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Circadian feeding rhythm of green-belly stink bug in corn cultivation

Abstract – The objective of this work was to evaluate the circadian feeding rhythm of the green-belly stink bug (*Dichelops melacanthus*) on corn (*Zea mays*), and to correlate it with meteorological parameters. The experiments were conducted in corn fields in the first (summer) and second (fall) crop seasons, in the municipality of Chapecó, in the state of Santa Catarina, Brazil. Plants in the V3 and V5 stages were covered with cages and infested with adults of *D. melacanthus*. After 24 hours of acclimation, the number of insects feeding on the plant stem was counted every three hours (at 6:00, 9:00, 12:00, 15:00, 18:00, 21:00, and 0:00 h). At night, a red led flashlight was used to visualize the insects without interfering with their behavior. Meteorological parameters were monitored and correlated to the number of insects feeding on the plants. In the first and second crop seasons, the number of insects feeding on the corn stem differed among evaluation times, with peak in the afternoon, in the evening, and at night. Few insects were found feeding at 6:00 and 9:00 h. There was a positive correlation between air temperature and feeding in both seasons. In milder temperature regions, the preferred feeding time of *D. melacanthus* on corn is during the afternoon, evening, and night hours, which should be taken into account for a precise management of this insect.

Index terms: *Dichelops melacanthus*, *Zea mays*, feeding behavior, Pentatomidae, seedling pest.


Ritmo circadiano de alimentação do percevejo barriga-verde em cultivos de milho

Resumo – O objetivo deste trabalho foi avaliar o ritmo circadiano de alimentação do percevejo barriga-verde (*Dichelops melacanthus*) em milho (*Zea mays*), e correlacionar esse comportamento com parâmetros meteorológicos. Os experimentos foram realizados em cultivos de milho de primeira (verão) e segunda safras (outono), no município de Chapecó, no estado de Santa Catarina, Brasil. Para isso, plantas nos estágios V3 e V5 foram cobertas por gaiolas e infestadas com adultos de *D. melacanthus*. Após 24 horas de aclimatização, o número de insetos que se alimentava no colmo das plantas foi mensurado a cada três horas (às 6:00, 9:00, 12:00, 15:00, 18:00, 21:00 e 0:00 h). Para avaliações noturnas, utilizou-se uma lanterna com led vermelho, para visualizar os insetos sem interferir em seu comportamento. Parâmetros meteorológicos foram monitorados e correlacionados com o número de insetos que se alimentava nas plantas. Nas primeira e segunda safras, o número de insetos que se alimentava nos colmos de milho diferiu entre os horários avaliados, com maior abundância à tarde e à noite. Poucos insetos foram observados alimentando-se às 6:00 e 9:00 h. Houve correlação positiva entre a temperatura do ar e a alimentação, em ambas as safras. Em regiões de clima mais ameno, o período preferido para alimentação de *D.*

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melacanthus em milho é nas horas da tarde e da noite, o que deve ser levado em consideração para um manejo eficiente desse inseto.

Termos para indexação: *Dichelops melacanthus*, *Zea mays*, comportamento alimentar, Pentatomidae, praga inicial.

Introduction

In Brazil, the green-belly stink bug, *Dichelops melacanthus* (Dallas, 1851) (Hemiptera: Pentatomidae), is a serious problem in the early developmental stages of corn cultivation (*Zea mays*) (Bortolotto et al., 2016; Fernandes et al., 2020). Although *D. melacanthus* is considered a secondary soybean pest, the incidence of this species in corn cultivations has increased over the years with the massive adoption of no tillage system and second growing season of corn (Panizzi, 2015). Also, the continuous presence of cultivated and wild hosts along the year favors the survival of this insect and its occurrence on crops such as corn, on whose vegetative structures of young plants it feeds (Silva et al., 2013; Smaniotto & Panizzi, 2015).

With the expansion of commercial *Bt* corn over the past 10 years and, consequently, the reduction of the use of insecticides against the fall armyworm, the incidence and damage of *D. melacanthus*, which was indirectly controlled depending on the insecticide sprayed, has raised in corn culture (Crosariol Netto et al., 2015). As *Bt* corn is ineffective against the green-belly stink bug, this insect can reach a high population growth and cause strong economic damage in *Bt* and conventional crops, if it is not managed properly.

In cases of high infestations, management measures in corn postemergence period are necessary as a complement of seed treatment, to lessen the damage caused by *D. melacanthus*. The chemical control by spraying insecticides is the method most regularly used in the postemergence phase of corn, for the management of green-belly stink bug populations (Brustolin et al., 2011; Ávila & Duarte, 2012; Guerreiro et al., 2017). However, failures in the chemical control in the postemergence period of the culture may occur because of the lack of knowledge on some behavioral aspects of *D. melacanthus*. When not placed in the corn stem for feeding, this species usually stays on the ground, underneath crop residue, where it can find shelter against its natural enemies and adverse

conditions (Cruz et al., 2016). As many contact insecticides from the pyrethroid group, whether alone or combined with neonicotinoids, have been registered for *D. melacanthus* control in corn (Agrofit, 2020), the efficacy of chemical control in the postemergence period of this crop can be reduced, when most of the insects are protected in crop residue, preventing the insecticide to reach the target pest. Besides, in face of this habit, the visual observation of *D. melacanthus* on young corn crops is hindered, and the population of this insect may be underestimated during monitoring.

Most farmers and pest samplers face difficulty to visualize *D. melacanthus* in corn fields, and only detect and manage the insect when symptoms of attack are already apparent on plants. An adequate sampling and control timing are crucial for an effective management of *D. melacanthus* in the postemergence period of corn (Sosa-Gómez et al., 2020). For this, knowing the habits of this insect in corn cultivations is essential. Several studies on the bioecology of *D. melacanthus* have been carried out (Chocorosqui & Panizzi, 2008; Bortolotto et al., 2016; Oliveira et al., 2019), and some aspects of *D. melacanthus* feeding on corn stems –such as body position, ingestion site, frequency, duration, and ingestion and excretion rates – were elucidated in laboratorial studies, by using the electropenetrography (EPG) technique (Lucini & Panizzi 2017a, 2017b, 2018; Panizzi & Lucini, 2019). In Brazil there is a prevailing “belief” that the green-belly stink bug is more active and feeds in the early morning hours, while taking shelter under the straw layer in the warmest hours of the day; however, this “belief” cannot be entirely sustained, since there is a lack of scientific information on the circadian feeding rhythm of this species in corn fields.

A clear understanding about the preferred feeding time of *D. melacanthus* on corn plants along the day is essential for a precise monitoring and an effective chemical control of this pest, in the postemergence period of corn, which is optimized when insects are feeding and, consequently, more exposed to insecticide spraying.

The objective of this work was to evaluate the circadian feeding rhythm of the green-belly stink bug on corn, and to correlate it with meteorological parameters.

Materials and Methods

The experiments were carried out in the municipality of Chapecó (27°05'19"S, 52°38'13"W, at 658 m altitude), in the state of Santa Catarina, Brazil, during the 2017/2018 crop year in the first (summer) and second (fall) growing seasons.

The soil of the area is classified as Latossolo Vermelho distroférrico (Potter et al., 2004), which corresponds to Ferralsol (IUSS Working Group WRB, 2015), and it was managed under no-tillage, showing the following characteristics: 610 g kg⁻¹ clay; pH water (1: 1), 5.8; 9.3 mg dm⁻³ P; 66.0 mg dm⁻³ K; and 30 g kg⁻¹ of organic matter (OM). The climate in this site is humid subtropical (Cfa), according to Koppen-Geiger's classification, with hot summers (Pandolfo et al, 2002).

In the first and second growing seasons, triticale (*x Triticosecale* Wittmack) and common bean (*Phaseolus vulgaris*) were the predecessor crops, respectively. Thirty days before sowing, spontaneous plants were desiccated with the use of glyphosate (Roundup Original, 480 g L⁻¹ a.i.), and 2,4-dichlorophenoxy acetic acid (2,4-D Nortox, 806 g L⁻¹ a.i.), at 5 and 2 L ha⁻¹, respectively. Sowing was carried out in October 5th, 2017 [first growing season (summer)] and in February 2nd, 2018 [second growing season (fall)], using the corn hybrid Pioneer 30F53 VYHR [Leptra (a transgenic event expressing the proteins Cry1F, Cry1Ab, and VIP3Aa20 (from *Bacillus thuringiensis*), PAT (from *Streptomyces viridochromogenes*), and CP4-EPSPS (from *Agrobacterium tumefaciens*)]. The spacing between rows was 0.8 m, and average sowing density was 6 seed per linear meter of row.

The basic fertilization consisted of 400 kg ha⁻¹ of N-P₂O₅-K₂O 09-33-12, according to previous analysis of the soil. In the V2 and V8 plant stages, two applications of glyphosate (Roundup Original, 480 g L⁻¹ a.i.) + 0.25% soybean oil methyl ester (Aureo, 720 g L⁻¹ a.i.) adjuvants were performed at the dosage of 3 L ha⁻¹, in a mixture volume of 150 L ha⁻¹. The application of N under cover was done in the V5–V6 stages, using 112.5 kg ha⁻¹ N as common urea. The other cultural treatments followed the technical recommendations for corn production in Brazil (Reunião Técnica Anual de Pesquisa de Milho, 2017), except for the application of insecticides, which was not carried out during the entire crop cycle.

In both growing seasons, 10 tulle fabric cages with 0.5 m² (1 m length × 0.5 m width × 0.5 m height)

were installed, each one on five selected plants (about 1-meter row) in field plots, when plants reached the V3 and V5 stages (Ritchie et al., 1986). The straw layer in the area with cages was standardized prior to the installation of the cages.

One day before starting the evaluations (to allow of acclimation of insects in the field), each cage was infested with 5 pairs of insects (10 individuals), obtained from a laboratory colony that was maintained using the procedure described by Ribeiro et al. (2018). On each assessment day (V3 and V5 stages, in both first and second growing seasons), the infested plants were checked every 3 hours (at 6:00, 9:00, 12:00, 15:00, 18:00, 21:00 and 0:00 h), in order to visually verify the number of stink bugs feeding on the plant stems, following the feeding behavior described by Panizzi & Lucini (2019). For the nocturnal evaluations, a red LED flashlight (Limatec, Cruz das Almas, BA, Brazil) was used to visualize the insects on plants without interference. Air temperature, air relative humidity, and radiation data were obtained from a meteorological station of Epagri/Cepaf, located at 50 m from the experimental fields.

Generalized linear models (GLM) from the exponential family of distributions (Nelder & Wedderburn, 1972) were used to analyze the data. In all cases, the quality of fit was checked, using the half-normal probability plot with simulation envelope (Hinde & Demétrio, 1998). When significant differences were found among the treatments, multiple comparisons (by the Tukey's post hoc test, at 5% probability) were performed using the *glht* function of the *multcomp* package, with p-value adjustments for treatments with qualitative levels. The possible relationships between the feeding behavior and meteorological parameters were tested, using the nonparametric Spearman correlation analysis at 5% probability. All analyses were carried out using the statistical software R, version 3.4.3 (R Core Team, 2017).

Results and Discussion

In the first crop season, the number of *D. melacanthus* feeding on corn plants in the V3 stage, in the afternoon period (12:00–18:00 h), was significantly higher than that observed at dawn (6:00 h) (Figure 1 A). The highest number (>3.0) of stink bugs

feeding on corn plants were observed at 12:00 and 15:00 h, and differed significantly from the lowest values observed at dawn and morning. In the V5 plant stage, in the first growing season, few insects were found feeding on plant stems at 6:00 and 9:00 h; however, the feeding activity was higher from 12:00 to 0:00 h, with more than 4.2 insects feeding on plants (Figure 1 B). For both vegetative stages, in the first growing season, the peak of insects feeding on plants occurred at 15:00 h.

For corn at the V3 stage, in the second growing season, 15:00 and 21:00 h were the times of the day when the highest numbers of *D. melacanthus* were found feeding on plant stems (3.5 and 3.6 insects, respectively), with significant difference from the values obtained from 6:00 to 12:00 h (Figure 1 C). At the V5 plant stage, the insect feeding activity started

to increase at noon, with peak at 18:00 h (Figure 1 D). The number of stink bugs feeding on corn stem at this time was significantly higher than that observed at 6:00 and 9:00 h.

Findings from the present study show that *D. melacanthus* exhibits a circadian feeding behavior that results in different counts of stink bugs on corn stem throughout the day. In general, counts on corn plants were low at dawn and morning evaluations, and they increased at noon and afternoon with the temperature rise, evidencing that a heat uptake and raise in body temperature by solar exposure are necessary for the beginning of foraging and feeding activity (basking behavior). This behavior was reported for the first time for pentatomid bugs by Waite (1980), who verified that nymphs and adults of the phytophagous stink bug [*Nezara viridula* (Linnaeus, 1758) (Hemiptera:

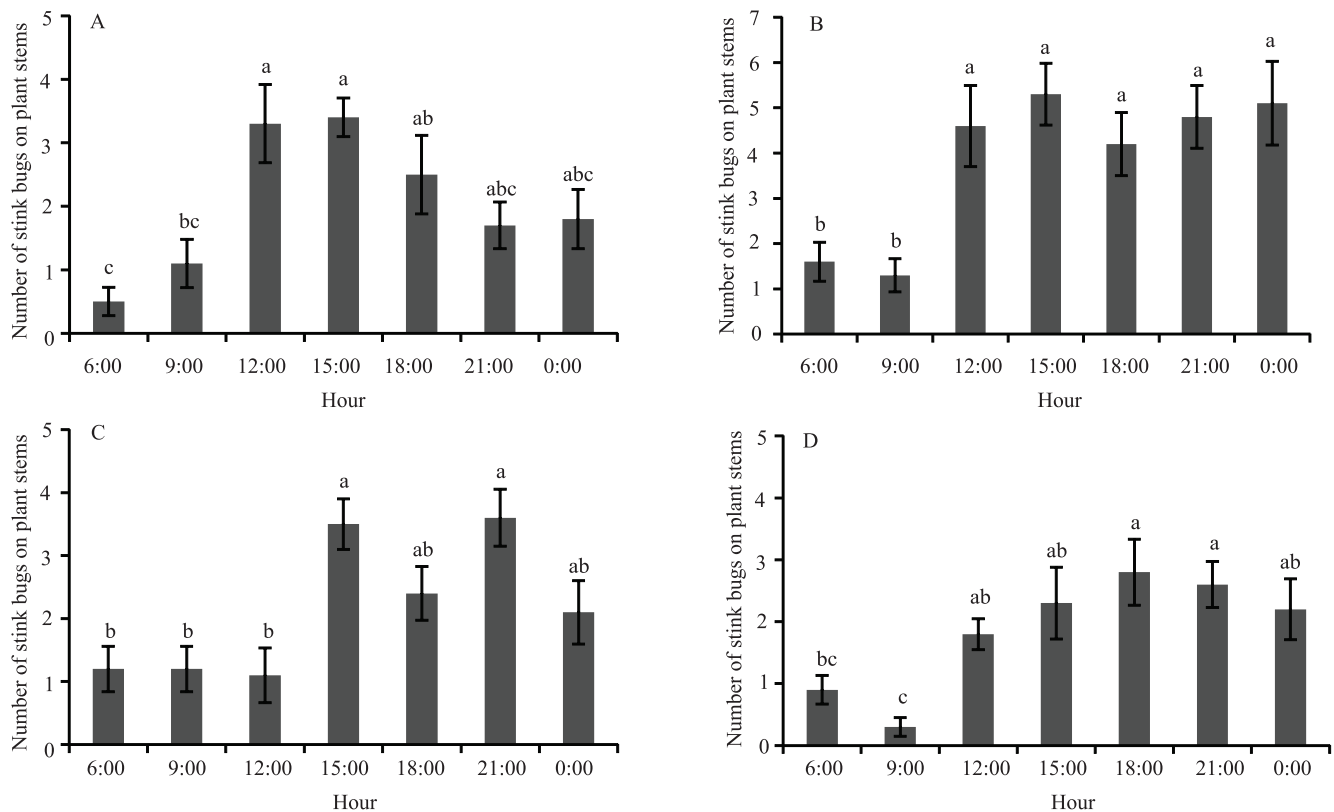


Figure 1. Number (mean±SE) of *Dichelops melacanthus* feeding on corn (*Zea mays*) stems in the first (A, B) and second (C, D) growing seasons, at different times of the day: A, first growing season, plants at the V3 stage (November 1st, 2017); B, first growing season, plants at the V5 stage (November 7th, 2017); C, second growing season, plants at the V3 stage (February 16th, 2018); D, second growing season, plants at the V5 stage (February 23rd, 2018). Means followed by different letters significantly differ (GLM with a quasi-Poisson distribution by the Tukey's post hoc test, at 5% probability. A, $F_{6,63} = 6.09$, $p < 0.0001$; B, $F_{6,63} = 7.03$; $p < 0.0001$; C, $F_{6,63} = 5.41$; $p = 0.00014$; and D, $F_{6,63} = 6.95$; $p < 0.0001$).

Pentatomidae)] moved to the canopy's surface in the morning, for sunlight exposure, and remained there for a period, before reentering into the soybean plant for feeding in warmest period. Our study corroborates the findings on other species of stink bugs, such as *Piezodorus guildinii* (Westwood, 1837) and *Bagrada hilaris* (Burmeister, 1835) (both Hemiptera: Pentatomidae), which were also reported to exhibit higher feeding activity in the warmest hours of the day (Guedes et al., 2009; Huang et al., 2013).

Most insects show daily cycles of activity, and feeding usually occurs in the active period (Saunders et al., 2002; Lam & Chiu, 2019). Because insects pose an ectothermic condition, they need an external source of heat to warm up their bodies and increment metabolism, which renders them particularly sensitive to environmental temperature (Lazzari & Insausti, 2008).

The average air temperatures registered in the first and second growing seasons were 21.4 growing 22.7°C, respectively, ranging from 12 to 26.2°C in the two days of evaluation of the first growing season, and from 15.2 to 27.4°C in the two days of evaluation of second growing season (Figure 2 A). The average air relative humidity in the two days of evaluation, in the first growing season was 55.9%, with minimum of 44.0 and maximum of 88.8%, while in the second growing season, the average air relative humidity was 66%, ranging from 45.4 to 90.4% (Figure 2 B). Among the meteorological parameters obtained in the evaluation days, air temperature and air relative humidity were those that most correlated with *D. melacanthus* feeding. The presence of *D. melacanthus* feeding on corn stems was positively correlated with air temperature in corn fields, in the first and second growing seasons (Table 1). Also, in the second growing season, there was a negative correlation between *D. melacanthus* feeding and air relative humidity. As stated by Jaworski & Hilszczański (2013), temperature and humidity are the main climate parameters that can directly influence insects by limiting, or stimulating, several activities of them.

The correlation between the number of *D. melacanthus* feeding on corn stem and solar radiation was not consistent and suggests that this parameter is not a meaningful indicator of feeding activity (Table 1). Solar radiation is a relevant factor that can influence trophic interactions; however, many insects

have a daily rhythm of feeding entrained mostly by temperature (Saunders et al., 2002). Although solar radiation is perceived by some insects, its effects on insect feeding are likely to be indirect, as they occur by increasing the expression of secondary metabolites and modifying the quality of plant tissues (Zavala et al., 2015).

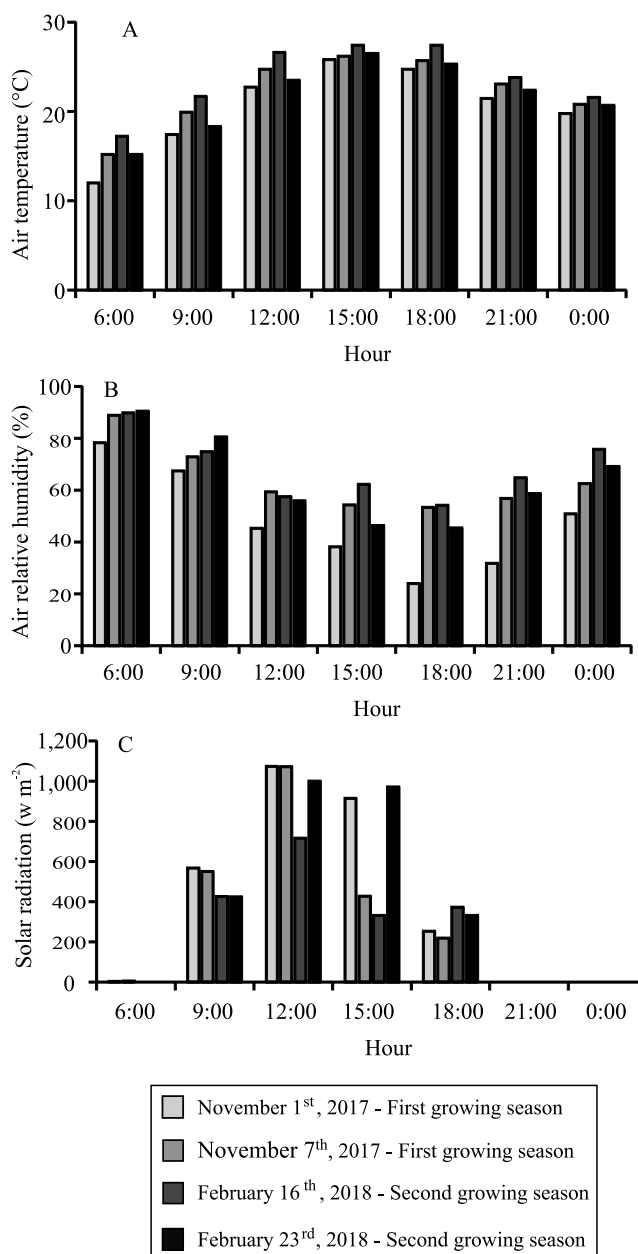


Figure 2. Meteorological parameters at the times of evaluation in the first and second growing seasons of corn (*Zea mays*): A, air temperature; B, air relative humidity; C, solar radiation.

Evaluations on both first and second growing seasons indicated that ambient temperature was the most important factor that influenced the feeding of *D. melacanthus* on young corn plants. Essentially, the number of stink bugs on corn stem increased according to temperature, with peak counts in the warmest period of the day, suggesting that *D. melacanthus* feeding is an activity triggered by temperature. The metabolism of insects is accelerated when temperatures from the surrounding environment increase to the thermal optima, directly influencing insect activities such as feeding (Jaworski & Hilszczański, 2013). Temperatures between 25 and 31°C are reported to be the most favorable ones for *D. melacanthus* development, whereas mild temperatures are detrimental for the biology and activity of this species (Bortolotto et al., 2016; Oliveira et al., 2019).

Temperatures at 6:00 and 9:00 h ranged from 12.01 to 21.69°C in the evaluation days of the first and second growing seasons (Figure 2); values below the thermal optima were recorded for this species (Bortolotto et al., 2016). As stated by Lemoine et al. (2014), the herbivory of insects is directly associated with environmental temperatures, and it tends to increase with temperatures between 20 and 30°C, as a result of the rise of metabolic demands of insects. Insects usually exert feeding in the most suitable period of the day. Feeding at temperatures below the thermal optima increases the energetic cost of movement from insects (Schowalter, 2016) which may explain the low number of green-belly stink bugs feeding from 6:00 to 9:00 h.

In our study, nighttime observations did not differ significantly from afternoon, showing that feeding by *D. melacanthus* on corn plants also occurs in nocturnal period. According to Saunders et al. (2002),

temperature can affect the expression of the circadian photoperiodic clock in insects. As temperatures observed in nocturnal evaluations of both growing seasons were not below the thermal optima for *D. melacanthus*, the feeding was not inhibited at nighttime. The feeding of *D. melacanthus* in corn along the day is not a continuous, but an intermittent activity (Lucini & Panizzi, 2017b), and our results suggest that feeding events occur both in photophase and scotophase. Therefore, spray of insecticides at night can be a viable option, since pesticides cannot be exposed to photodegradation by sunlight, which can affect the control effectiveness (Soliman, 2012).

Usually, pest samplers are recommended to monitor the green-belly stink bug at mild temperatures and to avoid the warmest hours of the day; however, this recommendation may be more suitable for warmest regions like the Brazilian savanna, where *D. melacanthus* is a serious problem in corn cultivated after soybean (Panizzi, 2015; Gomes et al., 2020). In the warmest regions of Brazil, like the Southeast, Midwest and Northeast, the diel feeding pattern of *D. melacanthus* might differ from that observed in our study, since increased temperatures can alter the feeding rhythm of herbivore insects (Lemoine et al., 2013). Temperatures above the optimum temperature range of a species can cause a mismatch between metabolism and consumption, leading to reductions of the ingestion efficiency and insect fitness (Lemoine & Burkepille, 2012). Since in corn fields from Brazilian savanna the temperatures around noon and early afternoon can easily exceed the utmost optimal temperature for *D. melacanthus* (30°C), the feeding of this species may occur more likely in the early hours of morning. Also, in unfavorable conditions, like extremely high temperatures and solar radiation, *D. melacanthus* find shelter under crop residue to avoid water loss (Panizzi, 2015), impairing the monitoring and chemical control when they are carried out in the afternoon.

Based on the results of the present study, the monitoring of *D. melacanthus* (visual observations on corn plants) could result in biased low estimates of population in the South of Brazil, where temperatures are relatively milder, if carried out during the coolest hours of morning (6:00 to 9:00), in the first and second corn growing seasons. The economic threshold level of *D. melacanthus* on corn is three

Table 1. Correlation between the observed number of *Dichelops melacanthus* feeding on maize plants and meteorological parameters.

Meteorological parameter	r ⁽¹⁾	p-value
First growing season		
Air temperature (°C)	0.771	0.002
Air relative humidity (%)	-0.314	0.273
Radiation (W m ⁻²)	-0.006	0.982
Second growing season		
Air temperature (°C)	0.596	0.024
Air relative humidity (%)	-0.558	0.037
Radiation (W m ⁻²)	-0.230	0.428

⁽¹⁾Spearman's coefficient correlation.

adults per meter of row (Gomes et al., 2020), and an inaccurate monitoring may lead to significant plant damage or unnecessary insecticide spray. Hence, the monitoring and the attainment of reliable results of *D. melacanthus* densities are essential for precise control decisions. Because corn growers usually rely on contact insecticides for managing *D. melacanthus* outbreaks, for their postemergence application to corn, sprays carried out in the afternoon, evening, and even at night, when insects are more active and feeding on corn stem, are probably more effective than during morning hours.

Conclusions

1. The green-belly stink bug (*Dichelops melacanthus*) exhibits a circadian feeding rhythm on corn (*Zea mays*) plants, with few insects feeding early in the morning and with a more pronounced feeding activity in the afternoon, evening and night hours.

2. Air temperature is the main meteorological parameter that influences the feeding activity of *D. melacanthus* on corn crop.

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