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Article

Leaf Consumption and Preference to *Conyza* sp., Conventional and Bt Soybean by *Helicoverpa armigera*

Consumo Foliar e Preferência Alimentar de ***Conyza*** *sp., Soja Convencional e Bt por* ***Helicoverpa armigera***

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ABSTRACT - *Helicoverpa armigera* caterpillars are polyphagous and exhibit high migratory potential. Given the traits of this pest, alternative hosts located in or near the crop stand may support the survival and permanence of *H. armigera* in the field. The aim of this study was to investigate *Conyza* sp. as a food source for *H. armigera* caterpillars in scenarios with conventional and Bt soybean. Two experiments were conducted in biochemical oxygen demand incubators. The first assessed consumption of conventional soybean, Bt soybean and *Conyza* sp. leaves by 3rd and 5th instar caterpillars, with no choice given (no-choice test). The second test evaluated the food preference of 3rd instar *H. armigera* larvae, with two choices given (dual-choice test). Fifth instar larvae favored *Conyza* sp. leaves to Bt soybean, but preferred conventional soybean to both of these options. The survival of 3rd instar caterpillars did not differ statistically regardless of the plant species used as a food source. Fifth instar larvae fed Bt soybean exhibited lower survival rates when compared to *Conyza* sp. and conventional soybean. In dual-choice test arenas, soybean leaf consumption was superior to *Conyza* sp. Conventional soybean consumption was also greater when offered in conjunction with Bt soybean. Caterpillars offered *Conyza* sp. and Bt soybean leaves only consumed the weed species. *Conyza* sp. plants can serve as a food source for *H. armigera* larvae, which showed a preference for *Conyza* sp. leaves to Bt soybean.

Keywords: weed, *Glycine max*, alternative host.

RESUMO - Lagartas de Helicoverpa armigera apresentam alta capacidade de migração e hábito alimentar polífago. Devido às características dessa praga, hospedeiros alternativos localizados nas proximidades ou interior da lavoura podem dar suporte para sua sobrevivência. O objetivo deste trabalho foi investigar a possibilidade de plantas de buva serem alimento para lagartas de ***H. armigera*** *em cenários com soja convencional e Bt. Foram conduzidos dois experimentos em câmaras do tipo biochemical oxygen demand. O primeiro avaliou o consumo foliar de soja convencional, soja Bt e buva, sem chance de escolha, de lagartas de 3º e 5º ínstares. No segundo, avaliou-se a preferência de alimentação de lagartas* ***H. armigera*** *de 3º ínstar, com dupla chance de escolha. Quando avaliado o consumo foliar de lagartas de 5º ínstar, observou-se preferência por buva em relação à soja Bt, porém ambas inferiores à soja convencional. A sobrevivência de lagartas de 3º ínstar não diferiu estatisticamente, independentemente da espécie fornecida como alimento. Lagartas de 5º ínstar alimentadas com soja Bt apresentaram menor sobrevivência em comparação com buva e soja convencional. Em arenas com*

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dupla chance de escolha, o consumo foliar de soja foi superior ao da buva. O consumo de soja convencional foi superior também quando ofertada em conjunto com a soja Bt. A oferta de buva e soja Bt apresentou consumo foliar apenas na espécie daninha. Plantas de buva podem servir de alimento para lagartas de ***H. armigera****. As lagartas têm preferência por consumir folhas de buva, em comparação à soja Bt.*

Palavras-chave: planta daninha, *Glycine max*, hospedeiro alternativo.

INTRODUCTION

Large populations of *Helicoverpa armigera* (cotton bollworm) were first recorded in Brazil during the 2012/13 growing season (Czepak et al., 2013), causing economic losses, particularly in soybean, maize and cotton crops (Embrapa, 2016). *H. armigera* caterpillars exhibit high migratory potential (Nibouche et al., 1998) and are polyphagous, which favors their success in different crops (Fitt, 1989). The *H. armigera* can feed on approximately 180 different plant species distributed among 45 families, including Asteraceae, Fabaceae, Solanaceae and Poaceae, which may be economically important or not (Pawar et al., 1986; Fitt, 1989; Pogue, 2004; Srivastava et al., 2005; Ali and Choudhury, 2009). The limited chemical control options also hamper management of the pest, since the species is resistant to 640 insecticides worldwide (Wyckhuys et al., 2013).

Given the traits of this pest, alternative hosts located in or near the crop stand may support the survival and permanence of *H. armigera* in the field. The availability of different host plants plays a vital role in population outbreaks of polyphagous insects (Singh and Parihar, 1988). In 2013 farmers and technicians found *H. armigera* caterpillars at an advanced stage of development feeding on *Conyza* sp. plants in Mato Grosso do Sul state during preparation for soybean planting. However, to date there are no scientific studies confirming that the *H. armigera* feeds on this weed species. Studies conducted in soybean growing areas found several other insect pests on *Conyza* sp. plants, including *Anticarsia gemmatalis*, *Chrysodeixis includens*, *Spodoptera frugiperda*, *Edessa meditabunda*, *Piezodorus guildinii* and *Euschistus heros* (Dalazen et al., 2016). Additionally, the trichomes present on stem surface of *Conyza* sp. serve as food for the spider mites, causal agent of citrus leprosis (*Brevipalpus phoenicis*) (Andrade et al., 2012) and dry *Conyza* sp. plants can serve as hosts for the oviposition of *Quesada gigas* (giant cicada), a coffee crop pest (Maccagnan et al., 2017).

The problem will be further exacerbated due the selection of *Conyza* sp. resistant biotypes. To date have been identified resistant biotypes to glyphosate (Lamego and Vidal, 2008), chlorimuron-ethyl herbicides (Santos et al., 2014), paraquat, atrazine, 2,4-D and saflufenacil (Heap, 2018) increasing the frequency of this weed in soybean crop fields. The difficult control of this species together with the possible indirect damages to the cultures by the interaction with *H. armigera* would exacerbates the negative effect of *Conyza* sp. in agricultural systems and is the essential need to eliminate these plants. This study aimed to investigate *Conyza* sp. leaves as a food source for *H. armigera* caterpillars and the food preference in scenarios with and without the choice of conventional and Bt soybean.

MATERIAL AND METHODS

The experiments were conducted in a laboratory, in Jan/Feb 2015. A biochemical oxygen demand (BOD) incubator was used, at a temperature of 25 ^{o}C and petri dishes as the experimental units. The caterpillars were obtained from a nursery and had been exclusively fed an artificial diet adapted from Greene et al. (1976), without anti-contaminants. The soybean leaves were removed from the upper third of plants grown in a greenhouse. *Conyza* sp. leaves were collected from random plants found in the field.

No-choice leaf consumption test

Two no-choice experiments were carried out to assess leaf consumption by *Helicoverpa armigera* caterpillars, one with 3rd instar and the other with 5th instar larvae. In both cases the caterpillars were individually submitted to diets of *Conyza* sp., conventional soybean (non-Bt) or Bt soybean (BRS 1001IPRO with *cry1Ac* protein). A completely randomized design was used, with 30 repetitions. Each repetition was consisted by one caterpillar.

One caterpillar and one leaf segment were allocated to each dish, with 56.71 cm^2, according to the respective treatments The caterpillars were released in the center of the plate, and they were feeding artificial diet before infestation. The filter paper used as substrate was moistened with 2 mL of distilled water. Third instar larvae were offered a 6.17 cm^2 leaf area of conventional soybean, 6.17 cm^2 of Bt soybean or 1.43 cm^2 of *Conyza* sp. over a 46 hour period and 5^{th} instar larvae 7.71 cm^2 of conventional soybean, 7.71 cm^2 of Bt soybean or 5.37 cm^2 of *Conyza* sp. over 60 hours.

Leaf consumption was assessed using a leaf area meter immediately after the end of the experiment, when the experimental units had been removed from the BOD incubator. The leaf area consumed was calculated by subtracting the final area from the initial area, considering a correction factor representing shrinkage of the leaf disks due to water loss. To estimate this factor, leaf disks were allocated to test arenas without caterpillars and the initial and final areas were measured to calculate reduction not related to the effect of larvae. After the end of the experiment caterpillars' survival were evaluated. Leaf consumption and caterpillars' survival data were submitted to analysis of variance, transformed when necessary ($\sqrt{x+1}$), and in the event of significance analyzed by Tukey's test ($p \leq 0.05$). The preference index (PI) was calculated according to Kogan and Goeden (1970), using the following formula: $PI = 2A / (M + A)$, where: PI = preference index; A = consumption of the test plant; and M = consumption of the standard plant. The results were interpreted based on the PI value obtained, as follows: PI > 1, the insect preferred the test plant to the standard plant; PI = 1, similar preference for the test and standard plant; PI < 1, the standard plant was preferred to the test plant.

Dual-choice food preference test

Food preference was evaluated using 3^{rd} instar *Helicoverpa armigera* caterpillars in each petri dish (56.71 cm^2). Two food choices were offered, in three possible combinations (*Conyza* sp./ Conventional soybean; *Conyza* sp./Bt soybean; Conventional soybean/Bt soybean). A completely randomized design was applied, with 30 repetitions. Each repetition was consisted by three caterpillars, that were released in the center of the plate, and they were feeding artificial diet before infestation. A 1.30 cm^2 leaf disk of each species was used, in addition to filter paper moistened with 900 µL of distilled water as substrate. The dishes were then incubated in a BOD incubator at 25 °C for 24 hours.

The leaf consumption of each plant species was analyzed after 24 hours, as well as the position of the caterpillars at assessment. The leaf area was adjusted using a correction factor representing the effect not related to ingestion by the caterpillars, as described for the previous experiment. Data on leaf consumption and caterpillar position were submitted to analysis of variance and transformed when necessary ($\sqrt{x+1}$). In the event of significance, data were analyzed by Tukey's test ($p \leq 0.05$). The preference indices were calculated in line with Kogan and Goeden (1970).

RESULTS AND DISCUSSION

In no-choice test arenas, leaf ingestion by 3^{rd} and 5^{th} instar caterpillars was greater for conventional soybean when compared to Bt soybean and *Conyza* sp. (Table 1). Average consumption of conventional soybean in the 3^{rd} instar was 5.014 cm^2 in 46 hours, and 6.821 cm^2 after 60 hours in the 5^{th} instar. Bt soybean ingestion was higher (4.756 cm^2) than *Conyza* sp. (0.010 cm^2) for caterpillars in the earlier instar, whereas 5^{th} instar larvae

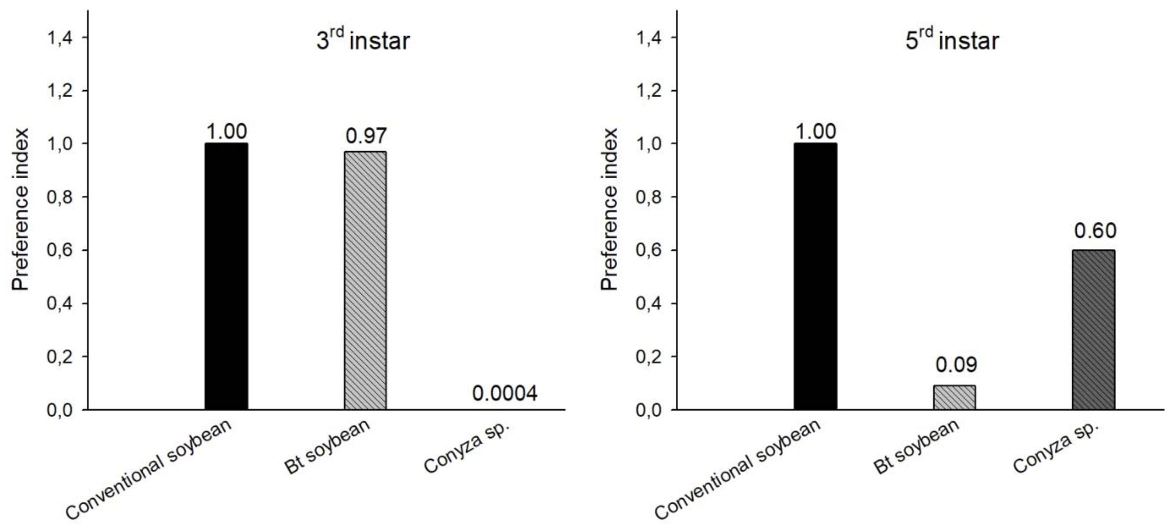
Table 1 - Consumption of *Conyza* sp., conventional and Bt soybean leaves (cm^2) by 3^{rd} and 5^{th} instar *Helicoverpa armigera* caterpillars in no-choice test arenas

Host	Instars	
	3^{rd} instar	5^{th} instar
Conyza sp.	0.010 C	2.900 B
Conventional soybean	5.014 A	6.821 A
Bt soybean	4.756 B	0.322 C
Mean	3.26	3.348
CV (%)	5.84	29.39

Means followed by the same uppercase letter in the column do not differ statistically, according to Tukey's test at 5% probability.

consumed more *Conyza* sp. than Bt soybean, with values of 2.900 and 0.322 cm², respectively.

Third instar *H. armigera* caterpillars showed a similar preference for conventional and Bt soybean, whereas preference for *Conyza* sp. was lower (Figure 1). Conventional soybean was considered standard food of *H. armigera*, with a PI of 1.0, while the respective PIs for Bt soybean and *Conyza* sp. were 0.97 and 0.0004, respectively. For 5th instar caterpillars, an increase in PI was observed for *Conyza* sp. in relation to Bt soybean, although both were lower than that of conventional soybean (standard). The PI of *Conyza* sp. (0.60) increased 1500 times when compared to 3rd instar larvae, but declined approximately 11 fold for Bt soybean, with a PI of 0.09.



Where: PI > 1: the insect preferred the test plant to the standard plant; PI = 1: similar preference for the test and standard plant; PI < 1: the standard plant was preferred to the test plant.

Figure 1 - Preference index (PI) of 3rd and 5th instar *Helicoverpa armigera* caterpillars in independent no-choice test arenas. The conventional soybean treatment was considered the standard.

The survival of 3rd instar caterpillars did not differ statistically, regardless of the plant species used as food source (Table 2). However 5th instar larvae fed Bt soybean exhibited lower survival rates when compared to those fed *Conyza* sp. and conventional soybean. In other studies, the survival of *H. armigera* caterpillars was also affected in accordance with the food offered and was greater when canola and maize were used, similarly to soybean. However, caterpillars fed white oats exhibited lower survival than that observed for soybean and none of those fed ryegrass survived (Suzana et al., 2015).

The leaf consumption of 3rd instar caterpillars with a dual choice after 24 hours corroborates the data already presented. In dual-choice test arenas containing conventional soybean and *Conyza* sp. leaf disks, soybean ingestion was 22 times higher, with average consumption of 0.891 cm² (Table 3). Conventional soybean ingestion was also greater when offered in conjunction with Bt soybean, which showed no decline in leaf area. Caterpillars offered *Conyza* sp. and Bt soybean leaves only consumed the weed specie.

Analysis of the physical position of the caterpillars after 24 hours demonstrated that most were on conventional soybean as opposed to *Conyza* sp. leaves at the moment of assessment. However, the number of caterpillars on conventional and Bt soybean leaf segments did not differ statistically, whereas comparison between Bt soybean and *Conyza* sp. indicated fewer larvae on the weed species.

Table 2 - Survival (%) of *H. armigera* caterpillars fed *Conyza* sp., conventional or Bt soybean leaves in no-choice test arenas

Species	Instars	
	3rd instar	5th instar
Conyza sp.	83.33 A	93.33 A
Conventional soybean	96.67 A	90.00 A
Bt soybean	80.00 A	20.00 B
Mean	86.67	67.78
CV (%)	43.13	

Means followed by the same uppercase letter in the column do not differ statistically, according to Tukey's test at 5% probability.

Table 3 - Preferred leaf consumption and position on leaf disks of *Helicoverpa armigera* caterpillars in dual-choice test arenas, offered combinations of *Conyza* sp., conventional and Bt soybean

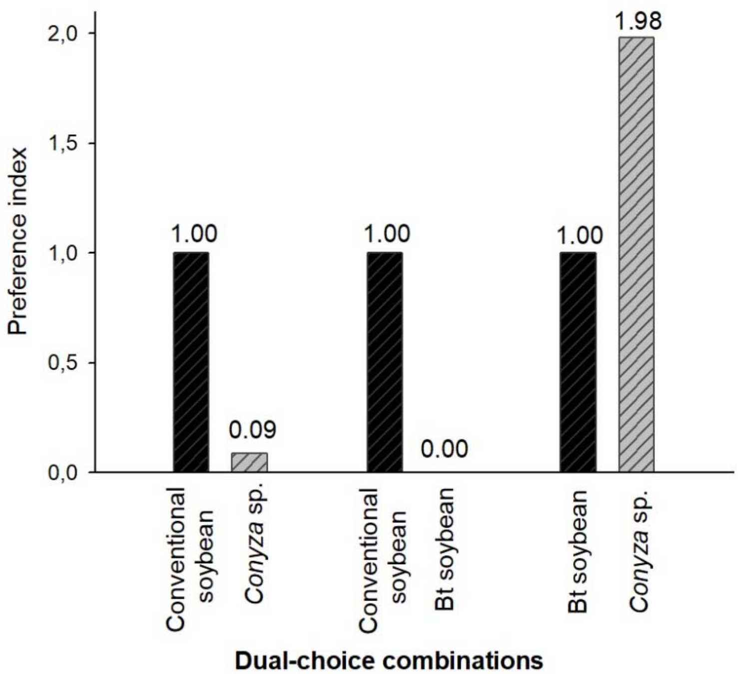
Preference – leaf consumption (cm^2)					
Conyza sp. x Conv. soybean		*Conyza* sp. x Bt soybean		Conv. soybean x Bt soybean	
Conyza sp.	0.040 B	*Conyza* sp.	0.027 A	Conventional soybean	0.103 A
Conventional soybean	0.891 A	Bt soybean	0.000 B	Bt soybean	0.000 B
Mean	0.466		0.014		0.052
CV (%)	6.86		2.42		6.10
Preference – position (unit)					
Conyza sp.	0.13 B	Bt soybean	0.7 B	Bt soybean	0.27 B
Conventional soybean	1.43 A	*Conyza* sp.	0.2 C	Conventional soybean	0.50 B
Paper	1.43 A	Paper	2.1 A	Paper	2.17 A
CV (%)	17.05		18.12		15.89

Means followed by the same uppercase letters in the column do not differ statistically according to Tukey's test at 5% probability. The data were transformed (√(x+1)) to comply with ANOVA assumptions.

The PI, calculated based on dual-choice leaf ingestion, demonstrates that conventional soybean is preferred by 3rd instar caterpillars when compared to *Conyza* sp. and Bt soybean (Figure 2). However, when the choice was between Bt soybean and *Conyza* sp., the PI was approximately double for the weed species. Bt soybean was considered the standard food (PI = 1.0) in comparisons with *Conyza* sp., which displayed a PI of 1.98.

The data presented indicate that conventional soybean is the preferred food of *H. armigera* caterpillars. However, *Conyza* sp. is preferred when compared to Bt soybean. These data suggest that in Bt soybean fields, and possibly conventional soybean during leaf senescence, *H. armigera* caterpillars could migrate to *Conyza* sp. plants that have survived pesticides control in search of food.

The results obtained on *Conyza* sp. leaf consumption demonstrate its potential as a resource for *H. armigera* in the absence of a preferred food source, with the risk of serving as a green



Where: PI> 1: the insect preferred the test plant to the standard plant; PI = 1: similar preference for the test and standard plant; PI< 1: the standard plant was preferred to the test plant.

Figure 2 - Preference index (PI) of 3rd instar *Helicoverpa armigera* caterpillars in dual-choice tests. Conventional soybean (standard) x *Conyza* sp.; Conventional soybean (standard) x Bt soybean; Bt soybean (standard) x *Conyza* sp.

bridge between crops. The weed species is preferred to Bt soybean by early instar caterpillars. This is important because Bt genetic modification technology is more efficient against larvae in the early stages of development, albeit in the form of species suppression. However, late-stage larvae showed a preference for *Conyza* sp.

Weed control becomes even more important with the prospect of serving as a possible host for pests. Other studies have found that soybean crops lacking proper weed control provide favorable conditions for *Anticarsia gemmatalis*, *Chrisodeixis includens* and *Spodoptera eridanea* (Stecca, 2011). Research on insect pests in *Conyza* sp. plants demonstrated peak *A. gemmatalis, S. frugiperda* and *Helicoverpa gelotopoeon* occurrence during the V0-V2 stages of soybean, indicating the role of the weed as a food source for early generations of lepidopteran species (Dalazen et al., 2016). Following crop maturity and harvesting, the authors observed another peak in the presence of the pests on *Conyza* sp. plants, indicating that the caterpillars migrated from soybean plants to the weeds after senescence (Dalazen et al., 2016).

The insects have a detection mechanism of volatile compounds through neural receptors, from which they are able to detect host plants for feeding and oviposition (Cui et al., 2018). In *Helicoverpa armigera* were identified 60 olfactory receptors, 19 ionotropic receptors, 34 olfactory binding proteins, 18 chemosensory proteins and 2 neuron-sensory membrane proteins (Zhang et al., 2015). In addition, *H. armigera* previous feeding experiences can positively influence future food preference (Hu et al., 2018). The knowledge of the nutritional needs of pests contributes to understanding their population dynamics and potential for damage (Slanky Junior and Wheeler, 1992; Parra, 1991; Cohen, 2004). The amount and quality of the food consumed determines the nutritional conditions for their growth and proliferation (Ruan and Wu, 2001; Barton Browne and Raubenheimer, 2003). In this respect, studies on the biology of *H. armigera* feeding *Conyza* sp. are needed to confirm successful completion of the pest's biological cycle. Different food sources affect the development of *H. armigera* larvae (Suzana et al., 2015), since the antibiosis mechanisms present in plants can directly affect their survival, size and weight (Bernays, 1998; Sarfraz et al., 2006). Host plant consumption as a food source is a significant factor that also influences pre-adult development time and length of the larval period of *H. armigera* (Razmjou et al., 2014). Cotton and soybean resulted in greater growth stage variability and higher survival rates among adult females during the reproductive period, while maize and wheat prompted high mortality rates in larvae and pupae, as well as low biotic potential (Gomes et al., 2017).

The presence of *Conyza* sp. plants in crop stands may support the survival and permanence of *H. armigera* populations in the field. Thus, precautions must be taken during the off season, since these weeds can become alternative hosts for the pest, facilitating access to successive crops. Weeds must be eliminated during this period to prevent this species from serving as a green bridge and providing caterpillars with a continuous food source. Weed management in crops is important not only to reduce competition for resources, but also because weed species can serve as hosts for insects (Dalazen et al., 2016) and nematodes (Kaspary et al., 2017).

Conventional soybean is the preferred food of *Helicoverpa armigera*, although *Conyza* sp. plants also serve as a food source. Caterpillars showed a preference for *Conyza* sp. leaves and higher survival rates when compared to Bt soybean, indicating that *H. armigera* in Bt soybean crops could seek shelter on *Conyza* sp. plants in search of food. As such, weed management is important not only to eliminate resource competition with the crop, but also to insect pests management. Monitoring pests in weeds may be useful to planning strategies to control *H. armigera* in timely.

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