2) could be observed mainly in the borders of the experimental area when the plants were at R3 and R4 (Figure 2a, b). However, there is a trend in which the insects are distributed towards the center of the experimental area and aggregated points as the evaluations progress (Figure 2c, d, e, f, g), corroborating the frequency distributions (Table 2).

The result obtained for the movement of nymphs in this study corroborates with that obtained by Weber et al. (2018) when studying the distribution of *E. heros* nymphs in cotton, and Silva et al. (2021), when studying the distribution of *E. meditabunda* nymphs in cotton, who observed a trend of infestation by the borders and randomness at the beginning of the infestation. However, the nymphs moved to the center of the experimental area in the following evaluations and remained aggregated.

Figure 2. Surface maps based on the inverse distance weighting (IDW) interpolation method, demonstrating the spatial distribution of *E. heros* nymphs on soybean at R3 (A), R4 (B), R5 (C), R5.4 (D), R6 (E), R7 (F), and R8 (G). The lowest density is indicated in green, while the highest density is indicated in red.



Source: Authors.

Random distribution is observed for adults at R3 (Figure 3a). Subsequent evaluations (Figure 3b, c, d, e, f, g) showed dispersion of adults for all cardinal points of the experimental area and aggregation sites, with the results of surface maps corroborating with those obtained by frequency distributions (Table 2).

The strong aggregation of *E. heros* adults may be related to the plant phenology, as the provision of more nutritionally adequate structures (pods and grains) made their dispersal to other areas in search of new food sources unnecessary (Reisig et al., 2015).

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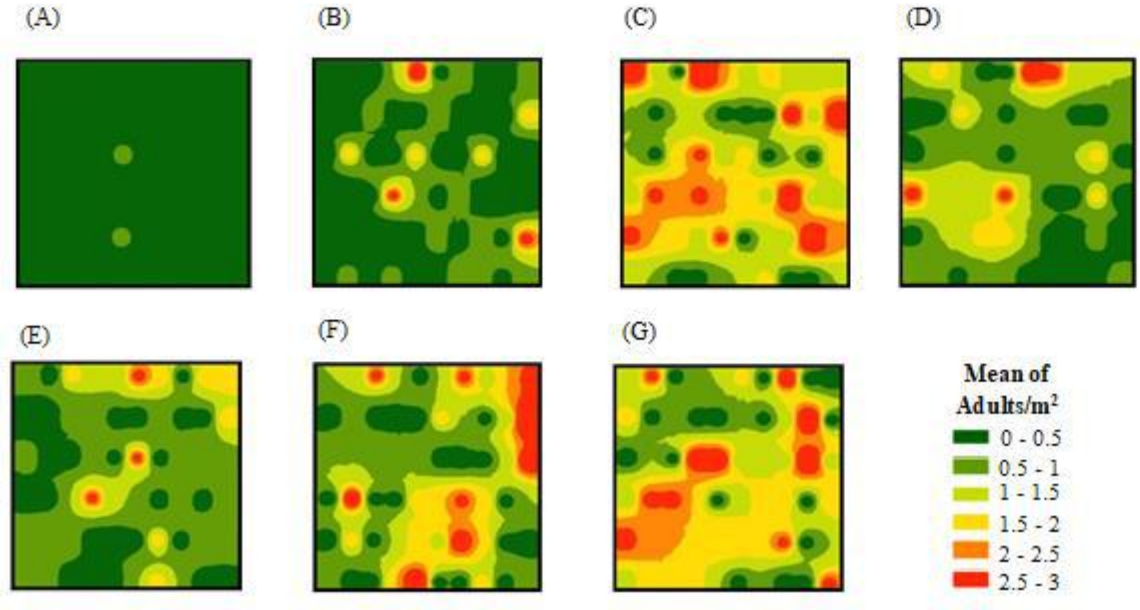
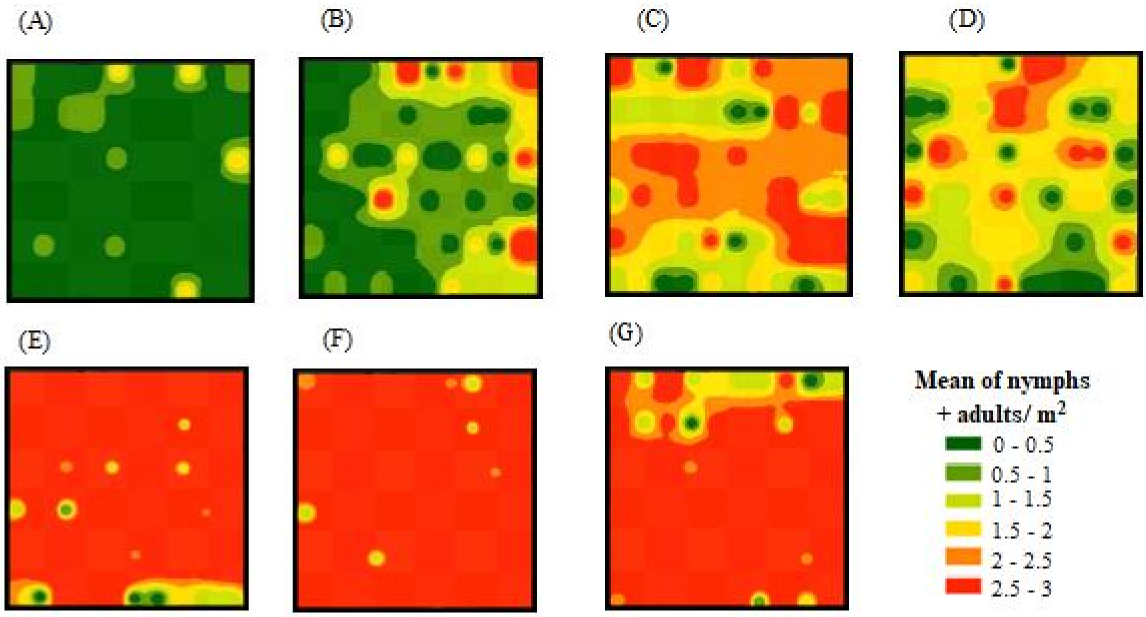
Figure 3. Surface maps based on the inverse distance weighting (IDW) interpolation method, demonstrating the spatial distribution of *E. heros* adults on soybean at R3 (A), R4 (B), R5 (C), R5.4 (D), R6 (E), R7 (F), and R8 (G). The lowest density is indicated in green, while the highest density is indicated in red.



Source: Authors.

The total population distribution (Figure 4) in the first two evaluations at R3 and R4 (Figure 4a and 4b) showed a behavior tending between randomness and aggregation. However, a trend towards the aggregation of the total population is observed after pod formation (Figure 4c), which is similar to what occurred with adult distribution, indicating the presence of adequate nutritional structures for the development of this insect on the crop.

Figure 4. Surface maps based on the inverse distance weighting (IDW) interpolation method, demonstrating the spatial distribution of the total population (nymphs + adults) of *E. heros* on soybean at R3 (A), R4 (B), R5 (C), R5.4 (D), R6 (E), R7 (F), and R8 (G). The lowest density is indicated in green, while the highest density is indicated in red.



Source: Authors.

This study suggests the control of *E. heros* only at the border at the beginning of the infestation. However, control in the total area will be necessary in case the population increase and its distribution to the inside of the cultivated area occurs.

Furthermore, the results show the possibility of using geostatistics in the evaluation of the spatial distribution of *E. heros* and the definition of sampling plans, aiming at more rational management of this insect pest.

4. Conclusion

In soybean, *E. heros* occurred throughout the reproductive stage and physiological maturation.

The spatial distribution of nymphs and adults is best described as aggregate based on aggregation indices. Regarding the theoretical distribution of frequencies, the occurrence of this pest in soybean was best described by the negative binomial distribution.

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