

Relationship Between Stink Bug Populations in Winter Shelters and Atmospheric Variables in Soybean Growing Areas in Southern Brazil

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Abstract

Stink bugs (Pentatomidae) are among the main entomological problems in the international farming. Their ability in using alternative plants (refuges) during the off-season is one of the reasons that led them to the status of key pests in several crops. Like other insect species, stink bugs are subject to atmospheric variations. Therefore, the objective of this experiment was to evaluate the abundance, the co-occurrence, and its variations according to the weather in the off-season. The work was conducted between 2014 and 2018, in the municipality of Cruz Alta, state of Rio Grande do Sul (RS), Brazil. Every year, refuges formed by Poaceae and located around the cropped area were evaluated in the second fortnight of June, corresponding to the beginning of the winter solstice. Atmospheric variables corresponding to the evaluation period were used to explain the variation in the populations. In short, our results demonstrated interannual variations in the population abundance of stink bugs in the evaluated refuges. We also found variations in the co-occurrence between species. Finally, we demonstrate the trend in the increase in these refuges in years with cold and dry off-seasons.

Introduction

The Neotropical region is characterized by its abundance in areas cropped with soybean and corn (Lugtenberg 2015, Prasad *et al* 2018). It is not different in Southern Brazil where soybean is grown between the periods of October until mid-March, and corn is grown between the months of August and December (CONAB - Companhia Nacional de Abastecimento 2019). In addition, autumn and winter crops such as turnip, oats, canola, and wheat are common in these cropping areas (CONAB - Companhia Nacional de Abastecimento 2019).

This conformation of agricultural landscape has a great importance on the population dynamics of several pest insects. Among these, stink bugs are notable for their polyphagia and adaptive capacity in the face of changes in the agricultural and climatic landscape, causing severe damage

to soybean and corn crops (Panizzi & Grazia 2015). Thus, they are responsible for a large part of the costs with insecticides for neotropical agriculture (Sosa-Gómez *et al* 2020). The main species of stink bugs occurring in Southern Brazil are the following: *Euschistus heros* F.; *Dichelops furcatus* F.; *Dichelops melacanthus* F.; *Edessa meditabunda* F.; and *Piezodorus guildinii* Westwood (Hemiptera: Pentatomidae) (Panizzi *et al* 2012).

The population dynamics of these species in the soybean and corn crops in the neotropics is well described (Panizzi *et al* 2012, Silva *et al* 2013). It occurs from the vegetative phase to the senescence of the soybean crop, then starting the colonization in the autumn and winter crops (in this case, particularly for the genus *Dichelops* spp.) such as crops of forage turnip, canola, corn, and wheat, besides weeds such as buva (Smaniotto & Panizzi 2015, Panizzi *et al* 2016, Bianchi *et al* 2019). Afterwards, there is a period of quiescence in

June, when there is a drop in the photoperiod and air temperature (Pasini et al 2018). After the rise in air temperature and photoperiod, stink bugs tend to leave these refuges, finding nutrients in corn and other associated plants, resulting in the multiplication of these species, which start to colonize the soybean crop again (Panizzi et al 2012). Its importance is reduced if, when they leave the quiescence, they find corn in the initial stage of development, and its damages are related to the reduction in the plant stand for this crop. This is especially true for the species *D. melacanthus*, as it has a greater damage capacity in this crop to the detriment of other species such as *E. heros* (Gomes et al 2020).

The plants belonging to the Poales phylogenetic clade are among the principal refuges for the stink bugs in the off-season in southern Brazil (Fuentes-Rodríguez et al 2019). Some of the main species of this clade associated with stink bugs are *Andropogon bicornis* L., *Eustachys retusa*, and *Saccharum angustifolium* (Klein et al 2013, Engel et al 2018, 2019, Pasini et al 2018). These plants form clumps in the vicinity of crops, so stink bugs are sheltered inside them in search of microclimate conditions that provide their survival during the off-season of the crops (Panizzi 1997, Klein et al 2013).

The understanding of the causes related to the variation in the population abundance of the stink bugs during the off-season is important for the integrated pest management (IPM), as it allows the detection and prediction of populations of the species that are harmful to the cropped plants (Panizzi 1997, Santos et al 2006). Therefore, the objective of this work was to describe the changes in population abundances and the co-occurrences of different species of phytophagous stink bugs according to the atmospheric variables in the off-season period of the soybean.

Material and Methods

Study site

The population survey of the economically important stink bugs was carried out in the vicinity of a cropped area of approximately 10 ha, located in the municipality of Cruz Alta, state of Rio Grande do Sul (RS), Brazil (Time Zone 22.244138; 6835737 UTM). The climate in Cruz Alta is of the Cfa-type, according to Koppen and Geier (Valério et al 2018).

Sampling

The sampling of the stink bugs was carried out in the second fortnight of June in 2014, 2015, 2016, 2017, and 2018, which corresponded to the off-season of soybean and corn crops. The second half of the month of June is characterized by the

beginning of the winter solstice in the southern hemisphere, a period when the air temperature (°C) and the photoperiod are lower; thus, the assessment of winter refuges at this time is appropriate because most surviving stink bugs will be sheltered in these sites (Panizzi 1997, Tougeron 2019). The three main stink bug refuges occurring in cropped areas in southern Brazil were sampled. These refuges are characterized by clumps of plants of the Poaceae family, particularly the *E. retusa* (Lag.) Kunth, *A. bicornis*, and *S. angustifolium*, (Poales: Poaceae). All stink bugs occurring in these plants were collected manually and taken to the laboratory for identification following the taxonomic classification of Garbelotto & Campos (2014). The number of stink bugs considered pests was organized by insect species, plant, and year. For each species of bug, 10 vouchers were stored in the entomological collection of the University of Cruz Alta.

Data analysis

To describe the variation in the population, data were grouped into years and species of stink bugs, with their abundances plotted as response variables. The variations in co-occurrence among the species were investigated using probabilistic matrices generated by using the model proposed by Veech (2013) through the 'cooccur' package (Griffith et al 2016). The significance of each co-occurrence was tested through the method proposed by Veech (2013), since binomial models are not appropriate because the data violate the premise of independence of the samples.

The years were classified according to the climatic characteristics in June using the variables minimum, maximum, and average temperature and minimum, maximum, and average air relative humidity. They were submitted to Pearson's correlation analysis, and the variables with a correlation greater than 0.5 ($\rho > 0.5$) were removed. Hence, we used the average temperature and air relative humidity variables to characterize the years. Once the atmospheric variables were selected, the years were subjected to the non-metric multidimensional analysis (NMDS) to group the years by their climatic similarities based on the distance matrix. After this analysis, the months of June in the years were classified as "cold" and "mild," which were the categories used to represent the data in the principal components analysis (PCA).

The first PCA was carried out to group the years according to the selected atmospheric variables, following the methodology of Tougeron et al (2018). Next, a second PCA was carried out to group the years according to the population abundance of the stink bug species. Finally, a canonical correspondence analysis (CCA) was performed to evaluate the relationships between the stink bug abundance matrices and atmospheric variables. To evaluate the significance of CCA, an analysis of variance with permutations (ANOVA.CCA) was performed.

A community approach was used to explain variations in the stink bug abundance matrix as a function of atmospheric variables. For this analysis, Bray-Curtis dissimilarity matrices were generated in the relative abundances in each sampling year and the data adjusted to linear models as response variables through the Adonis-Permanova command available in 'vegan' (Oksanen *et al* 2019). All analyses were performed using software R version 4.0.0 (<https://cran.r-project.org/>), using the level of $p < 0.05$ as the significance threshold.

Results

Variations in the richness and relative abundance of economically important stink bugs were observed between 2014 and 2018 (Fig 1A). In general, *E. heros* was the species with the largest number of individuals collected (4955 specimens), followed by *E. mediatubunda* (4577), *D. furcatus* (3818), and *P. guildinii* (222). In this scenario, interannual variations were also observed for the atmospheric variables selected in June (Fig 1 B and C). The average air temperature ranged between 11.14 and 14.93°C (Fig 1B). The average air relative humidity showed values between 75.43 and 85.2% (Fig 1C).

Variations in the co-occurrence probability of stink bug species have been observed over the years. The probability values are summarized in Table 1. Overall, *E. heros*,

D. furcatus, and *E. mediatubunda* had been showing probabilities of co-occurrence above 60% since 2015. The species *P. guildinii* was the one with the lowest probability of co-occurring with peers, arising only in the winter of 2016, and reaching maximum co-occurrence probability values for 2018 (greater than 30%).

The non-metric multidimensional scaling grouped the years 2014, 2015, and 2017 as "mild winters" years, while the years 2016 and 2018 were classified as "cold winters" years. The PCA corroborated this cluster with 75% of variation explained in the first axis of the biplot (Fig 2A). In general, years with winters considered to be cold were associated with greater abundance and co-occurrence of stink bugs in the refuges. This pattern can be observed through the vector arrangement in the biplot, with a total explained variation greater than 75% in the first two axes (Fig 2B).

No significant influence of the selected climate variables matrix (average temperature and air relative humidity) was found on the global abundance of stink bugs over the years (CCA ANOVA-like permutation test, $F = 0.85$, $df = 2$, $p = 0.57$). In more details, the abundance of stink bugs was not influenced by the average air temperature (Permanova, $F = 4.77$, $df = 1$, $R^2 = 0.15$, $p > 0.05$). However, our results indicated that the air relative humidity had a significant effect on the dissimilarity of the stink bug abundances (Permanova, $F = 15.31$, $df = 1$, $R^2 = 0.50$, $p < 0.05$).

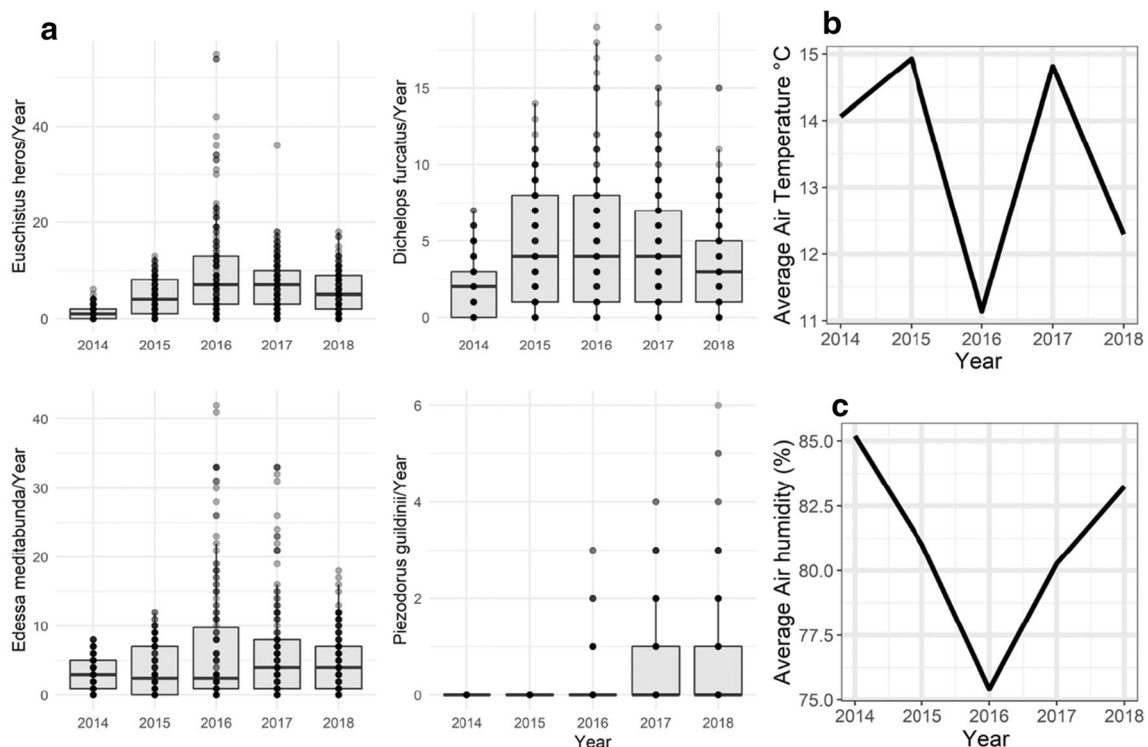


Fig 1 (A) Population abundance of stink bugs (Hemiptera: Pentatomidae) in alternative plants, average air temperature (B) and average relative humidity (C) in June in different years. Cruz Alta, RS, Brazil, 2014–2018.

Table 1 Co-occurrence probability of economically important stink bugs in alternative plants during winter in different years. Cruz Alta, RS, Brazil, 2011–2018.

Species cooccur	2014	2015	2016	2017	2018
Eh × Df	0.41*	0.77*	0.82*	0.85*	0.68*
Eh × Em	0.46*	0.62*	0.72*	0.75*	0.67*
Eh × Pg	-	-	0.12	0.31*	0.35*
Df × Em	0.54*	0.62*	0.68*	0.72*	0.65*
Df × Pg	-	-	0.11	0.30	0.34*
Em × Pg	-	-	0.10	0.26	0.34*

*Probability of a significantly higher expected co-occurrence than that observed at the $p < 0.05$ level. Eh, *Euschistus heros*; Df, *Dichelops furcatus*; Em, *Edessa meditabunda*; Pg, *Piezodorus guildinii*

Discussion

In short, the results obtained in this experiment demonstrate how the climatic variation during the off-season of the soy-bean crop rapidly translated into changes in abundances and co-occurrence of stink bugs in refuges. The searching pattern for economically important stink bugs by alternative plants during the off-season in the Neotropical region has been widely corroborated (Smaniotto & Panizzi 2015; Pasini et al 2018, Engel et al 2019). Nevertheless, few studies are dedicated to determining which variables influence their abundance within this period (Santos et al 2006, Klein et al 2013, Fuentes-Rodríguez et al 2020).

The occurrence of *P. guildinii* over the years cannot be related to any change in its distribution range, since its occurrence in the Neotropical region has been already established (Zerbino et al 2016, 2020). Thus, the results suggest the use of *A. bicornis* and *E. retusa* during the off-season as a

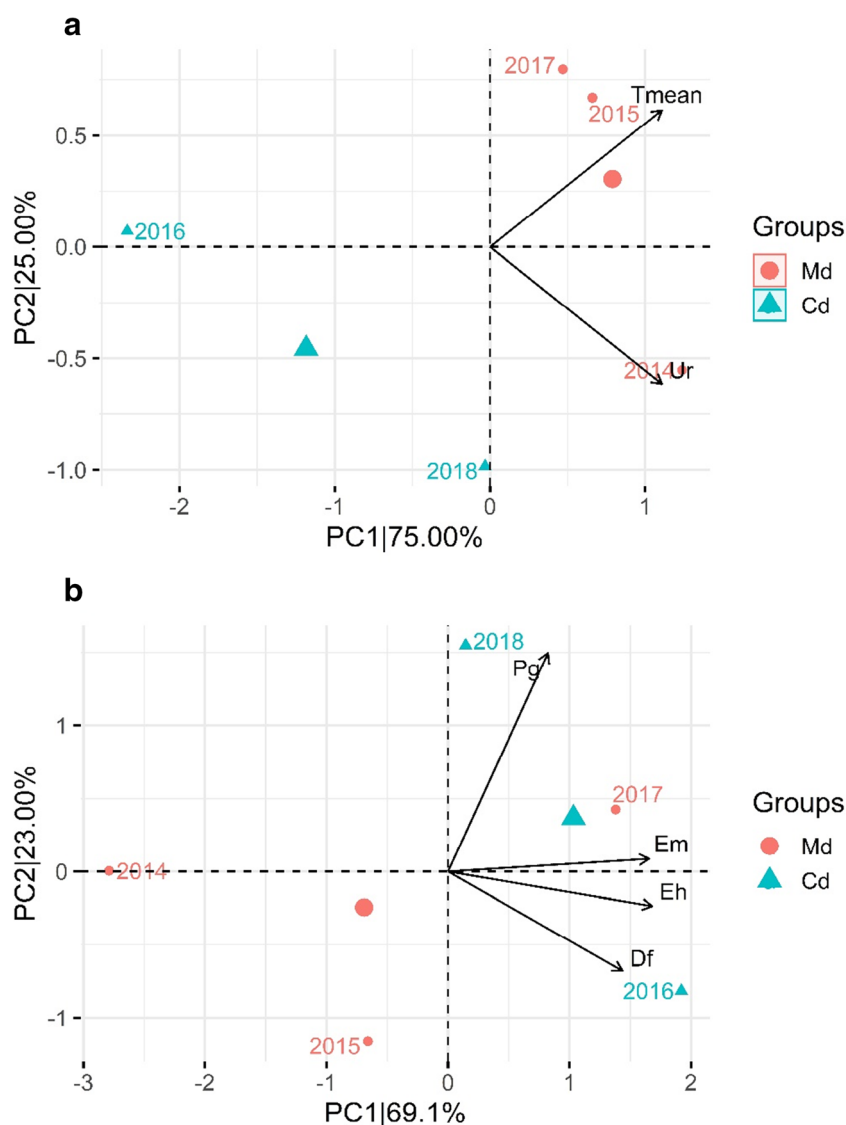


Fig 2 (A) PCA partitioning the variables average air temperature (Tmean) and average relative humidity (Ur) as a function of the sampling years. (B) PCA partitioning the relative abundance of stink bug species according to the years of sampling. Red and blue colors indicate “mild” (Md) and “cold” (Cd) winters, respectively.

refuge. Another factor that was observed was the non-occurrence of the bug *N. viridula* over the sampling years, considering that this species was regarded to have occurred in soybean crops in the Cruz Alta region in the past (Kogan *et al* 1974, Panizzi & Slansky 1985, Sosa-Gómez *et al* 2005, Vivan & Panizzi 2006). The absence of *N. viridula* over this study is corroborated by the drop in this species in recent years, reported by Panizzi & Lucini (2016).

The probability of co-occurrence of the stink bugs under study suggests a similar search pattern for refuges for different species. In addition, the co-occurrence between economically important species of stink bugs as those evaluated in the present study has important implications for agriculture. This result suggests that these species do not present any negative relations among each other during the off-season; therefore, they are able to survive in greater diversity and to colonize the crop area again. This result is corroborated mainly by the fact that these insects are not using these plants for feeding or reproduction purposes, but only for survival purposes during unfavorable periods (Klein *et al* 2013, Engel *et al* 2018).

Because there are no indications of negative relationships between species during the off-season, population variations can occur in these plants through differences in atmospheric requirements. Our results indicate that the years with cold off-season tend to have a greater abundance of stink bugs in the refuges. In a greater detail, we demonstrated that not the average temperature, but the relative humidity of the air, had an influence on the population abundances found. Although not significant, the effect of air temperature is particularly related to the search behavior of the stink bugs for quiescence sites. This occurs in conjunction with other factors, such as photoperiod and low availability of food in the area, which induce the diapause of stink bugs (Saulich & Musolin 2012).

However, air relative humidity has an important contribution to the survival of these bugs in these refuges. We found a greater population abundance for the 2016 off-season, which is considered to be cold and with a low relative humidity. The hypothesis of the greater abundance for this year due to the low relative humidity of the air is attributed to the potential mortality of entomopathogenic agents that occur in these places, such as *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* Bals. (Vuill), which accounts for about 30 to 70% of the mortality of pentatomids in these locations (Santos *et al* 2006, Fuentes-Rodríguez *et al* 2019). Thus, off-season with low values of air temperature and relative humidity tends to contribute to the rise in the search and survival, respectively, of stink bugs in refuges, generating an accumulation of populations.

It is shown in this work that the air relative humidity during the off-season is an important predictor of the population abundance of stink bugs in quiescence sites. Based on the results obtained in this work and in others that evaluated the

population dynamics of stink bugs, it can be observed that the changes in the patterns of abundance and co-occurrence during the off-season are not random (Engel *et al* 2019, Panizzi 1997, Panizzi *et al* 2012, Silva *et al* 2013). This has important implications for agriculture and for ecology, with regard to the generation of predictive models for pest bugs in southern Brazil, being an aid in the determination of the possible causes in climatic terms.

Nevertheless, these variables (temperature and relative humidity) cannot be considered isolated factors. Therefore, parameters such as the presence of suitable hosts throughout the summer and autumn, in addition to the level of management used during the soybean growing period and the presence of predators and parasitoids during the growing period and in the refuges must be taken into account during the off-season. These variables are reflected in the population abundance of stink bugs that will disperse to *A. bicornis*, *S. angustifolium*, and *E. retusa* plants in addition to others in the border of cultivation areas (Panizzi 1997, Engel *et al* 2018, 2020, Fuentes-Rodríguez *et al* 2019, Zerbino & Panizzi 2019, Sosa-Gómez *et al* 2020).

Finally, we conclude that intraseasonal population variations in the stink bug community are sensitive to climatic changes during the off-season. Hence, predictive analyses for the community of economically important stink bugs must now consider local changes in patterns of co-occurrence and abundance in response to climatic variations experienced in the short term.

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