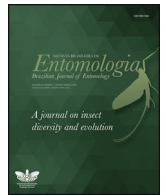




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## Interseasonal variation of *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae) populations in the Brazilian Savanna



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### ABSTRACT

*Chrysodeixis includens* (Walker, [1858]) is currently the main plusiine pest in the Americas, not only because of the damage caused to soybean, but also with several crop species in a broad geographical range. However, its population dynamics is still poorly understood, despite outbreak records that are common across different locations in the Americas. The current study aimed at identifying phenological patterns of *C. includens* emphasizing its differences among the three years of sampling effort in an intercropping area of the Brazilian Savanna. Thereafter, we tested whether the El Niño size effect, meteorological factors, or soybean, corn and wheat cycles, are better predictors of its monthly abundance. The insects were collected with a light trap during five consecutive nights (repetitions) during 35 new moons. In total, 2026 specimens were collected in all months of the year although not consecutively. Across each year, monthly abundance of *C. includens* was non-uniform, characterized by sharp population peaks concentrated in the rainy season. These peaks varied from January until March, depending on the year sampled. We found that the local soybean cycle and El Niño effect to significantly influence the species abundance across the entire period of study. These results aid in understanding the species population dynamics and its status as a pest, providing evidence of factors that determine its phenological patterns. Although it presents a very defined phenology, the population dynamics of *C. includens* varies significantly between years and locations, which demonstrates the importance and need to monitor local populations of larvae and adults for its management.

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### Introduction

Since the beginning of last century, *Chrysodeixis includens* (Walker, [1858]) has been considered the main pest of soybean, occurring with higher frequency and intensity in different locations in Brazil (Rolim et al., 2013; Sosa-Gómez and Omoto, 2013; Sosa-Gómez et al., 2010). However, even though its polyphagia and wide distribution across the Americas (e.g. Herzog, 1980; Lafontaine and Poole, 1991; Wagner et al., 2011; Specht et al., 2015), population increase of *C. includens* is commonly related to seasonal phenomena (i.e. hot and humid periods), or due to the period of increased host-plant availability (Alford and Hammond, 1982; Moraes et al., 1991; Marsaro et al., 2010).

Plant phenology (Bencke and Morellato, 2002; Ting et al., 2008; Morellato et al., 2010) is of particular importance to phenological patterns exhibited by herbivore insects, especially when these plants are cultivated (Fietz and Rangel, 2008; Freitas et al., 2010). The leaf flush period and the development of reproductive organs are of main concern to temporal distribution of specialized herbivores, but seeding and harvest periods of annual agricultural crops can drastically constrain their abundance, or even presence of insects in a short time period. The contrasting seasons of the Brazilian Savanna are marked by differences of water availability, which requires an intensification of primary agricultural production during the period of highest rainfall (usually soybean and corn). The dry period is usually reserved for the growth of secondary crops (intercropped with soybean, corn or cotton), such as grasses (wheat, sorghum, millet and signalgrass) or forage legumes (pencil flower, common jack bean and pigeon pea), whose production is for feeding cattle during the dry season, or to simply protect the soil from

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solar radiation and to increase organic matter (Hoffmann et al., 2004; Franchini et al., 2011). These crop management needs led to the development of cultivars with characteristics such as drought resistance and long, medium, early and extra-early cycles. Crops in the Brazilian Savanna are therefore always present throughout the year, though constantly changing in species composition according to season. This scenario favours the presence and abundance of polyphagous insects whose populations make use of different cultivated hostplants (Paula-Moraes and Specht, 2013). Although *C. includens* larvae are not very mobile, as Plusiinae in general (Bernarys and Singer, 2002), *C. includens* presents a high degree of polyphagia, being reported feeding on at least 175 species of plants (Baldin et al., 2014; Specht et al., 2015).

Local climate factors are reported as one of the main drivers in yield oscillations (Marchiori et al., 1999; Bergamaschi and Matzenauer, 2014). Likewise, the weather has been associated with population variations of pest insects (Moraes et al., 1991; Heineck-Leonel and Corseuil, 1997). Temperature, for example, has an important influence over the development of crops such as soybean, corn and wheat (Marchiori et al., 1999; Fietz and Rangel, 2008; Bergamaschi and Matzenauer, 2014), which in turn affects development and reproduction rates of insects (Porter, 1991). In the tropics however, a great part of the seasonal climatic variations is related to water availability during the year, evidenced by ecosystems with well defined dry and rainy seasons (Silva et al., 2014). Thus, insect dynamics in natural environments and agroecosystems are highly dependent on tropical dry/wet seasons (Silva et al., 2011; Kishimoto-Yamada and Itoioka, 2015).

Phenological oscillations also depend on supra-annual factors that influence the Earth's climate, such as hurricanes, earthquakes, cyclones and global temperature oscillations (i.e., La Niña and El Niño) (Torres, 1992; Bravo et al., 2010). The El Niño effect, depending on the locality, is known to cause excessive rainfall, high winds, drought and temperature increase (Bravo et al., 2010), and is responsible for related agricultural losses (Sivakumar et al., 2005) and insect outbreaks (Rouault et al., 2006). Nonetheless, effects caused by inter-annual phenomena are rarely investigated in insect population dynamics (Kishimoto-Yamada and Itoioka, 2015), and even less so when related to agricultural pest species (Sivakumar et al., 2005).

In order to contribute to the knowledge of pest-insect population dynamics in agroecosystems of the Brazilian Savanna (i.e. Cerrado), the current study aimed at identifying seasonality patterns of *C. includens* in an intercropping area during three years, testing: (1) significant major occurrence to a certain period of the year; (2) the same monthly abundance distribution among different years; (3) predictive power of the annual crop cycle, weather factors and El Niño effect on monthly moth abundance.

## Material and methods

### Sampling area

The experiment was performed in the 'Estação Experimental da Embrapa Cerrados', located in the city of Planaltina, Distrito Federal, Brazil (15°35'30" S and 47°42'30" W, altitude: 1007 m). The area is located in the 'Cerrado' biome (Ab'Sáber, 2003), also known as the Brazilian Savanna. According to the climate classification of Köppen, it has a Subtropical-Dry Winter climate (Cwa), with average temperature of the coldest month below 18 °C and from the hottest month above 22 °C. The region is marked by two seasons that are defined by the differences in rainfall accumulation. The rainy period starts in September and extends until April, with the rainiest months being November, December and January. The dry period starts in May and ends in September, resulting in a hot and rainy summer and mild and dry winter (Silva et al., 2014).

The sample landscape is totally devoid of natural areas, where several species of agricultural importance predominate, especially soybean, corn and wheat that occupies about 25%, 15% and 10% of the landscape. Part of the area is occupied with buildings and, to a lesser extent, crops of other species such as *Andropogon*, coffee, cassava, *Crotalaria*, *Eucalyptus*, oil palm, *Panicum*, passion fruit, cane. Wheat stands out because of its presence is restricted to the dry period, while soybean and corn cultivation is restricted to the rainy season. It is assumed that the other crops do not affect the phenology of *C. includens* because they are perennial, are not preferential hosts or are cultivated in small areas.

### Collecting method

Because *C. includens* is strongly attracted to light sources (Wagner et al., 2011), we used a Pennsylvania light trap (Frost, 1957) as a collection method, set with a black fluorescent light model BL T8 15W (Tovalight), with wavelengths varying between 290 and 450 nm with a peak around 340 nm. A plastic cone was set in the lower portion of the trap, with the widest diameter of 32 cm and narrowest of 16 cm, to which a plastic bucket was set with three litres of 96GL ethanol. The trap was set on a post, with its lower part approximately 3.5 m above the ground, and was lit from nightfall until the following morning.

The trap was lit 35 times during three years to represent all months during that period. The only month whose samples could not be considered was November of 2013, since the excess rainfall and a large number of Coleoptera, Hymenoptera and Isoptera attracted to the trap damaged the specimens preventing species identification. To minimize the effects of moonlight and meteorological effects on traps efficiency, each collecting event had five samples (nights) collected at the end of the waning moon and start of the new moon (Zanuncio et al., 1995).

*Chrysodeixis includens* specimens are preserved in 96GL ethanol, and representative vouchers were pinned and deposited in the 'Coleção de Insetos da Embrapa Cerrados'. Specific identification was based on the specialized bibliography (Eichilin and Cunningham, 1978; Lafontaine and Poole, 1991).

### Statistical analysis

Rao's Spacing test was used to test the uniformity of the temporal distribution of *C. includens* for each year. Taking into consideration that the Rao test rejected the hypothesis of an uniform distribution for *C. includens*, the estimated average angle of abundance in a certain period of the year was used to indicate the period of highest occurrence. The length of the average angle ( $r$ ), which may vary from 0 to 1, was used to indicate how clustered the abundance data were around the average (Zar, 2010). This value is correlated to the angular standard deviation calculated, which is used to represent the amplitude of a phenological pattern.

Once evidence of a phenological pattern of moth occurrence was observed, annual distributions (between harvests) were compared to each other to determine if the species maintains its patterns, even between distinct years. In this case, the average angles and abundance distributions were used as statistical parameters, associated to the Watson William ( $f$ ) and Watson- $U^2$  tests, respectively.

We also tested for different variables as predictors of the phenological abundance variation of *C. includens* during the three years. Because abundance distribution is not normal and data showed overdispersed residuals, we used the generalized linear model using a negative binomial distribution. The variables tested include hostplant availability, El Niño size effect, and weather

**Table 1**  
Monthly abundance of *Chrysodeixis includens* moths collected with light traps in three agricultural crop seasons in the 'Estação Experimental da Embrapa Cerrados', Planaltina, DF, Brazil.

Crop season	July	August	September	October	November	December	January	February	March	April	May	June	Total
2013/2014	1	5	3	0	<sup>a</sup>	5	1218	205	4	5	1	1	1448
2014/2015	2	1	1	0	0	1	57	374	22	4	0	0	462
2015/2016	0	0	0	1	0	0	3	52	58	0	0		114

<sup>a</sup> The samplings were not considered.

**Table 2**  
Descriptive statistics of the circular analysis of the abundance of *Chrysodeixis includens* in each harvest in the 'Estação Experimental da Embrapa Cerrados', Planaltina, DF, Brazil. *n*, number of moths;  $\mu$ , mean vector; CSD, circular standard deviation; *r*, length of mean vector; Conc., Concentration; *U*, Rao's spacing test;  $U^2$ , Watson's  $U^2$  test.

Crop season	<i>n</i>	$\mu$	CSD	<i>r</i>	Conc.	<i>U</i>	$U^2$
2013/2014	1452	34.597°	15.907°	0.96	13.49	357.52	<sup>a</sup> 73.32
2014/2015	462	44.279°	17.208°	0.96	11.61	353.77	<sup>a</sup> 20.52
2015/2016	112	49.225°	21.586°	0.93	7.58	347.14	<sup>a</sup> 2.49

<sup>a</sup> Significant values: 0.01.

<sup>b</sup> 0.001.

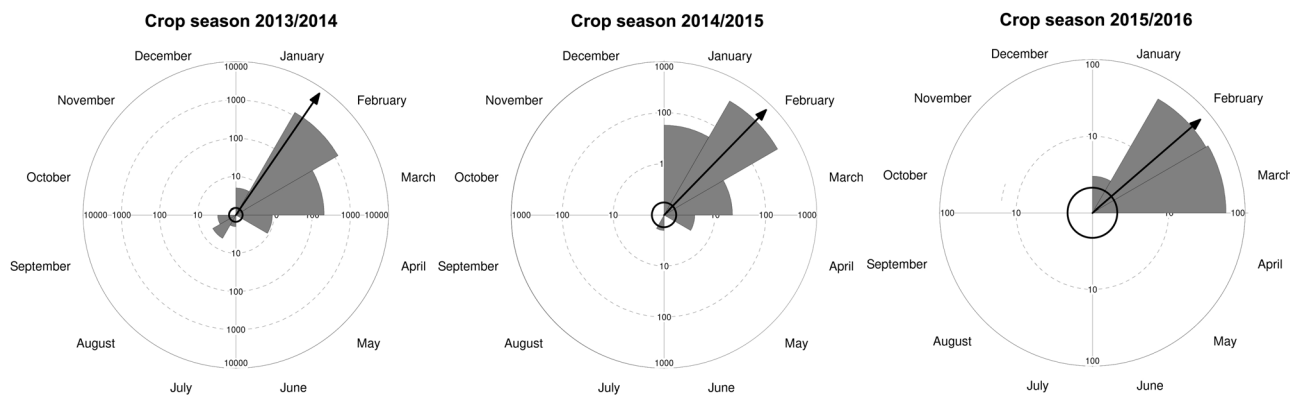
measurements. Since we could not access a quantitative estimate of available hostplants, soybean/corn and wheat were monthly coded as presence/absence according to local management of these cultures in the study area. Soybean and corn effect could not be distinguished because they are cultivated during the same season. However, since corn is only sporadically reported as a hostplant of *C. includens* (Herzog, 1980), we discussed the effect of this factor by being solely provided by the soybean. Soybean and corn crops were available for *C. includens* between December and March, the months of its sowing and ripening, respectively. During April, *C. includens* cannot use the leaves of the soybean and corn because they dry and fall when the pod is ripening (Moscardi et al., 2012). The wheat cycle, however, starts in March and extends until September. These hostplant cycles were selected because they represent non-perennial crops cultivated in the study area that are used by *C. includens* as hostplants.

Measurements from the previous month of average, maximum and minimum relative humidity, rainfall, average, maximum and minimum temperature, and insolation were selected to represent two main factors that could influence on the development of *C. includens* and, therefore, its abundance. Thus, to improve the interpretation of results and avoid collinearity in our data we used the interaction of the first four variables to represent water availability and the last four variables to represent temperature affecting abundance of the soybean looper. The linear model was fitted using glm function, available in R environment R Development Core (R Core Team, 2013).

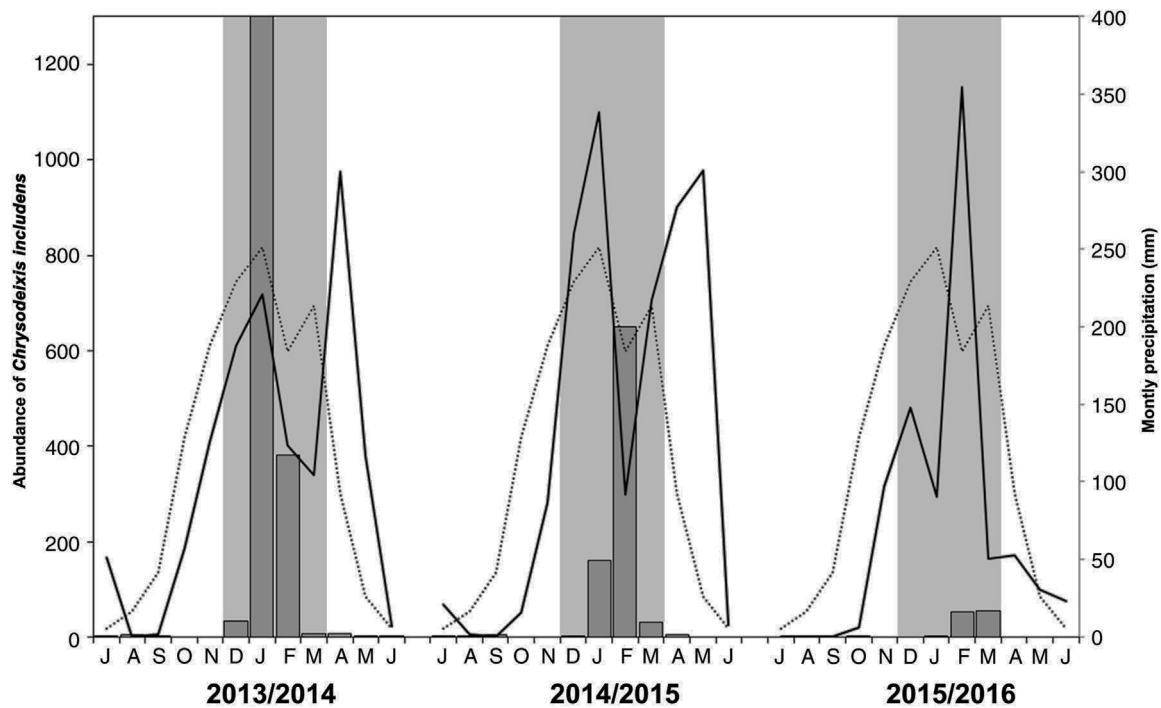
## Results

During the three years (July/2013 until June/2016) 2026 specimens of *C. includens* were collected (Table 1). The monthly abundance was marked by an exceptional peak occurring in February, elevated abundances between January and March depending on the year, and only occasional records in other months, when present. The species was not detected in October of the first and second crop seasons, and in May, June and November of the second and third crop seasons. A non-uniform pattern was observed for *C. includens* in all sampled years, as confirmed by the Rao Spacing test (Table 2).

Abundance and phenological patterns of *C. includens* differed between years. Although total abundance decreased through the years (1452 in the 2013/2014, 462 in the 2014/2015 and 112 in the 2015/2016), the average vector angle (the circular measurement that represents the phenological mean distribution of the species) delayed slightly from the first to the third year. In the first, the vector angle was directed towards the beginning of February. In the following years the average angle delays slightly to the middle of February and March, accordingly (Fig. 1), although always concentrated in a small portion of the rainy season and almost synchronic to soybean availability (Fig. 2). Circular statistical analyses confirmed the differences between the average angles and the abundance distributions between years (Table 3). When all variables are included to predict *C. includens* abundance, only soybean availability and El Niño effect showed significant influence, although both factors acts conversely. While soybean availability raises *C. includens* abundances, higher effects of El Niño reduces



**Fig. 1.** Monthly abundance of *Chrysodeixis includens* moths during three crop seasons. Vectors angles represents circular mean abundance while vectors length represents how clustered is the abundance data around the average.



**Fig. 2.** Population variation of *Chrysodeixis includens* (bars) sampled through light traps, compared to monthly normal rainfall (mm/month) (dotted line), monthly accumulated rainfall (mm/month) (continuous line) and period of corn and soybean cultivation (grey area) during five nights each month (new moon), in the 'Estação Experimental da Embrapa Cerrados', Planaltina, DF, Brazil.

**Table 3**

Comparison of abundance distribution of *Chrysodeixis includens* between crop seasons, based on Watson–Williams *F*-test and Watson's  $U^2$  test.

Crop season (no. moths)	Watson–Williams <i>F</i> -test		Watson's $U^2$ test	
	<i>F</i>	<i>p</i>	$U^2$	<i>p</i>
2013/2014 (1452) × 2014/2015 (462)	125.07	<sup>a</sup>	47.67	<sup>a</sup>
2013/2014 (1452) × 2015/2016 (112)	80.94	<sup>a</sup>	4.92	<sup>a</sup>
2014/2015 (462) × 2015/2016 (112)	6.64	<sup>a</sup>	6.69	<sup>a</sup>

<sup>a</sup> Significant values: 0.001.

**Table 4**

GLM coefficients from predictive power of weather and crop cycle on monthly abundances of *Chrysodeixis includens*. Theta value = 0.564 (±0.163SE).

	Coefficients	Std. error	<i>z</i>	<i>p</i> value	Sign.
Intercept	−0.420	7.695.094	−0.055	0.957	ns
Wheat cycle	0.664	1.311.244	0.506	0.613	ns
Soybean cycle	5.385.903	1.261.145	4.271	<0.001	<sup>a</sup>
El Niño effect	−119.125	0.354	3.367	<0.001	<sup>a</sup>
$I(T_{max} + T_{med} + T_{min} + Ins)$	−0.005	0.093	−0.052	0.959	ns
$I(UR + UR_{MAX} + UR_{MIN} + Prec)$	0.002	0.003	0.794	0.427	ns

ns, not significant.

<sup>a</sup> Significant values: 0.001.

its populations. All other factors influence did not differ from zero (Table 4).

## Discussion

The phenological dynamic patterns of insect species has so far been focused on species with natural distributions (Wolda, 1988), although such patterns can be easily translated to pest species. Recognizing the phenological patterns exhibited by pest species, such as *C. includens*, is particularly important to applied crop sciences, as this kind of information is necessary to develop and/or recommend methods to minimize economic losses (Hudson and Keatley, 2010). As it occurs to species in natural habitats, phenological patterns are dependent of the range of hostplant used by herbivorous species. For monophagous, oligophagous, or species particularly associated to certain annual agricultural crops,

a higher degree of phenological association is expected due to the limited available resource (Novotny and Basset, 1998). Thus, from harvest to the beginning of the next crop growth cycle, populations are expected to be absent. On the other hand, polyphagous or generalist species can also present significant major occurrence (population peaks) for certain periods of the year, but low abundance can still be recorded in the absence of the preferred hostplant (Wolda, 1988; Kishimoto-Yamada and Itoika, 2015), as they will feed on inter-annual crops, weeds or cover plants.

Throughout the Americas, *C. includens* presents sharp population peaks in different periods of the year (Alford and Hammond, 1982; Moraes et al., 1991; Marsaro et al., 2010). During the three studied years, its abundance was always concentrated in a narrow period of the rainy season (between January and March), showing a strong phenological pattern. This pattern was here showed to be influenced by the soybean cycle, which is relatively short compared

to other crop species (Freitas et al., 2010). However, it is important to highlight that *C. includens* maintained small populations surviving in alternate hostplants throughout much of the year, probably due to its high degree of polyphagia. Its absence during occasional months may be explained by low population numbers, and stochastic factors related to light attraction traps (Muirhead-Thompson, 1991; Vilarinho et al., 2011). In the presence of soybean and favourable conditions, these populations might multiply rapidly, occasionally causing outbreaks, massively infesting other crops like bean and castor bean (Baldin et al., 2014). Other characteristics of this species, such its migratory behaviour in certain regions (Ferguson et al., 1991; Wagner et al., 2011), could also explain the dynamics of these population peaks. Nevertheless, other methodologies (e.g. molecular approaches) are required to detect the migratory influence on population dynamics (Palma et al., 2015).

The strong phenological pattern of the adults of *C. includens* in central Brazil also corroborates observations from immature data. Evidently, larval populations also increase in the presence of soybean crops in very distinct Brazilian agriculture systems (Moraes et al., 1991; Didonet et al., 1998; Conte and Corrêa-Ferreira, 2014). Because of this direct association with the soybean cycle (Herzog, 1980; Moscardi et al., 2012), the period when species abundance is higher also follows the period when soybean is cultivated in different regions of America. In the northern hemisphere, for example (latitude around 30°), the soybean phenology is inverted. There the higher abundance peaks of *C. includens* occurs from July to September (e.g. Harding, 1976; Alford and Hammond, 1982). The same occurs to regions close to the Equator, though still further north. In the state of Roraima, Brazil (latitude around 2°), Marsaro et al. (2010) reported the higher abundance of larvae of *C. includens* in the month of June. Together, these reports highlight the phenological plasticity of this pest species, whose population variation is extremely adapted to the seasonal conditions of Earth's hemispheres.

Circular statistical analyses already demonstrated that different taxa of the Brazilian Savanna, including plants, present high mean vectors and concentration values (Pinheiro et al., 2002; Silva et al., 2011; Pilon et al., 2015; Velasque and Del-Claro, 2016). This means that this ecosystem is broadly characterized by plant species with strong phenological patterns, such as observed in *C. includens*. Vector length of reproductive phenology of plants varies from 0.101 to 0.919 (Velasque and Del-Claro, 2016), 0.07 to 0.81 (Morellato et al., 2000), and 0.08 to 0.59 (Pilon et al., 2015). The values obtained for *C. includens* (0.93 to 0.96) indicate sharper population peaks in a shorter period when adults are present. Although these values are unknown for the majority of pest-species, the population dynamics already described for a few of them suggest smoother phenology patterns (Tarragó et al., 1977; Moraes et al., 1991).

The attempt to correlate different annual crop cycles with the abundance of *C. includens* allows for the verification of different aspects of these relationships. Although it can feed on more than 175 species of plants, including soybean, corn, cotton, wheat, bean and tobacco (Specht et al., 2015), the population peaks of *C. includens* in the Brazilian Savanna are clearly related to soybean availability, as mentioned elsewhere (e.g. Herzog, 1980; Moraes et al., 1991; Moscardi et al., 2012; Specht et al., 2015). Likewise, other insect pests with preference for soybean present similar population peaks, as in *R. nu* (Guenée, 1852) and *Anticarsia gemmatilis* Hübner, 1818, *Eushistus heros* (Fabricius, 1794), *Piezodorus guildinii* (Westwood, 1837), *Nezara viridula* (Linnaeus, 1758) and *Cerotoma arcuata tingomariana* (Bechyné, 1951) (Tarragó et al., 1977; Heineck-Leonel and Corseuil, 1997; Marsaro et al., 2010). The use of wheat as an intercropping species did not influence *C. includens* abundances, although it could have a fundamental role in allowing the maintenance of *C. includens* populations during the dry season, even in low numbers. This population dynamic is particularly

important to insect pest management since it has been hypothesized that the populations of *C. includens* benefit from crop rotation, especially in irrigated crops, to remain throughout the year (Fritz et al., 2008). In Brazil, there has been an increase in the use of wheat as an alternative for the production of grains and intercropping (Franchini et al., 2011). Cotton and bean, on the other hand, are examples of preferred hostplants of *C. includens*, which are also used in intercropping with soybean. The use of these species could therefore reveal different population dynamics than observed in the present study, but more study is necessary to clarify this inference.

Despite the importance of crop cycles, weather factors can also be determinant in regulating the abundance of pest species (Tarragó et al., 1977). In ecosystems marked by a dry and rainy season, such as the Brazilian Savanna (Silva et al., 2014), it is expected that water availability factors play a more important role driving species abundances (Kishimoto-Yamada and Itioka, 2015). Moreover, insects often present richer fauna during the rainy season (Pinheiro et al., 2002; Silva et al., 2011). However, neither water availability (measured by humidity and precipitation) nor temperature were correlated to the abundances of *C. includens*. This lack of response is probably because their high abundance peaks concentrated during a narrow temporal distribution. Therefore, there are long periods in the year marked of high variability of weather conditions, when population abundance is zero or varies slightly above it.

Weather variation was predictive of *C. includens* population variations when measured as an inter-annual factor (e.g. effect of El-Niño). Among years sampled, the El Niño effect was considered “weak to moderate” in 2014/2015, but “very strong” during 2015/2016, affecting specially the rainfall and temperature levels in the southern hemisphere (Null, 2016). This clearly resulted on the decrease of *C. includens* monthly abundances. Thus, considering that both excess and lack of rain are known to raise larval mortality, especially in the first instars (Zalucki et al., 2002), supra-annual phenomena such as the El Niño can cause substantial variations in species populations (Rouault et al., 2006; Kishimoto-Yamada and Itioka, 2015), including those with economic importance. Such phenomena should not be omitted in studies that aim to predict the factors responsible for the population dynamics of pests. With this additional information, agricultural techniques will be able to estimate more precisely how pest populations should behave during unexpected weather oscillations.

Other factors responsible for population variations of pest species, such as natural enemies and agricultural management techniques (Lima et al., 2009), could not be estimated in the current study. However, the populations of *C. includens* are known to be negatively affected by pathogens, parasitoids and predators (Harding, 1976; Maruyama et al., 2001; Sosa-Gómez et al., 2003), as well as positively by management techniques, such as the use of pesticides which directly affect these natural enemies (Ferron, 1978; Sosa-Gómez et al., 2003). Studies including this kind of information are required, especially in the case of *C. includens*, where a considerable fraction of its population variation is still unexplained by weather and crop cycle factors.

### Conflicts of interest

The authors declare no conflicts of interest.

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