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ORIGINAL ARTICLE

Temporal variation and spatial distribution of *Piezodorus guildinii* (Weestwood, 1837) in soybean (*Glycine max* (L.) Merrill)

Rafael Azevedo Silva[1]*, Paulo Eduardo Degrande[2], Bruno Souza Martins[1], Ellen Patricia Souza[2], Marcos Gino Fernandes[2]

[1] Department of Plant Protection, Federal Institute of Mato Grosso do Sul, Brazil

[2] Department of Agricultural Science, Federal University of Grande Dourados, Dourados, Brazil

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*Corresponding address:
rafael.silva@ifms.edu.br

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Abstract

The red-banded stink bug, *Piezodorus guildinii*, is an important pest in soybean. Information on its distribution is needed to determine the most effective timing for pest control and strategies to avoid yield losses, such as adequate monitoring. The present study was aimed at examining the temporal variation and spatial distribution of *P. guildinii* in soybean. The experimental design comprised an area of 5400 m² planted with soybean, with 54 plots of 100 m² each, in which plants were examined weekly for nymphs and adults of *P. guildinii* with a beating sheet. Evaluations were carried out from soybean emergence to harvest; however, *P. guildinii* occurred only during the reproductive stage. Based on aggregation indices, theoretical frequency distributions, and semivariograms, nymphs and adults were randomly distributed at the beginning of infestation but, tended to be aggregate during pod setting and seed filling. Our findings have a contribution to improving pest sampling systems and infestation mapping, including future semiochemicals studies.

Keywords: dispersal indices, frequency distribution, infestation, red-banded stink bug, spatial analysis

Introduction

The red-banded stink bug, *Piezodorus guildinii* W. (Hemiptera: Pentatomidae), is a neotropical pest associated with several species of Fabaceae, Solanaceae, Poaceae, Brassicaceae, Malvaceae, as well as many native plants and weeds (Bundy *et al.* 2018). It has been recorded from southeastern United States to the Southern Cone region of South America (Souza *et al.* 2013; Zerbino *et al.* 2015).

Phytophagous stink bugs can cause quantitative and qualitative losses, such as lower seed weight, slower maturation, reduction of oil content and germinating power of seeds (Bundy *et al.* 2018). Among phytophagous stink bugs, *P. guildinii* has the greatest potential to cause these types of damage (Zerbino *et al.* 2015). In addition, it can spread bacteria (some phytopathogenic) to their hosts as *Pseudomonas flectens, Brenneria nigrifluens, Erwinia persicina* and *Ralstonia picketti* by red-banded stink bug feeding (Husseneder *et al.* 2017).

To reduce losses, chemical control is the most usual strategy for stink bug management in soybean crops, and the insecticide groups used are restricted to neonicotinoids, organophosphates and pyrethroids, carried out by many farmers during the vegetative stage, when it is not always effective and selective for natural enemies (Marques *et al.* 2019). The indiscriminate use of pesticides can also cause ecological and economic problems. Therefore, measures aimed at providing guidelines for the use of insecticides are essential (Silva *et al*. 2021).

The establishment of reliable sampling is needed to define effective monitoring for an early assessment of pest populations, which is essential for effective pest control, as well as damage and time of occurrence (Cocco *et al*. 2014). Thus, characterizing the spatial distribution of *P. guildinii* is the first step to establish sampling plans to reduce sampling time and increase reliability (Grigolli *et al*. 2012), providing information on the occurrence of this pest in the field, when aggregation indices and theoretical frequency distribution present similar results (Fernandes *et al*. 2018).

Based on these results, the species distribution can be described as aggregate, random and uniform. However, for confirmation of this distribution it is necessary to know how to adjust theoretical frequency for each species and for each specific crop (Silva *et al*. 2021).

Although these indices provide information to characterize the distribution of a population, they disregard the spatial location of sampling points, not differentiating distinct spatial patterns. In addition, they are dependent on sample size (Liebhold *et al*. 1993). However, geostatistics is a complementary tool that provides quantitative descriptions of natural variables distributed in space or in time and space, making it possible to quantify spatial dependence among samples collected in the field and constructing maps from these dependencies (Liebhold *et al*. 1993).

In view of the above, information on spatial distribution is the first step to assist in the decision-making process of pest control and provide guidelines for insecticide application when needed for application in specific sites in the field (Silva *et al*. 2021). Therefore, the present study was aimed at examining the temporal variation and spatial distribution of *P. guildinii* in soybean.

Materials and Methods

The study was carried out under field conditions at the Federal Institute of Mato Grosso do Sul, located in the municipality of Nova Andradina/MS-Brazil, during the 2018–2019 crop season, coordinates 22°04’48.4” and S 53°27’58.7” W.

The perimeter of the area was delimited with a global positioning system (GPS), with interface for a Pocket PC. Each plot had a planting area of 10 × 10 m, thus comprising 100 m^2, with 54 sample plots, giving a total planting area of 5,400 m^2.

The experimental field was planted with the cultivar SWBriza® on 17 October 2018, with a spacing of 0.45 m between rows, resulting in 13 plants per linear meter. Diseases and weeds were managed according to recommendations for the crop.

The characterization of the developmental stages of soybean plants followed Matsuo *et al*. (2015) by dividing into vegetative (V1 to Vn) and reproductive (R1 to R8) stages.

A population survey of the red-banded stink bug was carried out weekly from V2 until harvest with a white beating sheet. After quickly putting the beat cloth on the ground, the plants were vigorously shaken to dislodge the stink bugs onto the beat cloth for enumeration. Two points were taken at each plot, corresponding to 1 m^2/plot, when nymphs and adults of *P. guildinii* were visually counted.

Insects were observed in nine of the 15 evaluations, between 55 days after emergence (*DAE*) and 112 *DAE*, and the number of nymphs and adults counted were used to mathematically describe the distribution of the stink bug population, based on the mean, variance, and aggregation indices.

The aggregation indices used for *P. guildinii* were variance/mean ratio, Morisita index, and exponent *k* of the negative binomial (Fernandes *et al*. 2018). In addition, theoretical frequency distributions (Poisson, negative, and positive binomial distributions) were used, as described by Fonseca *et al*. (2017).

To visualize the calculated statistical models, surface maps were constructed by using the Inverse Distance Weighting (IDW) for data exhibiting a nugget effect. Data without a nugget effect were submitted to geostatistical analysis by using semivariograms, from which models were adjusted for interpolation of population data and subsequent construction of maps generated by kriging (Carvalho *et al*. 2015). The analysis of semivariograms, kriging, and the construction of surface maps were carried out with the software Gamma Design GS+. Surface maps thus describe the density of stink bugs in the experimental field.

Results

Temporal variation of *Piezodorus guildinii*

Throughout this study, 487 specimens of *P. guildinii* (nymphs + adults) were recorded. Figure 1 illustrates the population dynamics of this stink bug in soybean. Assessments were carried out from emergence to crop harvest. The infestation began with the first soybean flowers (R1), 55 *DAE*, and increased to 2.98 stink bugs/m^2 (R7) at 105 *DAE*. However, despite the population decrease in the following evaluation (R8), the mean remained at 1.5 stink bugs/m^2.

Dispersion indices

The mean, variance, and aggregation indices [variance/mean ratio (*I*), Morisita index (*Iδ*), and exponent *k*) were presented in Table 1. Based on aggregation

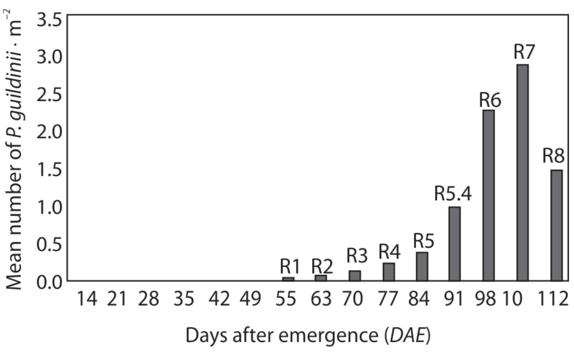

Fig. 1. Temporal variation of *Piezodorus guildinii* in soybean with time. Nova Andradina-MS, Brazil

indices, variance/mean ratio (*I*), the distribution of *P. guildinii* was random in all evaluations. However, based on the *Iδ*, the population was randomly distributed at 91 and 105 *DAE*, but aggregate at 70, 77, 84, 98, and 112 *DAE*. According to the exponent *k* of the Negative Binomial (Table 1), the evaluations at 55, 63, 91, and 105 *DAE* indicated a uniform distribution. However, in the remaining evaluations (70, 77, 84, 98, and 112 *DAE*), the distribution of the population was aggregate.

Frequency distribution

The chi-square values indicated that the *P. guildinii* population fitted with three models of theoretical frequency distributions (Table 2); twice for the Poisson distribution (84 and 91 *DAE*), twice for the binomial negative distribution (84 and 91 *DAE*), and once for the positive binomial distribution (91 *DAE*). In ecological statistics, the best fit was characterized by frequency distributions with the lowest chi-square value.

In the present study, the number of frequency classes from *P. guildinii* was insufficient to detect the distribution of stink bugs at 55 and 63 *DAE*, due to

Table 1. Phenology, time of evaluation (in days after emergence, *DAE*), mean infestation and dispersion indices of *Piezodorus guildinii* in soybean, Nova Andradina-MS, Brazil

Phenology	*DAE*	$\dot{m}$	S^2	*I*	*Iδ*	*k*	χ^2
R1	55	0.05	0.05	0.96 ns	–	–1.47 un	53.00
R2	63	0.09	0.08	0.93 ns	–	–1.23 un	51.00
R3	70	0.16	0.02	1.06 ns	1.56*	2.55 ag	59.44
R4	77	0.25	0.30	1.20 ns	1.85*	1.25 ag	66.00
R5	84	0.39	0.50	1.27 ns	1.70*	1.48 ag	69.64
R5.4	91	1.02	0.70	0.34 ns	0.32 ns	–3.26 un	23.00
R6	98	2.33	3.03	1.30 ns	1.07*	7.782 ag	63.61
R7	105	2.98	2.38l	0.80 ns	0.93 ns	–14.81un	43.92
R8	112	1.52	1.89	1.25 ns	1.16*	6.18 ag	68.51

*significant at the 5% level with 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*; χ^2 – chi-square

Table 2. Chi-square test of adherence (χ^2) to the theoretical frequency distributions of Poisson, negative binomial, and positive binomial of *Piezodorus guildinii* throughout time (in days after emergence, *DAE*) in soybean, Nova Andradina-MS, Brazil

Phenology	*DAE*	Poisson		Negative binomial		Positive binomial	
		χ^2	*df* (nc-2)	χ^2	*df* (nc-3)	χ^2	*df* (nc-3)
R1	55	0.002	0	0.001	*i*	0.001	*i*
R2	63	0.012	0	0.001	*i*	0.033	*i*
R3	70	0.828 ns	1	0.039	*i*	0.340	*i*
R4	77	0.162 ns	1	0.917	0	0.258	*i*
R5	84	7.441*	1	7.096*	1	26.426	0
R5.4	91	33.912*	3	9.837*	1	26.274*	2
R6	98	4.897 ns	5	1.515 ns	4	7.714 ns	3
R7	105	10.504 ns	5	6.663 ns	4	9.282 ns	6
R8	112	6.364 ns	4	3.024 ns	3	6.677 ns	2

*significant at the 1% level; *i* – insufficient number of classes; ns – not significant at the 5 and 1% level; nc – number of classes; *df* – degrees of freedom the low occurrence of this species in these periods. However, based on Table 2, the distribution pattern of *P. guildinii* at 70 and 77 *DAE* adjusted only to the Poisson distribution but, in the following evaluations, the best fit was the negative binomial distribution (84, 91, 94, 105, and 112 *DAE*). According to the theoretical frequency distribution at the beginning of the stink bug infestation, *P. guildinii* was randomly distributed but, from seed filling until harvest, the distribution was aggregate.

Spatial analysis

Surface maps illustrate the patterns described by aggregation indices and theoretical frequency distributions. These patterns are of fundamental importance to define guidelines for the management of this pest under field conditions.

Geostatistical analysis (Table 3) revealed that the spherical model was the best fit for the data, as the range of spatial dependence (*a*) throughout the evaluations varied between 31.4 m at 77 *DAE* and 16.2 m at 98 *DAE*. With these data the aggregation area from *P. guildinii* ($a = \pi .\ r^2$, where $r = a$) was estimated. In the present study, it ranged from 3,907 m² at 77 *DAE* to 824 m² at 98 *DAE*.

The adjustments to the models were obtained for most evaluations, except for 55, 63, 70, and 112 *DAE* (Table 3 and Fig. 2), when a pure nugget effect was observed, which means there was no spatial dependence among the sampled insects, and the infestation was random. In addition, the parameters of the semivariogram were adjusted based on the models that provided the highest values of coefficient of determination (r^2), ranging between 0.98 and 0.34 (Table 3).

The $C_0/(C_0 + C)$ ratio corresponds to the estimate of the degree of randomness from samples. In the present study, the values varied between 0 and 0.22, indicating that the maximum variation between the samples was 22%.

After the adjustment of semivariograms, kriging interpolation was carried out, but, not when plants were

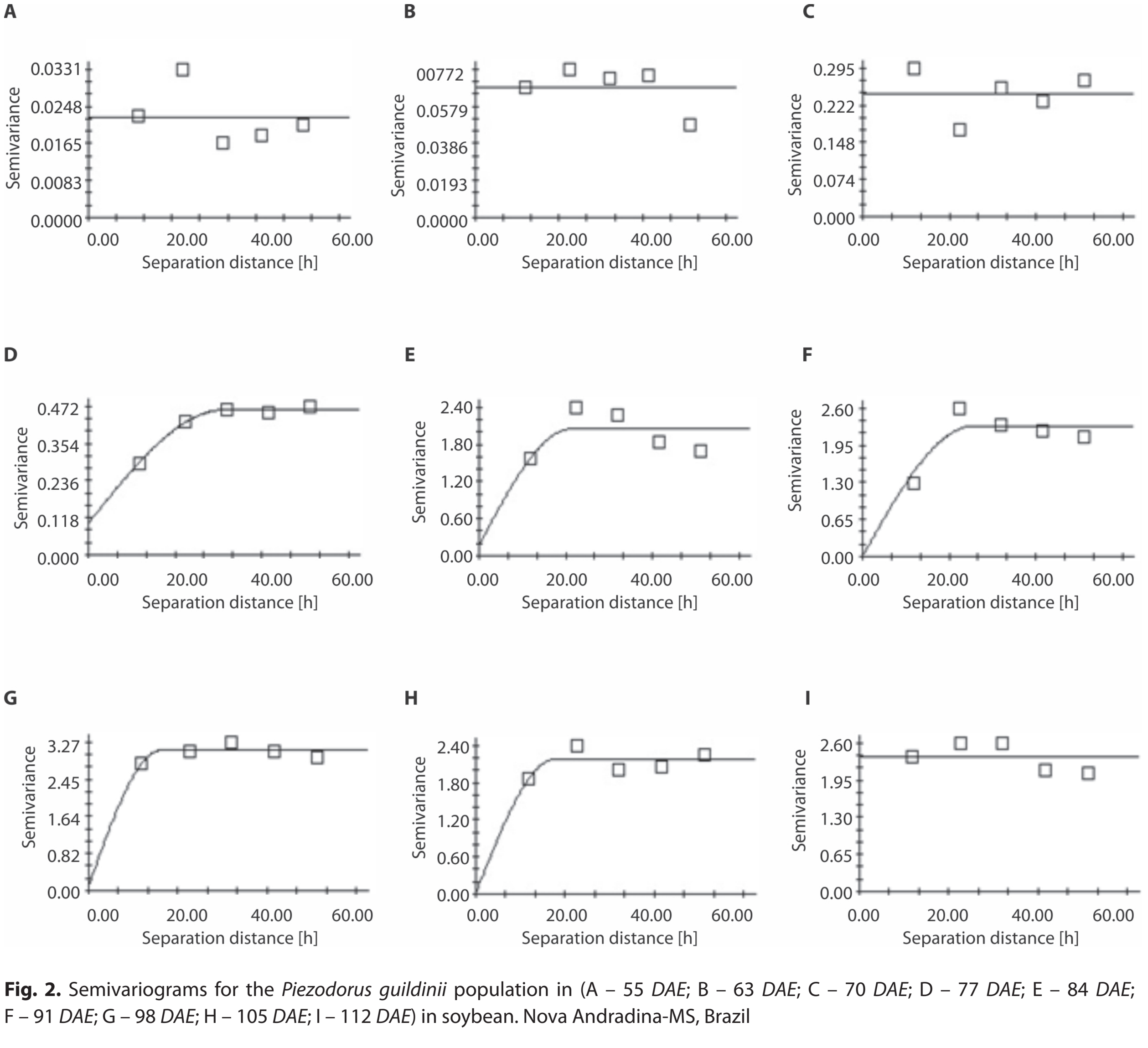

Fig. 2. Semivariograms for the *Piezodorus guildinii* population in (A – 55 *DAE*; B – 63 *DAE*; C – 70 *DAE*; D – 77 *DAE*; E – 84 *DAE*; F – 91 *DAE*; G – 98 *DAE*; H – 105 *DAE*; I – 112 *DAE*) in soybean. Nova Andradina-MS, Brazil

Table 3. Estimates for semivariogram parameters of the spatial distribution of *Piezodorus guildinii* in soybean. Nova Andradina-MS, Brazil

DAE	C_0	$C_0 + C$	$C_0/(C_0 + C)$	*a* [m]	r^2	Area [m²]*	Mathematical model
55	0.02	–	–	–	–	–	nugget effect
63	0.07	–	–	–	–	–	nugget effect
70	0.24	–	–	–	–	–	nugget effect
77	0.10	0.46	0.22	31.4	0.98	3097	spherical
84	0.05	0.62	0.08	21.3	0.34	1425	spherical
91	0.01	0.35	0.03	25.4	0.82	2026	spherical
98	0.08	3.09	0.02	16.2	0.57	824	spherical
105	0.00	2.18	0.00	17.7	0.46	984	spherical
112	2.35	–	–	–	–	–	nugget effect

C_0 – nugget; $C_0 + C$ – sill; *a* – range
*area calculated as $\pi \cdot r^2$, where π – 3.1416 and *r* – *a* [m]

at 55, 63, and 70 *DAE*. However, to understand the dynamics of *P. guildinii* in the field, Inverse Distance Weight interpolation was carried out for the abovementioned dates. Temporally, no infestation was observed in soybean plants until 49 *DAE* (Fig. 1). Starting at 55 *DAE* (Fig. 3A), an infestation was observed on the eastern side of the experimental field, where it remained until 63 *DAE* (Fig. 3B). The distribution was characterized as random and the infestation occurred on the field edge in both evaluations, confirming the results obtained by aggregation indices and frequency distributions (Tables 1–3).

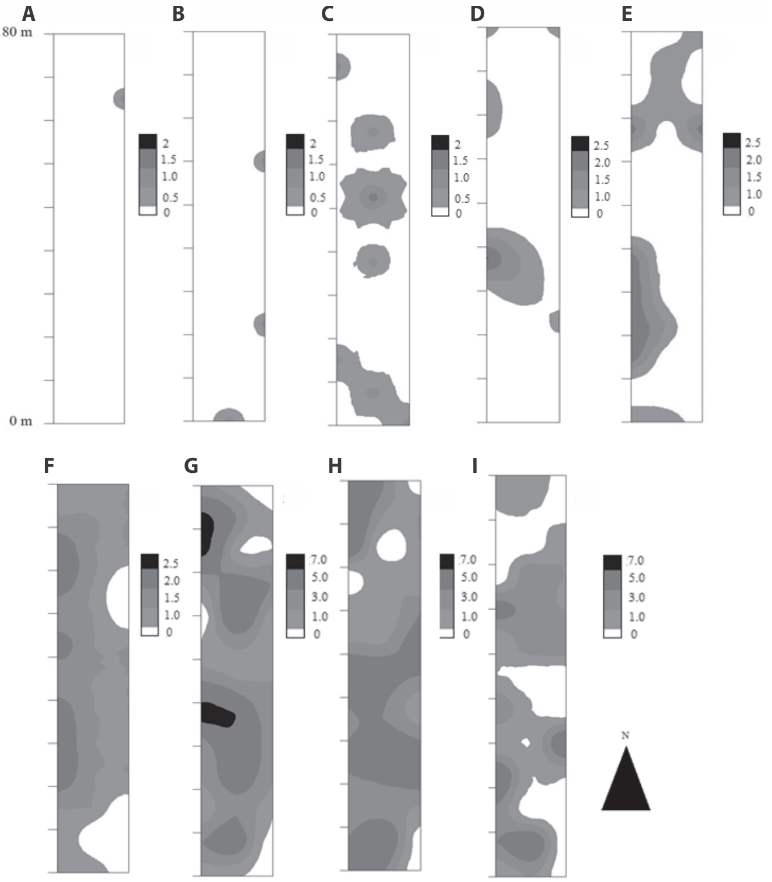

Fig. 3. Surface maps based on the Inverse Distance Weighting (A – 55 *DAE*; B – 63 *DAE*; I – 112 *DAE*) and kriging (C – 70 *DAE*; D – 77 *DAE*; E– 84 *DAE*; F – 91 *DAE*; G – 98 *DAE*; H – 105 *DAE*) of the spatial distribution of *P. guildinii* in soybean. Nova Andradina-MS, Brazil

At 70 *DAE*, stink bugs dispersed to the entire experimental area (Fig. 3C) however, the mean infestation remained relatively low (0.16 stink bugs/m²). The distribution of *P. guildinii* in the field was considered to be random and confirmed by frequency distributions (Table 2), according to estimates of the parameters from the semivariogram (Table 3).

As pods completed their development at 77 *DAE* (R4), an aggregation of *P. guildinii* was observed on the west side of the experimental field (Fig. 3D), and at some points there was more than one stink bug/m². However, as observed at 70 *DAE*, the mean infestation was low (0.25 stink bugs/m²) and random.

At 84 *DAE*, at the beginning of seed filling (R5), the population did not rise much (0.39 stink bugs/m²) proportionally compared to the previous evaluations. However, the aggregation on the west side of the field increased and the population dispersed to other points in the experimental field (Fig. 3E). At this evaluation, stink bug distribution best fitted to the negative binomial model (Table 2), and thus was characterized as aggregate. This was also confirmed by the $C_0/(C_0 + C)$ ratio (Table 3), with a value below 25%, an indication of strong aggregation.

In subsequent evaluations, a large increase in the *P. guildinii* population was observed, reaching a mean of 2.98 stink bugs/m² (105 *DAE*). In these evaluations, the distribution was aggregate at 91 (Fig. 3F), 98 (Fig. 3G), and 105 *DAE* (Fig. 3H), confirming the results obtained for the frequency distribution (Table 2) and the estimates for the parameters of semivariogram (Table 3), which also indicated an aggregate distribution.

In the evaluation in R8 (112 *DAE*), however, due to the population decrease, geostatistics was not able to detect a spatial dependence among samples, which indicated a random infestation, contrary to what the aggregation indices and theoretical frequency distributions would suggest.

Discussion

This study, which described the occurrence of *P. guildinii* in soybean, confirmed its status as one of the main pests in this crop. This stink bug has often been reported in South and Central America as one of the main soybean pests (Zerbino *et al.* 2015). In this analysis, this insect was observed only when soybean plants began flowering, demonstrating its preference for reproductive structures (flowers, pods, and seeds). Furthermore, a prominent increase was observed after pod setting and seed filling. Based on frequency distributions and spatial analysis, the distribution of *P. guildinii* was random at the beginning of infestation, tending to aggregate after pod setting.

Temporal variation of *Piezodorus guildinii*

The occurrence of *P. guildinii* in soybean began during flowering (R1) and increased over time. The highest occurrence was observed when plants reached the phenological stage R7 (105 *DAE*). This is in accordance with the results of Defensor *et al.* (2020), who observed a predominant infestation of *P. guildinii* in soybean during the reproductive period, with the largest number of sampled individuals observed at the end of the soybean cycle.

More frequent occurrence which began during seed filling might have been due to the presence of more suitable structures for the development of stink bugs (pods and seeds), as *P. guildinii* was highly attracted to soybean areas during flowering and at the beginning of seed filling. However, the highest occurrence was usually observed at the end of seed filling (Musser *et al.* 2011). In addition, *P. guildinii* is known to actively move around soybean areas, even during the reproductive stage, explaining the population increase with time (Olson *et al.* 2011).

Molina *et al.* (2012), studied the behavior of *P. guildinii* when selecting soybean pods and observed that nymphs were strongly attracted to mature pods (R7 and R8). However, Panizzi and Alves (1993) reported a high mortality of nymphs when fed during the phenological stage R8. This might be explained by the hardness and density of hairs on pods, as well as a high rate of lignin (Saes Zobiole *et al.* 2010).

It should be pointed out that in this study, during the most vulnerable time of the attack of *P. guildinii*, the infestation remained above the level of economic damage (one stink bug for seed production or two for grain production/m) (Panizzi *et al.* 2012). Moreover, due to the rapid reproduction in soybean observed in this study, in which from R5 to R5.4, the mean infestation increased nearly threefold, attention is recommended when monitoring this insect-pest, using intervals of no more than 7 days between evaluations.

Dispersion indices

As the experimental area was not surrounded by other fields planted with soybean, movement between fields was not reported in this analysis. Therefore, no aggregation pattern was observed at the beginning of the common infestation, mainly in areas sown late with soybean, which can serve as shelter, feeding, and reproduction sites of migrant stink bugs (Silva *et al.* 2021)

For the variance/mean ratio index, the distribution of *P. guildinii* can be described as random, mainly due to the low incidence of this insect in the field compared to the number of sampling points. Souza *et al.* (2018) obtained similar results for this index of *Euchistus heros* F. (Hemiptera: Pentatomidae), under conditions similar to those in the present study.

According to the *Iδ,* most evaluations had values higher than one, confirming that the distribution of *P. guildinii* in soybean was aggregate. Regarding the exponent *k* of the negative binomial, the result was in agreement to those obtained for the *Iδ*, in which in most evaluations, the population was aggregate throughout the development of soybean. These results also corroborate those obtained by Fernandes *et al.* (2018), on phytophagous stink bugs in soybean and Fonseca *et al.* (2017) on *Edessa meditabunda* F. (Hemiptera: Pentatomidae) in *Bt* and non-*Bt* soybean.

Frequency distribution

According to the theoretical frequency distributions (Table 2), the distribution of *P. guildinii* was random at the beginning of the infestation and aggregate from pod setting to harvest.

Infestation in soybean areas by stink bugs usually occurs on field edges due to migration from one soybean area to another or from alternative hosts. In this study, on the west side of the experimental field there was an area planted with *Crotalaria* sp. which was in advanced developmental stages. This plant is considered to be a reproductive host of *P. guildinii* (Bundy *et al.* 2018). The movement and spatial distribution of insects can therefore occur for reproductive purposes, in response to meteorological changes, such as temperature, humidity, wind, luminosity, aggregation pheromones or the presence of neighboring host plants, and the availability of new feeding sources (Tillman and Cottrell 2016).

As soybean plants develop, they become more nutritionally suitable for stink bugs, resulting in a population increase during seed filling and maturation (Fonseca *et al.* 2017). Consequently, an aggregate distribution pattern of *P. guildinii* was observed, corroborating Fonseca *et al.* (2017) who reported similar results for *E. meditabunda* in soybean.

Thus, when the spatial arrangement is random, any size of sampling unit is equally efficient for detecting the population distribution. Its efficiency is defined by the number of sampling points, and large samples are recommended in populations with random distribution (Fernandes *et al.* 2003). However, when spatial distribution is aggregate, a larger number of sample units were required than for any other type of spatial distribution during the monitoring process. In addition, the number of samples is extremely important, since within the complex of phytophagous stink bugs, *P. guildinii* has the greatest potential for damage (Zerbino *et al.* 2015).

Our study described the occurrence and spatial distribution of *P. guildinii* in soybean to assist in the decision-making process to control this insect pest. In addition, it provided the framework for the development of a sequential sampling process to reduce sampling time, especially in regions that lack monitoring in large soybean growing areas (Allen 2014).

Spatial analysis

The areas with the highest infestation densities of *P. guildinii* were identified with distribution maps. Adjustment to the spherical model occurred in most evaluations, except for 55, 63, 70, and 112 *DAE*. This can be due to two factors: random pest distribution on these dates or distances from sampling points that were greater than what was necessary for the detection of spatial dependence. As the variance was lower than the mean at 55, 63, and 70 *DAE*, the distribution was considered random. However, at 112 *DAE*, the variance was greater than the mean, thus the distribution was characterized as aggregate. However, spatial dependence was not observed, because the distance between samples was too large (Carvalho *et al.* 2015).

A pure nugget effect is commonly reported in entomological studies, since spatial dependence can occur on a scale smaller than that carried out in some studies (Liebhold *et al.* 1993). However, on dates when the nugget effect was not observed, the $C_0/(C_0 + C)$ ratio (Table 3) indicated that the maximum variation in the samples was 22%, suggesting that the spatial dependence among samples was high.

Data obtained allowed for the creation of maps (Fig. 3) and the estimation of the spatial dependence of samples. Based on IDW (Figs 3A, B and C), the initial infestation occurred on the edge, which is an infestation pattern commonly observed for stink bugs in the field, as reported in several studies (Weber *et al.* 2018; Silva *et al.* 2021).

Starting at 77 *DAE*, the spatial dependence among samples increased and kriging was thus applied, indicating a strong aggregation of the sampled population, such as in those with a level of economic damage higher than that indicated for stink bugs in soybean, which is approximately one to two stink bugs/m (Panizzi *et al.* 2012).

Kriging maps can be very useful for analyzing the dynamics of a given variable in the field over time. In our study, these maps were used to observe the movement of *P. guildinii* from the surrounding areas to the center throughout the soybean phenology. As pods developed, the population increased and forced the dispersion of stink bugs to search for new plants for feeding (Reisig *et al.* 2015).

These results suggest the control of *P. guildinii* only on the field edge at the beginning of the infestation until pod setting. However, after that and especially during seed filling, the sampling effort needs to be intensified to evaluate the behavior of this pest in the field, since the population was aggregate and in high density. Combined, these could result in major losses in large-scale farming.

Our findings indicated that usage of geostatistics was feasible to evaluate the spatial distribution of *P. guildinii* and define sampling plans, aiming at developing guidelines for the management of this stink bug. However, more studies are needed to define this parameter, because at the beginning of the infestation, it was not possible to detect spatial dependence among the samples. Moreover, only one experimental area was analyzed, as there are several *Bt* and non-*Bt* soybean varieties, cultivated in the most diverse environments, which are known to interfere in the spatial distribution of stink bugs.

Conclusions

In soybean, *P. guildinii* occurred only during the reproductive stage of plants. Regarding aggregation indices, the spatial distribution was random at the beginning of the infestation but, tended to become aggregate after pod setting. The theoretical frequency distribution of this species was best described by the negative binomial distribution.

The use of geostatistics, therefore, is a viable tool to describe the aggregate spatial distribution of *P. guildinii* in soybean in an experimental area, with an aggregation radius ranging between 16.2 and 31.4 m.

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