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CROP SCIENCE

Temporal dynamics of *Apis mellifera* (Hymenoptera: Apidae) during flowering in indeterminate soybean (*Glycine max*)

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Abstract: Soybean varieties with indeterminate growth habit are widely used in the state of Mato Grosso do Sul and are characterized by not having well-defined phenological stages. Due to the importance of bee conservation, studies on the frequency and time of visitation in indeterminate soybean are needed. Samples were collected in a 4-ha plot of soybeans not treated with insecticides. Sampling was carried out at random spots, throughout the flowering period, starting at 08h:00min, during 15 min/hour, successively until 16h:00min. Generalized Linear Models were generated and the Poisson regression model provided the best fit, reflecting the significant differences revealed by the deviance analysis. The association between bees and climatic variables was tested with the Spearman's correlation. The temporal pattern of bee visitation was independently influenced by flowering period and time of day. However climatic variables did not influence significantly bee visitation rate. In the first days of flowering, bees were more abundant, especially between 10h and 15h. These results have practical implications in the maintenance of this species during soybean management practices, contributing to a balanced coexistence between beekeepers and soybean growers, by avoiding applications of phytosanitary products during the periods of high bee visitation.

Key words: foraging, bee, behavior, visiting time.

INTRODUCTION

Pollination is a natural ecosystemic service of great importance, although many plants do not rely on external agents to fecundate their flowers and perform self-pollination in the presence of self-compatible flowers (Rech et al. 2014). Bees provide this service and maintain relationships of dependence that may often be essential for crops that receives the visitation of these insects, or modest in some cases, such as soybeans (*Glycine max* (L.) Merr.), which have a reduced dependence on the honey bee *Apis mellifera* L. 1758 (Hymenoptera: Apidae) (Giannini et al. 2015a, b).

This modest relationship is due to the autogamous reproduction of soybeans and their self-pollinating flowers with female and male structures that synchronize stigma receptivity and pollen viability, resulting in higher yields (Miyasaka & Medina 1981). Some varieties have an indeterminate growth habit characterized by a flowering period that can be extended or shortened depending on climatic conditions and overlap with pod formation (Fehr & Caviness 1977, Miyasaka & Medina 1981).

Seed filling is the stage when soybeans are more vulnerable to the attack of herbivorous insects that damage fruits and seeds, decreasing their quality and interfering with the viability of

the seeds. However, an indeterminate plant still produces flowers in the apical region which may be attractive to pollinators and expose bees to contamination if they are present in the field during this period (Gazzoni 2017, Corrêa-Ferreira & Azevedo 2002) when phytosanitary products are applied.

Honey bees of the species *A. mellifera* are the most frequent floral visitors in soybeans, although other pollinating insects also occur (Giannini et al. 2015a). This high frequency can be attributed to the nutritional need to supply the demands of bee colonies and their generalist habit in search for the best floral resource that will provide the nutrients required for the development of their offspring and their physiological needs (Free 1980, Milfont et al. 2013, Santos et al. 2013). Its natural or introduced presence to collect pollen and nectar results in cross-pollination and can increase yields up to 18%, despite a lack of need for pollinators (Milfont et al. 2013, Blettler et al. 2018).

Honey bees forage throughout the day, but this behavior can be influenced by climatic variables and/or the availability and quality of floral resources that may vary during the day (Chiari et al. 2005, Abou-Shaara 2014). Studies that elucidate the foraging behavior of honey bees in crops of agricultural interest are essential to provide information to mitigate the risk of exposure of bees to phytosanitary products during applications (Jacob et al. 2019a, b, Tadei et al. 2019). The present study was aimed at investigating the temporal distribution pattern of *A. mellifera* in an indeterminate soybean field.

MATERIALS AND METHODS

The study was carried out at the Experimental Farm of the Federal University of Grande Dourados during the 2017/2018 harvest (22°23'55", 54°98'82"). A 4-ha plot was planted with indeterminate soybeans of the variety Monsoy 6410 IPRO® (Monsoy 2018). A fragment of native forest was 75.28m away from the soybean field with feral colonies of honey bees, and pasture and sugarcane fields also surrounded the area. A commercial colony of *A. mellifera* was located 300 m from the center of the soybean area. Sowing was carried out on 18 October 2017 and followed the practices for the crop, but phytosanitary products were not applied during the entire experiment.

Sampling started when the first flowers opened during the R1 stage and extended throughout the flowering period (Fehr & Caviness 1977), as follows: 14 December 2017 (52 DAE - days after emergence), 21 December 2017 (59 DAE), 27 December 2017 (65 DAE), 3 January 2018 (72 DAE), and 05 January 2018 (74 DAE), to determine when bees were more frequently observed during flowering until it ended. Bees were collected with an entomological net (with wooden handle, cable extension: 120cm; basket diameter: 35cm; basket depth: 80cm; network mesh: Voal), starting at 08h:00min for 15min, successively throughout the day until 16h:00min (08h, 09h, 10h, 11h, 12h, 13h, 14h, 15h, and 16h) 9 total sample throughout the day and 5 days collection (Oliveira & Fernandes 2016) (the time zone UTC -4:00 (Coordinated Universal Time)). The frequency of bee visitation during the day and the visitation period during the flowering were evaluated. Individuals were collected directly on the flowers in slow zigzag walking pattern from the edges to the center of the field and were placed in a killing jar with ethyl acetate. Samples were stored in labeled plastic containers containing the time and day of collection and maintained at -18°C until the identification of insects in the laboratory.

Statistical Analysis

Generalized Linear Models were generated with the Poisson, Quasi-poisson and Negative Binomial distributions. The Poisson model was the best fit for the temporal dynamics of bees. The Poisson model was assessed with Half-Normal Plots using hnp package (Moral et al. 2017). Significant differences were examined with deviance analysis. The Spearman Correlation analysis was carried out to evaluate the relationship between collected bees and climatic variables using the R Core Team (2017) statistical program for all tests.

RESULTS

The temporal dynamics of bees was influenced by time of day (P <0.00001) and DAE of the plant (P <0.00001), but no interaction was observed between time x DAE (P = 0.2902), therefore each factor influenced the temporal dynamics of bees collected in indeterminate soybeans independently. In the first days of the flowering stage, bee visitation was more intense and decreased as time progressed. At 52 DAE and 59 DAE, the frequency of bees was higher than at any other time during the flowering period (Fig 1). At 65 and 72 DAE, the number of foraging bees decreased considerably.

At 74 DAE, a small increase was observed, but in the days that preceded it, flowering ended as seed filling had already begun. Thus, bee visitation continued low until ceasing, since soybean plants did not have available resources for bees to collect.

However, the temporal dynamics of bees in relation to visitation time varied considerably. The times with the highest mean frequencies predicted by the model were 11h, 12h, and 13h (Fig 2), indicating when bees are most likely to be present in the indeterminate soybean field. Bee frequency was highest in the field from 11h

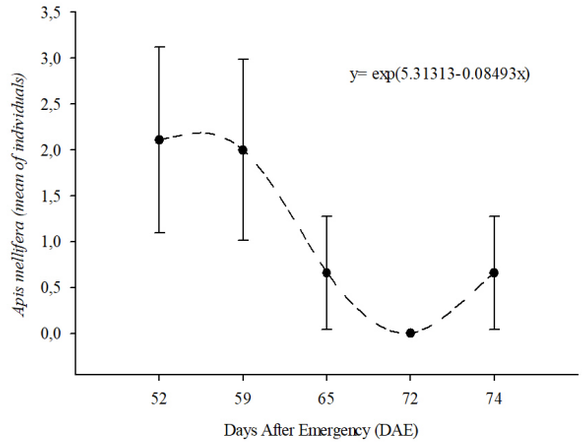

Figure 1. Temporal dynamics of *A. mellifera* bees in an indeterminate soybean field, during the flowering period estimated by the generalized Poisson regression model.

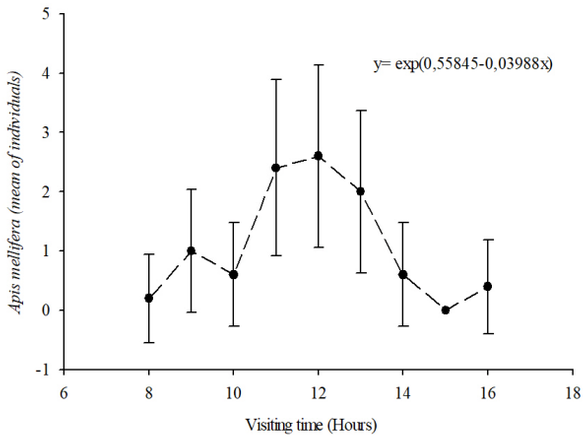

Figure 2. Temporal dynamics of *A. mellifera* throughout visiting hours in an indeterminate soybean field, estimated by the generalized Poisson regression model.

to 13h, then declined after 14h. Nonetheless, our observations indicated that bees are present in the field during the entire period evaluated in the study.

The frequency of bees in the field was not influenced by climatic factors in the present study during visitation times (Table I). However, although not significant, precipitation and relative humidity showed a weak negative correlation with bee frequency indicating that as they increased, the number of bees decreased.

Table I. Spearman's correlation coefficient between the number of *A. mellifera* bees collected during the day in the flowering indeterminate soybean field with climatic variables in Dourados-MS, 2017/2018 harvest.

	Average temperature (°C)	Relative humidity (%)	Wind Speed (m/s)	Precipitation (mm)	Liquid solar radiation (W/m²/hour)
Apis mellifera	rho= 0,184 *P*= 0,61	rho= -0,184 *P*=0,61	rho= 0,156 *P*=0,67	rho= -0,413 *P*=0,24	rho=0,393 *P*= 0,26

***P* value: probability of significance. rho=coefficient of correlation. Source of climate data: INMET.**

For the variables mean temperature, wind speed, and solar radiation, the correlation was weakly positive but also not strong enough to influence bee visitation in the field.

DISCUSSION

The pattern of temporal distribution of *A. mellifera* bees in an indeterminate soybean field was influenced by the variables: time of day and the flowering period of soybean plants (DAE), independently. Bee abundance was highest in the field in the first days of flowering, at 52 DAE and 59 DAE, while during the day, the period with the highest abundance of bees was between 10h and 15h, corroborating with Blettler et al. (2016) who recorded a greater abundance of bees in the middle of the day and early soybean flowering. Thus, application of phytosanitary products should be avoided, as this is the most critical period due to the intense activity of bees in the soybean field.

After 65 and 72 DAE, the number of forage bees decreased considerably, Blettler et al. (2016) also reported a similar behavior and observed that the flowering period of the crop influences the foraging of bees that decrease their visits to the crop according to availability of food decreases. *Apis mellifera* bees have been reported as visit the soybean field in higher numbers starting 10h, whereas in the early hours of the day, they forage with less intensity (Oliveira & Fernandes 2016) supporting our findings.

Bees are considered the most frequent pollinators of soybeans, especially *A. mellifera* (Chiari et al. 2005, Oliveira & Fernandes 2016), foraging intensely in the field in search of nectar and pollen. However, the most sought floral resource by *A. mellifera* in soybeans is nectar, which is often collected in isolation, while grains of pollen alone are rarely collected (Chiari et al. 2005). Chiari et al. (2005) observed that *A. mellifera* activity is more intense around 13h, and that in the first hours of the day and after 16h, bee frequency is lower, and the peak of bee activity is in the middle of the day.

Bletter et al. (2018), evaluating the effect of *A. mellifera* on soybean yield, observed that the period of 12h had the highest rate of bee visitation, and that its presence in the crop increased yields up to 18% in the first year of evaluation. However, in the second harvest no significant differences were found between the plots visited by bees and those where bees were prevented from visiting. This could be due to differences in climatic conditions from one year to the next or soybean variety, since many cultivars have flowers that are pollinated even before they open, which could influence soybean yields.

This pattern of bee visitation in soybeans is associated with the quantity and quality of the resources offered. In addition, soybean nectar secretion is correlated with increase in air temperature, and around 28°C soybean plants tend to secrete larger quantities of this resource and become more attractive to bees, as water evaporation is greater and nectar tends to

become more viscous and more viscous nectar means a higher sucrose concentration (Robacker et al. 1983). This may explain why frequency of honey bees foraging is higher during the hottest hours of the day in the field since they usually visit soybeans most often for nectar. However, climatic variables were not correlated with the temporal pattern of bee visitation in the indeterminate soybean cultivar evaluated in our study, but temperature may have influenced it indirectly, acting on soybean plants which increase nectar production and consequently the attractiveness of flowers to bees.

The ability of *A. mellifera* to visit soybean fields and provide the pollination service has already been reported, which can increase yields through higher number of seeds and pods per plant and consequently higher profits (Milfont et al. 2013, Bletter et al. 2018). Along with the implementation of IPM (Integrated Pest Management), they can contribute to reduce the use of phytosanitary products in the environment by favoring biological control, promoting an equilibrium in the agroecosystem and reducing expenses to farmers (Pedigo 1995, Ávila & Santos 2018).

Soybean productivity, however, does not depend solely and exclusively on the presence or absence of bees as it is an autogamous plant, but when present, bees do act as pollinating agents. Therefore, good practices such as timing the application of products at times when bees are not present or are less frequent and/or the use of selective products can contribute to their conservation and improve pollination performance. Studies on the temporal distribution pattern of pollinators in soybean fields can help increase yields and provide information on the behavior of bees in the field that can be used to develop measures to mitigate the risk of contamination of pollinators. Therefore, the use of phytosanitary products between 10h and 15h should be avoided in the first days of soybean flowering, when bee visitation is higher. These products should be used preferably after 75 DAE in indeterminate soybeans, in order to avoid the contamination of the bees in the field. Because, bee intoxication with phytosanitary products used in soybeans is most likely during the first days of flowering, as they are frequently foraging in the soybean field.

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Pre-project structuring, setting up and evaluating the experiments, collecting and analyzing data, writing and revising the article. Souza, E.P. Pre-project structuring, data analysis, writing and revision of the article. Degrande. P.E.; Alves Junior, V.V. Setting up and evaluating the experiments, collecting and analyzing data, writing and revising the article. Barbosa, V.O.; Malaquias, J.B.