ENTOMOLOGICAL Communications

*Entomological Communications, 4, 2022: ec04017*
*doi: 10.37486/2675-1305.ec04017*
*e-ISSN: 2675-1305*
*Open Access Full Text Article*

# Bioassay

# Population density, spatial patterns and sample size of *Edessa meditabunda* (F., 1794) (Hemiptera: Pentatomidae) on alternative plants during soybean off-season in southern Brazil

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***Edited by: Regiane C. de Oliveira***

***Received: December 25, 2021. Accepted: May 06, 2022. Published: June 20, 2022.***

**Abstract.** *Edessa meditabunda* (F., 1794) (Hemiptera: Pentatomidae) is considered a potential pest for soybean crops in the Neotropical region. During winter (i.e. the soybean off-season), the stink bugs seek alternative host plants such as *Saccharum angustifolium* (Nees) Trin. and *Andropogon bicornis* L. at the edge of crop fields for shelter. The objective of this study was to investigate the population density between plants and tussock diameter, spatial distribution and sample size of *E. meditabunda* on *A. bocornis* and *S. angustifolium* at the edge of cultivated areas during soybean off-season. In both plants, the increase of tussock diameter resulted in a higher population density of *E. meditabunda*. Tussocks of *S. angustifolium* had a higher average number of stink bugs.plant$^{-1}$ than *A. bicornis*. We observed an aggregation trend in both plant species. For practical applicability following Integrated Pest Management, a minimum sample size of 106 and 116 plants for *A. bicornis* and *S. angustifolium* respectively was obtained. Evaluation of host plants at the edges of crop fields during soybean off-season plays an important role in management strategies during the next cropping season.

**Keywords:** Population dynamics, Monitoring, Heteroptera, Stink bug, Management systems.

Stink bugs (Hemiptera: Pentatomidae) are the main pest of several crops around the world (Grazia et al. 2015). In Brazil, the main species occurring are *Euschistus heros* (F., 1798), *Diceraeus furcatus* (F., 1775), *Diceraeus melacanthus* (Dallas, 1851), *Piezodorus guildinii* (Westwood, 1837) and *Edessa meditabunda* (F., 1794). Soybean is the main host of these insects which are known to cause significant damage that result in high application of insecticides making crop cultivation expensive (Grazia et al. 2015). Among these stink bug species, *E. meditabunda* is classified as of lesser economic importance. However, its damage has already been confirmed for the soybean crop and due to its growing population abundance, it is necessary to understand its ecology on different hosts (Grazia et al. 2015).

The determining factor for the adaptive success of the stink bugs is the ability to switch hosts across the landscape (Fuentes-Rodríguez et al. 2019; Engel et al. 2020). This behavior is mainly caused by the perception of unfavorable conditions such as low air temperatures, shorter photoperiod and lack of food. Hence, stink bugs seek shelters that offer stable microclimate for survival during their quiescent period (Klein et al. 2013; Engel et al. 2020).

Plants located close to the cultivation area are of special importance for this purpose. This is due to the fact that these insects have limited flight capacity (Pasini et al. 2018). Among the plants that surround cultivation areas are the species *Andropogon bicornis* L. and *Saccharum angustifolium* Nees Trin. (Poales, Poaceae). These plants are native to the Americas and are widely distributed throughout the Neotropical region (Klein et al. 2013). Despite the record of the occurrence of *E. meditabunda* in these plants at the edges of the cultivationareas (Engel et al. 2020), no study has focused on its spatial distribution in southern Brazil.

The determination of stink bugs spatial distribution patterns can help in building located sampling plans and management, increasing efficiency and reducing costs and environmental impact during management (Reay-Jones 2014). To define the spatial of insects several indexes of dispersion and frequencies distribution are used (Pezzini et al. 2019). The confirmation of the type of insect distribution in an area occurs only with the knowledge of the probabilistic models that describe the frequency distributions of the numbers of individuals of the studied insect (Souza et al. 2013).

With knowledge of the spatial distribution patterns of an insect pest, procedures to determine an ideal sample size to estimate its population are possible (Arbab 2014). In this context, the minimum sample size based on different levels of precision is the first step towards the establishment of a sampling plan for an insect pest (Engel et al. 2021). In view of this, the objective of this study was to evaluate the population density, spatial distribution and to determine the minimum number of samples for estimating *E. meditabunda* population density on plants of *A. bicornis* and *S. angustifolium* during soybean off-season.

## Location and climate

The study was conducted in the municipality of Cruz Alta, Rio Grande do Sul, Brazil (GPS coordinates 22, 244138; 6835737 UTM). According to Köppen and Geier the climate of the study area belongs to the Cfa type. The average temperature is below 28°C in the coldest month and above 22 °C in the hottest month of the year (Valério et al. 2018).

## Sampling

Sampling was carried out during the second half of June in the years 2014, 2015 and 2016, the period corresponding to the beginning of winter. Each year, 50 perennial plants of *A. bicornis* and *S. angustifolium* were sampled, totaling 150 experimental units for each plant species. These plants sampled were located at a limit distance of 20 meters from the edge of the cultivation area (10,000 m²). For each plant, tussocks of 10 to 50 cm of diameter were evaluated. The minimum distance adopted was 15 meters between tussocks (Engel & Pasini 2019).

### Population density (stink bugs.plant$^{-1}$)

We used the structure of a Generalized Linear Mixed Model (GLMM) with quasi-Poisson errors distribution to compare populations between plants. In this model, the effects of the year were considered random, while the plant species, tussock diameter and its interaction effects were considered fixed. For this analysis, we used "*MASS*" package (Venables & Ripley 2002). The model's fit was assessed by the half-normal plots with envelopes simulated at 95% confidence interval, using the '*hnp*' package (Moral et al. 2017). After checking and confirming the model's residual adjustment, a deviance analysis was performed, and the averages were compared using the Tukey test HSD ($p < 0.05$). All analyzes were performed using software R version 4.0.0 (https://cran.r-project.org).

### Spatial distribution

For the analysis of the spatial distribution, the indexes of Morisita ($I_\delta$), $K$ of the negative binomial distribution, Green's coefficient ($Cx$) and the variance/mean ratio ($I_\delta$) was used, based on Morisita (1962), Green (1966), Rabinovich (1980) and Elliott (1990), respectively. Adherence to the Poisson and Negative Binomial distributions was assessed using chi-square tests, according to Souza et al. (2013).

Adjustments to the Poisson distribution were tested, with the hypothesis that all individuals are equally likely to occupy any place in space and that the presence of one individual does not affect the presence of another. This distribution, also known as random distribution has variance equal to the mean ($s^2 = \bar{x}$). In the Negative Binomial distribution, the occurrence of an individual increases the probability of occurrence of neighboring individuals in the same unit. This distribution presents variance greater than the mean ($s^2 > \bar{x}$) and represents the contagious or aggregate distribution.

### Minimum sample size

According to the central limit theorem, non-normal basic populations, common in counting data, tend to present approximately normal sample distribution for samples greater than 30 (Bussab & Morettin 2004). Therefore, considering the number of samples and the central limit theorem, the data in this study offer credibility for studies involving estimation of the sample size.

Using the 150 experimental units evaluated for each plant species ($n$ = 150), the sample size ($N$) was estimated for the semi-amplitudes of the confidence intervals at levels of 10, 20, 30, 40 e 50% ($D$) estimate of the mean ( population density (stink bugs.plant$^{-1}$), with 95% confidence level ($1$-α), through the expression (Bussab & Morettin 2004):

$$N = \frac{\left(t^2_{\frac{\alpha}{2}} * s^2\right)}{(D\bar{x})^2} \quad (1)$$

$t\alpha/2$ was used as the critical value of *Student*'s t distribution, whose area on the right is $\alpha/2$ , that is, $t$ such that $P\left(t > t_{\frac{\alpha}{2}}\right) = \frac{\alpha}{2}$ ,with (n-1) degrees of freedom, with α= 0,05 of probability, s² = estimated variance.

Then, locking as 150 sample points, corresponding to the sample size used in the study, the estimation errors of the means (in percentage for each plant were calculated using the equation:

$$D = (100 * t_{\alpha/2} * s) / (\sqrt{n} * \bar{x}) \quad (2),$$

where $s$ is the standard deviation.

Like the two plants species available, 1,736 adult individuals were sampled. The absence of nymphs in these plants indicates the adults are the purpose of quiescence from the cultivation area. In GLMM a significant effect of tussock diameter ($\chi^2$ = 629.429; df = 1; p-value < 0.001) and plant species ($\chi^2$ = 4.474; df = 1; p-value = 0.034), but no its interaction ($\chi^2$ = 0.898; df = 1; p-value = 0.343) was observed. The effect of tussock diameter was strongly positive, with greater abundance as a function of the increased tussock diameter (Fig. 1A). When comparing the estimated means, it was found that the *S. angustifolium* had a higher number of stink bugs.plant$^{-1}$ (Fig. 1B).

The spatial distribution indexes showed an aggregation trend, especially as the same result was found by all indexes for both plant species evaluated. This result was corroborated by the adjustments to the probabilistic models in which an adjustment was observed only for the Negative Binomial Distribution, confirming the behavior of *E. meditabunda* in both plant species (Tab. 1).

**Table 1.** Spatial distribution indexes and values of the test of adherence of the observed frequencies to the expected frequencies for the distributions of the probabilistic models (Negative Binomial, Positive Binomial and Poisson) for population of *Edessa meditabunda* (F.) in *Andropogon bicornis* L. and *Saccharum angustifolium* Nees. (Trin.) during the soybean off-season.

| Probabilistic indices and models | *Andropogon bicornis* | *Saccharum angustifolium* |
|---|---|---|
| $I_\delta$ | 2.25* | 2.40* |
| $K$ | 0.79$^{had}$ | 0.70$^{had}$ |
| $Cx$ | 2.79$^{ad}$ | 4.83$^{ad}$ |
| $I$ | 7.82* | 9.67* |
| **Negative Binomial** | **136.00**** | **150.00**** |
| **Poisson** | **1.02e-41$^{ns}$** | **1.18e-50$^{ns}$** |

$I_\delta$(Morisita Index), K (exponent K of the Negative Binomial distribution), $Cx$ (Green coefficient), (Variance/average ratio). *Significant by the $\chi^2$ test ($p < 0.05$); ad (aggregated distribution), had (highly aggregated distribution). **Significant by the $\chi^2$ test ($p < 0.01$), ns (not significant).

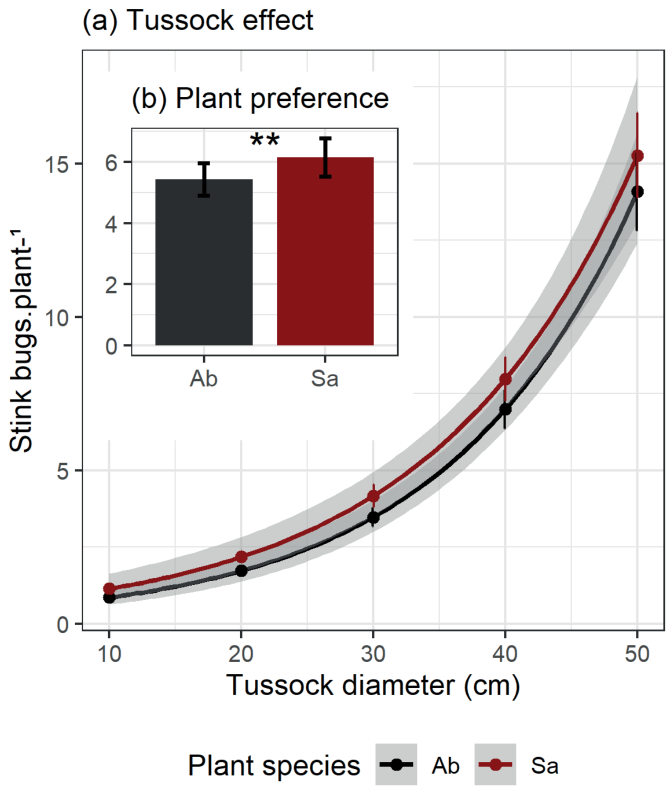


**Figure 1. (A)** Population density of *Edessa meditabunda* (F.) (stink bugs.plant$^{-1}$) as function of tussock diameter; **(B)** Population density of *E. meditabunda* between plant species. Bars indicate standard error; ** differ statistically between plant species by the GLMM model ($\chi^2$ = 4.475; df = 1; $p < 0.001$); Ab = *Andropogon bicornis* L.; Sa = *Saccharum angustifolium* Nees. (Trin).

When determining the minimum number of samples, we identified a great variation between the different levels of precision established. In *A. bicornis*, the values varied between 957 to 38 sample units, for *S. angustifolium* the variation was between 1044 to 42 units. For use within Integrated Pest Management programs, where the degree of accuracy adopted is generally 70% (Arbab 2014; Engel et al. 2021) we verified that the number of samples for both evaluated plants was 106 and 116 for *A. bicornis* and *S. angustifolium* respectively (Fig. 2).

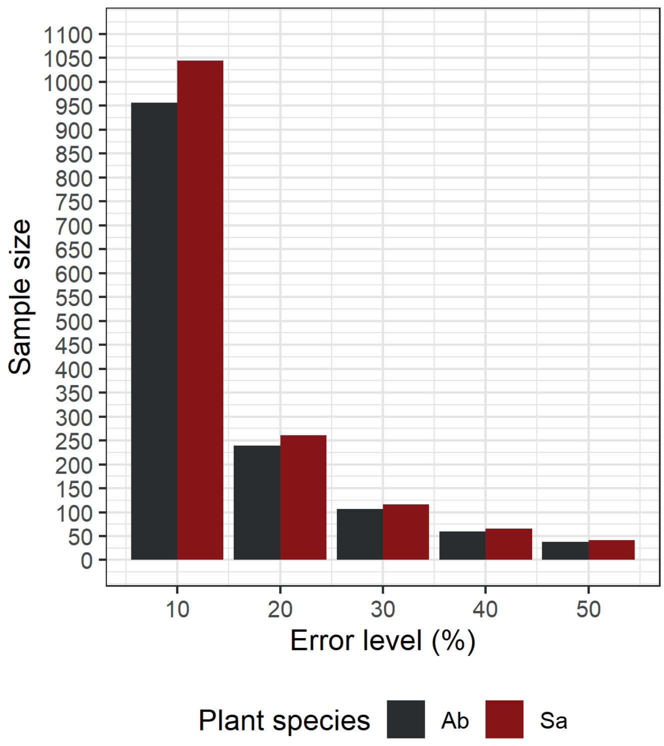


**Figure 2.** Minimum number of sample units (plants) to estimate the population density of *Edessa meditabunda* F. (Pentatomidae) in *Andropogon bicornis* (Ab) and *Saccharum angustifolium* (Sa) tussocks during the soybean off-season. Accuracy for estimating population density in *A. bicornis* and *S. angustifolium* = 75 and 74%, respectively.

In general, it was found that plants of *S. angustifolium* had higher populations of *E. meditabunda* compared to *A. bicornis*. These insects, despite not reproducing, can feed occasionally from these plants, since it has a preference for vegetative structures over reproductive ones (Silva et al. 2012). Despite this, further studies should be carried out to assess the reasons for this observed of preference pattern. The effect of the diameter of the tussock diameter on *E. meditabunda* is related to the offer of shelter by the plants. In this scenario, larger tussocks can offer more suitable microclimatic conditions, as well as greater protection against bad weather and predators (Klein et al. 2013; Engel et al. 2020). The same behavioral pattern was observed for *E. heros*, *D. furcatus*, *P. guildinii* and *Tibraca limbativentris* (Stal, 1860), suggesting a general pattern in Pentatomidae family (Pasini et al. 2018; Engel et al. 2021).

The results obtained showed aggregate pattern for *E. meditabunda* in both plants evaluated. The same behavior was found for others stink bug species in soybean and rice crops (Souza et al. 2013; Pasini et al. 2018; Engel et al. 2021). In this type of distribution, the presence of an individual increases the likelihood of the occurrence of other (Souza et al. 2013). However, in plants located on the edges of cultivation areas, there is still no report on the spatial distribution of stink bugs during the winter. The distribution and population flow of stink bugs depend on the distribution of their host and associated plants, which directly influence the degree of aggregation of populations (Costa et al. 2019).

Tussocks of *A. bicornis* and *S. angustifolium* tend to have a large number of stink bugs during the off-season of crops (Klein et al. 2013; Engel et al. 2020). These insects look for these plants due to the microclimate stability they can offer, thus increasing the probability of survival during the off-season (Klein et al. 2013; Engel et al. 2020). Tussocks with larger diameters tend to have a higher number of sheltered stink bugs, which can optimize the sampling process and help to draw up integrated management plans with greater sustainability (Engel et al. 2021).

When determining the minimum number of plants to be sampled, there was a high variation between the different degrees of precision adopted. This variability depends in theory on the population density present, in an inverse relationship, that is, the higher the population density the smaller the number of samples needed to estimate the population (Arbab 2014; Pezzini et al. 2019). In ecology studies in which degrees of accuracy between 90 and 95% are adopted, a large number of samples were observed, making labor expensive. However, for the adoption of Integrated Pest Management (IPM) practices, in which the degree of precision varies between 70 to 75%, we verified an acceptable number of samples, with 106 and 116 for *A. bicornis* and *S. angustifolium* respectively (Arbab 2014). This result makes it possible to estimate the population abundance of *E. meditabunda* during the winter, making it possible to determine what population pressure of *E. meditabunda* will occur in the next soybean harvest. This knowledge allows the formulation of more sustainable IPM strategies.

Studies have been conducted to understand the role of alternative plants in the life history of pentatomids stink bugs (Possebom et al. 2020; Zerbino et al. 2020). In addition, studies to estimate the stink bug abundance and patterns of the population fluctuation from the abundance found in alternative plants during the off-season have been developed (Pasini et al. 2018). Among the main results already obtained, it appears that a large part of these wild plants that shelter stink bugs in the off-season can be used as "sentinel plants", which allow to estimating the population occurring during the next crop, allowing to define IPM strategies in advance (Engel et al. 2021).

In conclusion, we found that the *E. meditabunda* population density was higher in *S. angustifolium* plants when compared to *A. bicornis*. Its spatial distribution pattern in *A. bicornis* and *S. angustifolium* is considered to be aggregated. The number of plants to be sampled to estimate the *E. meditabunda* population density is highly variable between the different levels of precision adopted. However, for IPM levels, the minimum number of samples estimated suggests a practical feasibility, with 106 and 116 plants to *A. bicornis* and *S. angustifolium*, respectively. Wild plants of *A. bicornis* and *S. angustifolium* located at the edges of crop areas can help to monitor *E. meditabunda* populations before soybean sowing.

## Authors' Contributions

EE planned, designed, executed experimental work, conducted data analyses, and wrote the manuscript. DAS, RPB, MPBP, ALPR and GAB executed experimental work and helped with data analyses and funding. All authors read and approved the manuscript.

## Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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