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Antixenosis and antibiosis to *Spodoptera frugiperda* (Lepidoptera Noctuidae) in sunflower genotypes

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Abstract

Sunflowers are grown in various countries throughout the world and used for cooking oil, biodiesel, animal feed and as ornamental plants. *Spodoptera frugiperda* (JE Smith) is a polyphagous insect species that is native to tropical America and that has spread rapidly around the world. The purpose of the current study was to evaluate resistance of sunflower genotypes to *S. frugiperda* . This was accomplished by assessing antibiosis and antixenosis in 25 sunflower genotypes. Were evaluated: free and no-choice attractiveness test, larval stage: period and viability of the larval stage and weight of caterpillars at ten days of age; pre-pupa stage: period and viability; pupal stage: period, weight at 24 hours of age and viability; complete cycle: period and viability; adult stage: daily longevity assessments and sex ratio; nutritional parameters The BRS 55 and BRS 64 genotypes showed antixenosis resistance in choice and no-choice tests. Biological and nutritional measures demonstrated that the BRS 323, BRS 56, BRS 65 genotypes were unfavorable to *S. frugiperda* development. These genotypes showed moderate resistance and could be used by sunflower growers as a way of controling *S. frugiperda*.

Keywords: Fall armyworm, *Helianthus annuus*, plant resistance to insects.
Abbreviations: AD_approximate digestibility; DCE_Digested food conversion efficiency; ICE_ ingested food conversion efficiency; MC_metabolic cost; PI_Preference Index; PVC_polyvinyl chloride**;** RCR_relative consumption rate; RGR_relative growth rate; UPGMA _Hierarchical Cluster Analysis.

Introduction

The sunflower (*Helianthus annuus*) is an important crop that is grown in most countries, covers 27.3 million hectares and yields 56.07 million tons per year (FAOSTAT, 2021). The planted area in Brazil has been increasing, primarily as a second crop, and is used for cooking oil, biodiesel, animal feed and as ornamental plants (Person, 2012).

In Brazil, sunflowers are grown between the growing seasons of major crops such as soybeans and corn and are affected by some of the same pests as these crops (Lazzarotto et al., 2005). The fall armyworm, *Spodoptera frugiperda* (JE Smith), is a prolific species that is native to tropical regions of the Americas, but that has spread rapidly to various African countries (Goergen et al., 2016), India (Mallapur et al., 2018) and China (Jing et al., 2020).

In Brazil, this species is an important pest in corn and other crops such as soybeans, cotton, rice, and sorghum (Carvalho et al., 2013). Sunflower crops may become an important host for *S. frugiperda* given the size of the planted area and polyphagous feeding habits of the pest (Gual, 2020).

Spodoptera frugiperda is usually controlled by synthetic pesticides which, if used indiscriminately, can lead to the selection of resistant individuals (Neri et al., 2005). Tactics compatible with Integrated Pest Management have been used to reduce the use of synthetic insecticides and keep pest populations below economic damage levels. Insect resistant plants are promising in this regard (Moreira et al., 2018). In addition, there are currently no synthetic insecticides registered in Brazil for the control of *S. frugiperda* in sunflowers (AGROFIT, 2021). Management programs for this pest are also lacking for this crop.

Plant resistance to insects is an important component of integrated pest management and is compatible with other control tactics such as chemical and biological controls (Boiça Júnior et al., 2015). Plant resistance to insects can be classified into three categories. The first of these is antixenosis, which is associated with aspects of plant morphology, such as the number of trichomes and leaf color, and chemical constituents, such as the volatile compounds that interfere with insect behaviors regarding oviposition, feeding and shelter (Smith, 2005; Quairoz et al., 2020). The second category is antibiosis, which is caused by plant chemical constituents and affects aspects of insect biology and physiology by reducing larval and pupal weights, prolonging life cycles and changing sex ratios, among others (Almeida et al., 2017, Paiva et al., 2018). The third category is tolerance, which is related to a plant's ability to resist, or recover from damage caused by an insect without affecting crop yields (Baldin et al., 2019, Almeida et al., 2021).

The CF 101 genotype showed the lowest leaf consumption and provided unfavorable conditions for the development of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). However, there are few studies that examine sources of

resistance to *S. frugiperda* in sunflowers. Thus, the aim of the current study was to evaluate antixenosis and antibiosis resistance to *S. frugiperda* in sunflower genotypes.

Results and Discussion

Antixenosis

The various sunflower genotypes influenced the attractiveness of *S. frugiperda* larvae in both the free-choice and no-choice tests (Table 1). The most attractive genotypes in the free-choice test were BRS 46, BRS 54, BRS 56, BRS 63, BRS 67, and BRS 323. The attractiveness indices of the BRS 46, BRS 54, BRS 56, BRS 63, BRS 67, BRS 72 and BRS 323 genotypes were classified as stimulants.

The genotypes that most attracted *S. frugiperda* in the no-choice test were BRS 46, BRS 53, BRS 54, BRS 55, BRS 56, BRS 62, BRS 63, BRS 65, BRS 67, BRS 68, BRS 69, BRS 70, BRS 71, BRS 72, Hélio 251 and SYN 045. The attractiveness index from the no-choice test classified the BRS 53, BRS 54, BRS 56, BRS 62, BRS 65, BRS 67, BRS 68, BRS 69, BRS 7, BRS 72, Hélio 251 genotypes as stimulants.

Insect antixenosis involves a complex of interactions that make plants less preferred by insects for food, oviposition or shelter (Ta-Liao and Chen, 2017). These preferences can be explained by factors such as plant volatiles (Mitchell et al., 2016) and physical barriers such as trichome number (Queiroz et al., 2020) and epidermal thickness and hardness (Silva et al., 2014). In the current study, BRS 64 and BRS 66 were classified as deterrent since they showed antixenosis, which was indicated by the lowest attractiveness indices in both the free-choice and no-choice tests.

Antibiosis

The larval period, larval viability, prepupal period, prepupal viability, pupal period and pupal viability were statistically influenced by the sunflower genotypes (Table 2). The larval period was longest in larvae fed on BRS 46, BRS 65, and BRS 70 genotypes, and shortest when fed BRS 323. The genotypes BRS 46, BRS 53, BRS 54, BRS 56, BRS 64, BRS 65, BRS 66, BRS 67, BRS 68, BRS 69, BRS 70, Hélio 251, SYN 045 showed the highest larval viability while BRS 55, BRS 57, BRS 62, BRS 63, BRS 71, BRS 72, BRS 323, Aguara 06 showed the lowest.

The pre-pupal period was longest in BRS 46, BRS 54, BRS 55, BRS 56, BRS 62, BRS 71, BRS 72, Aguara 06, Hélio 251 and shortest in BRS 53, BRS 57, BRS 62, BRS 63, BRS 64, BRS 65, BRS 66, BRS 67, BRS 69, BRS 70, BRS 323 and SYN 045. Prepupal viability was highest in BRS 46, BRS 53, BRS 54, BRS 56, BRS 64, BRS 65, BRS 66, BRS 67, BRS 68, BRS 69, BRS 70 and Hélio 251 and lowest in BRS 55, BRS 57, BRS 62, BRS 63, BRS 71, BRS 72, BRS 323, Aguara 06 and SYN 045.

The BRS 53, BRS 54, BRS 57, BRS 66, BRS 70, BRS 72, BRS 323, Aguara 06, Hélio 251, SYN 045 genotypes had the longest pupal periods while BRS 64 and BRS 65 had the shortest. Pupal viability was highest in the BRS 54, BRS 62, BRS 64 and BRS 65, BRS 68, BRS 69, BRS 70, BRS 71, BRS 323, Hélio 251 genotypes and lowest in BRS 46, BRS 53, BRS 55, BRS genotypes 56, BRS 57, BRS 63, BRS 66, BRS 67, BRS 72, Aguara 06 and SYN 045.

Larval weight, longevity, and the period and viability of the complete cycle were statistically influenced by the genotypes (Table 3). The sunflower genotypes did not have a significant effect on pupal weight and sex ratio. The larvae that fed on BRS 72 weighed more than those fed on all other genotypes. Adult longevity was greater in insects fed on BRS 67, BRS 68, BRS 70 and BRS 71 than those fed on the remaining genotypes.

The complete cycle was longest in insects fed on BRS 46, BRS 53, BRS 54, BRS 55, BRS 57, BRS 62, BRS 63, BRS 64, BRS 65, BRS 70, Hélio 251, Aguara 06, SYN 045, and shortest in those fed on BRS 56, BRS 66, BRS 67, BRS 68, BRS 69, BRS 71, BRS 72, BRS 323. The BRS 46, BRS 53, BRS 63, BRS 64, BRS 65, BRS 67, BRS 68, BRS 71, Aguara 06, Hélio 251, SYN 045 genotypes showed the highest total insect cycle viability, while BRS 54, BRS 55, BRS 56, BRS 57, BRS 62, BRS 66, BRS 69, BRS 70, BRS 72, BRS 323 were associated with the lowest.

The sunflower genotypes in this study negatively affected *S. frugiperda* biology by prolonging development and reducing insect viability and weight. Interference that affects an insect's biological cycle suggests inadequate nutrition, which results from ingesting chemical compounds from the food substrate that provides the plant with resistance to the insect (Silva et al., 2017). Truzi et al., 2017 found that *Helicoverpa armigera* (Lepidoptera: Noctuidae) showed low survival and low consumption rates when feeding on the CF101 sunflower genotype.

Nutritional Parameters

Consumption and relative growth rate (RGR) were influenced by the sunflower genotypes, while weight gain and the relative consumption rate (RCR) were not (Table 4). The digested food conversion efficiency (DCE), ingested food conversion efficiency (ICE), approximate digestibility (AD) and metabolic cost (MC) of the *S. frugiperda* caterpillars were influenced by the sunflower genotypes (Table 5). DCE was highest in caterpillars fed on BRS 46, BRS 323 and lowest in BRS 55, BRS 56, BRS 57, BRS 62, BRS 64, BRS 65, BRS 66, BRS 67, BRS 68, BRS 70, BRS 71, BRS 72 and Hélio 251. In general, low food consumption decreases the size and weight of insects and prolongs their life cycles (Hemati et al., 2012).

Caterpillars fed on genotypes BRS 54, BRS 55, BRS 56, BRS 57, BRS 63, BRS 64, BRS 65, BRS 66, BRS 69, BRS 70, BRS 71, BRS 72, BRS 323 and Hélio 251 had the highest AD while caterpillars that fed on genotypes BRS 46, BRS 53, BRS 62, BRS 67, BRS 68, Aguara 06 and SYN 045 had the lowest AD. Only the BRS 323 genotype showed lower MC than the remaining sunflower plants. Low DCE, ICE and AD values suggest that the *S. frugiperda* larvae spent more time feeding on these genotypes with suboptimal nutrition (Ramalho et al., 2011). The effect of inadequate nutrition on insect development may be caused by plant allelochemicals or associations between nutrients and these allelochemicals (Nogueira et al., 2019).

The sunflower genotypes were grouped according to resistance levels by UPGMA (Euclidean Distance) analysis (Figure 1). Group I consisted of highly susceptible genotypes: BRS 68, BRS 55, BRS 63, BRS 46, BRS 57, BRS 66, Hélio 251, BRS 72, BRS 53, BRS 54, Aguara 06 and SYN 045; groups II (BRS 67) and III (BRS 71, BRS 62, BRS 69, BRS 64, BRS 70) clustered susceptible genotypes and groups IV (BRS 323 and BRS 56) and V (BRS 65) consisted of genotypes with moderate resistance to *S. frugiperda*.

Multivariate analysis (UPGMA) separated the sunflower cultivars into different levels of resistance to *S. frugiperda*. This type of analysis can be used to complement univariate methods in selecting insect resistant plants (PITTA et al. 2010).

Table 1. Attractiveness (±SE) and attractiveness indexes of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to sunflower genotypes.

Genotypes	Attractiveness		Attractiveness index	
	Free-choice	No-choice	Free-choice	No-choice
BRS 46	0.50±0.07 a	0.46±0.11 a	1.14	0.81
BRS 53	0.28±0.03 b	0.90±0.10 a	0,85	1.15
BRS 54	0.70±0.10 a	0.68±0.06 a	1.30	1.01
BRS 55	0.18±0.02 b	0.34±0.06 a	0.64	0.67
BRS 56	0.45±0.15 a	0.69±0.07 a	1.08	1.01
BRS 57	0.26±0.03 b	0.32±0.03 b	0.81	0.65
BRS 62	0.31±0.04 b	0.75±0.09 a	0.90	1.06
BRS 63	0.54±0.13 a	0.66±0.02 a	1.17	0.99
BRS 64	0.23±0.06 b	0.12±0.02 c	0.75	0.30
BRS 65	0.25±0.04 b	0.76±0.09 a	0.79	1.06
BRS 66	0.33±0.05 b	0.09±0.03 c	0.93	0.24
BRS 67	0.72±0.10 a	0.69±0.08 a	1.31	1.01
BRS 68	0.32±0.08 b	0.89±0.06 a	0.91	1.14
BRS 69	0.20±0.04 b	0.80±0.04 a	0.69	1.09
BRS 70	0.25±0.05 b	0.60±0.05 a	0.79	0.99
BRS 71	0.23±0.06 b	0.91±0.11 a	0.75	1.15
BRS 72	0.42±0.05 b	0.93±0.09 a	1.05	1.16
BRS 323	0.61±0.08 a	0.37±0.02 b	1.23	0.71
Aguara 06	0.38±004 b	0.32±0.05 b	1.00	0.65
Hélio 251	0.31±0.05 b	0.76±0.09 a	0.90	1.06
SYN 045	0.26±0.03 b	0.67±0.02 a	0.81	1.00
F	2.69	10.30	-	-
p-value	0.0001	<0.0001	-	-

Means followed by the same letter within a column do not differ significantly from each other by the Tukey test at 5% probability.

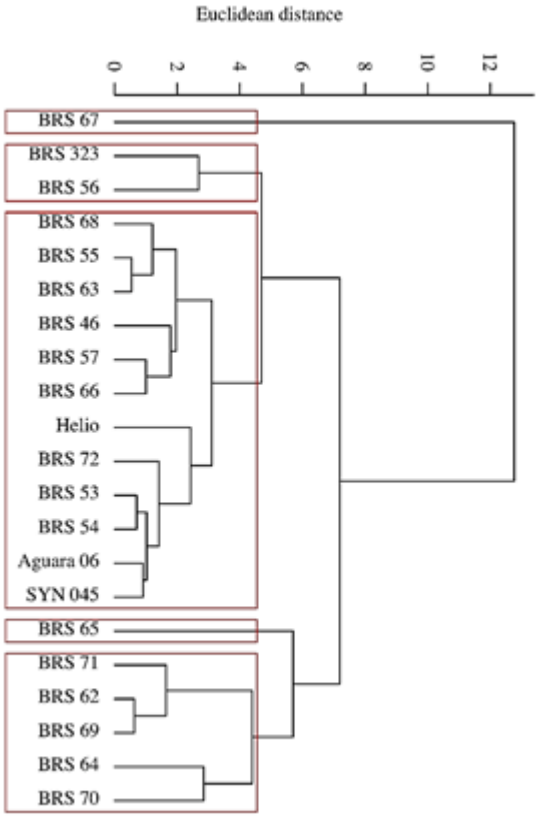

Figure 1. UPGMA dendrogram of sunflower cultivars based on biological variables of *S. frugiperda* caterpillars.

Table 2. Length of larval, prepupal and pupal periods (days) (± EPM) and viability (%) of S. *frugiperda* fed on leaves of sunflower cultivars/genotypes.

Cultivars	Larval stage		Pre-pupal stage		Pupal stage	
	Period	Viability	Period	Viability	Period	Viability
BRS 46	24.07±0.44a	90.00±4.63 a	2.07±0.15 a	90.00±3.33 a	10.38±0.34 b	43.33±9.26 b
BRS 53	23.37±0.73b	90.66±7.85 a	1.72±0.13 b	96.66±6.92 a	10.86±0.45 a	50.00±9.10 b
BRS 54	24.25±0.26b	93.33±3.33 a	2.03±0.16 a	93.33±6.31 a	10.68±0.33 a	63.33±8.95 a
BRS 55	23.60±0.56b	76.66±3.33 b	2.04±0.15 a	76.66±3.33 b	10.36±0.38 b	33.33±8.51 b
BRS 56	22.86±0.42b	96.66±6.92 a	2.00±0.12 a	96.66±7.85 a	10.20±0.51 b	50.00±9.26 b
BRS 57	21.47±0.41b	76.66±3.33 b	1.73±0.13 b	76.66±4.63 b	11.33±0.40 a	40.00±6.92 b
BRS 62	20.76±0.53b	86.66±7.85 b	1.44±0.15 b	83.3 3±3.33 b	10.19±0.39 b	70.00±9.26 a
BRS 63	21.13±0.51b	76.66±5.57 b	1.45±0.15 b	73.33±5.57 b	10.28±0.59 b	46.66±9.28 b
BRS 64	21.75±0.39b	96.66±0.00 a	1.65±0.17 b	96.66±7.43 a	9.33±0.39 c	70.00±9.26 a
BRS 65	24.59±0.38a	90.00±7.85 a	1.70±0.14 b	90.00±4.63 a	8.44±0.35 c	83.33±5.57 a
BRS 66	21.14±0.51b	90.00±6.31 a	1.74±0.14 b	90.00±7.85 a	11.60±0.38 a	50.00±8.75 b
BRS 67	20.58±0.36b	96.66±0.00 a	1.75±0.17 b	96.66±3.22 a	10.06±0.50 b	53.33±8.51 b
BRS 68	22.00±0.48b	96.66±5.57 a	2.03±0.19 a	93.33±5.57 a	9.72±0.45 b	83.33±8.95 a
BRS 69	20.92±0.53b	90.00±3.33 a	1.85±0.16 b	90.00±6.92 a	10.38±0.45 b	70.00±8.75 a
BRS 70	27.62±0.00a	100.0±0.00 a	1.83±0.19 b	100.0±0.00 a	10.96±0.54 a	90.00±8.75 a
BRS 71	21.48±0.35b	83.33±3.33 b	2.08±0.21 a	83.33±8.21 b	10.31±0.40 b	63.33±9.28 a
BRS 72	23.25±0.67b	76.66±5.57 b	2.21±0.13 a	76.66±5.57 b	11.14±0.36 a	46.55±6.92 b
BRS 323	19.34±0.32c	86.66±7.85 b	1.42±0.17 b	86.66±3.33 b	11.95±0.36 a	66.66±9.28 a
Aguara 06	22.37±0.37b	83.33±5.57 b	2.41±0.15 a	80.00±5.57 b	11.25±0.36 a	53.33±8.75 b
Hélio 251	21.44±0.44b	100.0±0.00 a	2.10±0.16 a	100.0±0.00 a	11.80±0.22 a	66.66±9.20 a
SYN 045	23.40±0.48b	100.0±6.92 a	1.76±0.10 b	86.66±7.85 b	11.40±0.51 a	50.00±9.28 b
F	9.80	2.27	2.70	2.06	4.86	3.05
p-valor	$2.20e^{-16**}$	0.0010^{**}	$1.02e^{-10**}$	0.0040^{**}	$1.65e^{-10**}$	$1.01e^{-05**}$

Means followed by the same letter within a column do not differ significantly from each other by the Tukey test at 5% probability.

Table 3. Larval and pupal weights (mg), longevity (days), period (days) and viability (%) of the total cycle and sex ratio of *S. frugiperda* fed on leaves of sunflower cultivars.

Genotypes	Weight		Longevity	Total Cycle		Sex Ratio
	Larval	Pupal		Period	Viability	
BRS 46	0.23±0.44 b	0.21±0.21	3.50±0.50b	36.75±1.74a	13.33±6.31a	0.69±0.90
BRS 53	0.27±0.46 b	0.16±0.20	3.40±0.64b	38.80±1.26a	16.66±6.92a	0.73±0.11
BRS 54	0.31±0.28 b	0.20±0.63	3.00±0.00b	39.00±2.77a	6.66±4.63b	0.42±0.10
BRS 55	0.23±0.41 b	0.22±0.19	3.50±0.50b	38.50±1.50a	6.66±6.92b	0.50±0.14
BRS 56	0.28±0.31 b	0.22±0.20	3.00±0.00b	35.50±1.50b	6.66±6.92b	0.66±0.12
BRS 57	0.40±0.24 b	0.23±0.21	3.50±0.50b	38.50±1.90a	6.66±3.33b	0.33±0.11
BRS 62	0.39±0.28 b	0.63±0.20	3.50±0.50b	38.00±2.56a	6.66±6.31b	0.57±0.12
BRS 63	0.34±0.23 b	0.21±0.21	3.80±0.26b	38.60±1.39a	16.66±8.75a	0.57±0.17
BRS 64	0.30±0.41 b	0.19±0.20	3.50±0.66b	39.50±1.94a	13.33±6.92a	0.47±0.14
BRS 65	0.45±0.44 b	0.20±0.21	3.00±0.33b	39.40±1.23a	16.66±7.85a	0.52±0.13
BRS 66	0.42±0.26 b	0.21±0.20	4.00±0.22b	36.00±1.49b	3.33±6.31b	0.46±0.10
BRS 67	0.42±0.33 b	0.22±0.21	5.66±0.33a	37.66±2.12b	13.33±4.63a	0.68±0.12
BRS 68	0.43±1.05 b	0.21±0.19	5.00±0.73a	37.50±2.40b	13.33±4.63a	0.64±0.13
BRS 69	0.40±0.27 b	0.20±0.23	2.00±0.20b	33.15±2.51b	6.00±6.31b	0.61±0.12
BRS 70	0.43±0.31 b	0.21±0.17	5.00±1.00a	38.50±0.63a	6.66±4.63b	0.66±0.14
BRS 71	0.30±0.34 b	0.19±0.22	4.80±0.60a	37.40±2.53b	16.66±6.92a	0.52±0.11
BRS 72	0.27±0.35 a	0.18±0.22	2.00±0.16b	33.60±1.93b	6.00±6.31b	0.42±0.12
BRS 323	0.35±0.32 b	0.19±0.20	3.66±0.28b	36.66±2.04b	10.00±4.63b	0.65±0.11
Aguara 06	0.32±0.39 b	0.20±0.22	3.60±0.24b	39.40±1.13a	16.66±0.00a	0.62±0.13
Hélio 251	0.26±0.43 b	0.19±0.22	3.60±0.00b	39.20±2.14a	33.33±5.57a	0.45±0.13
SYN 045	0.26±0.42 b	0.20±0.24	2.85±0.91b	39.57±2.49a	23.33±4.63a	0.46±0.11
F	2.32	0.82	2.05	2.55	1.83	0.77
p-valor	$9.64e^{-4**}$	0.682^{NS}	0.02*	$2.3e^{-04}$	0.014^{*}	0.74^{NS}

Means followed by the same letter within a column do not differ significantly from each other by the Tukey test at 5% probability.

Table 4. Consumption, weight gain, relative growth rate (RGR), and relative consumption rate (RCR) of *S. frugiperda* catepillars fed on sunflower genotypes, Urutai, Goias, Brazil, 2020.

Genotypes	Consumption	Weight gain	RGR	RCR
BRS 46	2.00±0.64b	1.66±0.3	0.458±0.05b	0.051±0.01
BRS 53	2.32±0.40b	1.80±0.3	0.539±0.03b	0.045±0.01
BRS 54	2.82±0.43b	2.80±0.5	0.620±0.09b	0.065±0.02
BRS 55	1.75±0.46b	1.22±0.3	0.355±0.06b	0.024±0.01
BRS 56	2.44±0.30b	1.40±0.2	0.613±0.08b	0.031±0.01
BRS 57	2.45±0.26b	1.50±0.4	0.541±0.07b	0.031±0.02
BRS 62	2.07±0.34b	1.11±0.2	0.391±0.08b	0.019±0.01
BRS 63	2.10±0.60b	2.12±0.5	0.437±0.05b	0.044±0.02
BRS 64	4.66±0.42a	1.85±0.3	0.990±0.07b	0.030±0.01
BRS 65	4.27±0.15a	2.50±0.4	0.823±0.09b	0.045±0.02
BRS 66	2.35±0.31b	1.11±0.2	0.530±0.05b	0.026±0.01
BRS 67	2.84±0.36b	1.33±0.3	0.535±0.02b	0.019±0.01
BRS 68	4.13±0.28a	1.82±0.4	0.955±0.10b	0.035±0.02
BRS 69	2.12±0.45b	1.85±0.1	0.475±0.08b	0.037±0.01
BRS 70	4.17±0.37b	2.74±0.5	1.952±0.24a	0.082±0.02
BRS 71	2.11±0.32b	6.56±0.7	0.551±0.06b	0.072±0.02
BRS 72	1.40±0.31b	5.00±0.3	0.199±0.04b	0.047±0.01
BRS 323	2.25±0.32b	3.00±0.4	0.418±0.07b	0.052±0.02
Aguara 06	3.09±0.40b	1.40±0.8	0.391±0.02b	0.046±0.01
Helio 251	2.28±0.34b	1.75±0.2	0.682±0.04b	0.041±0.02
SYN 045	1.70±3.96b	2.50±0.4	0.368±0.04b	0.06±0.01
F	4.06	1.14	4.99	1.32
p-valor	<0.0001	0.3092	<0.0001	0.1736

Means followed by the same letter within a column do not differ significantly from each other by the Tukey test at 5% probability.

Table 5. Digested food conversion efficiency (DCE), ingested food conversion efficiency (ICE), approximate digestibility (AD) and metabolic cost (MC) of *S. frugiperda* caterpillars fed on sunflower genotypes.

Genotypes	DCE	ICE	AD	MC
BRS 46	30.53±4.60 a	9.99±3.20b	34.81±5.5b	69.47±2.17a
BRS 53	17.29±3.18 b	7.51±2.70b	46.36±3.14b	82.71±3.14a
BRS 54	17.84±4.79 b	10.32±1.20b	59.33±5.10a	82.15±2.82a
BRS 55	12.90±3.81 c	7.58±1.36b	64.17±6.12a	87.09±4.07a
BRS 56	16.12±1.83 c	6.04±2.03b	60.08±7.60a	83.87±4.68a
BRS 57	8.26±2.15 c	7.26±0.99b	89.29±6.60a	91.74±5.13a
BRS 62	14.78±2.07 c	5.47±0.94b	41.84±5.10b	85.22±3.84a
BRS 63	17.95±3.27 b	11.36±1.36b	67.19±4.33a	82.05±2.57a
BRS 64	11.48±1.82 c	5.42±0.99b	62.53±6.20a	88.55±2.98a
BRS 65	8.72±1.57 c	5.82±1.26b	72.85±8.10a	91.27±6.18a
BRS 66	13.72±2.58 c	6.04±2.03b	63.66±5.35a	86.28±5.13a
BRS 67	10.84±2.82 c	4.36±3.22b	46.80±6.70b	89.15±4.74a
BRS 68	10.72±3.12 c	4.06±2.70b	47.55±5.30b	89.28±2.84a
BRS 69	17.84±2.82 b	10.67±3.90b	67.65±7.30a	82.15±2.83a
BRS 70	6.08±2.17 c	4.23±0.49b	70.30±5.24a	93.91±2.58a
BRS 71	3.98±1.12 c	31.02±4.10a	68.12±5.30a	96.02±6.18a
BRS 72	3.83±1.17 c	19.69±3.90a	67.14±4.56a	96.17±7.12a
BRS 323	43.30±4.50 a	15.37±2.90a	70.31±8.30a	56.70±1.08b
Aguara 06	22.17±3.60 b	14.12±2.70a	42.71±3.30b	77.82±2.57a
Helio 251	10.31±2.68 c	8.87±3.02b	85.08±8.01a	89.68±2.83a
SYN 045	21.30±3.27 b	16.86±2.03a	37.74±6.16b	96.72±7.14a
F	6.18	2.42	4.54	2.56
p	<0.0001	0.0010	<0.0001	0.0006

Means followed by the same letter within a column do not differ significantly from each other by the Tukey test at 5% probability.

Table 6. Sunflower genotypes selected for resistance to *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae).

Cultivar	Company	Cultivar	Company
BRS 46	Embrapa	BRS 67	Embrapa
BRS 53	Embrapa	BRS 68	Embrapa
BRS 54	Embrapa	BRS 69	Embrapa
BRS 55	Embrapa	BRS 70	Embrapa
BRS 56	Embrapa	BRS 71	Embrapa
BRS 57	Embrapa	BRS 72	Embrapa
BRS 62	Embrapa	BRS 323	Embrapa
BRS 63	Embrapa	Aguara 06	Atlântica sementes
BRS 64	Embrapa	Hélio 251	Heliagro
BRS 65	Embrapa	SYN 045	Syngenta
BRS 66	Embrapa		

Materials and Methods

The experiment was carried out at the Agricultural Entomology Laboratory of the Instituto Federal Goiano Campus Urutaí, Goiais, Brazil under controlled conditions: temperature (25°C±3), photoperiod (12 h) and humidity (70%±10). The sunflower genotypes were selected from materials in the commercial release stage (Table 6).

Plant production

Five seeds from each cultivar were sown in pots (5 liters) containing soil, sand and organic matter (3:1:1) and grown in a greenhouse under natural light and temperature and irrigated daily. Each pot was thinned to two plants at 30 days after emergence. The plants were fertilized according to crop recommendations (Sousa and Lobato, 2004), and no phytosanitary products were applied.

Spodoptera frugiperda production

Moth pairs were placed in polyvinyl chloride (PVC) tubes: diameter (10 cm), length (21.5cm). These cages were lined internally with sheets of bond paper that served as substrate for oviposition and closed with voile material. Cotton pads, soaked in a 10% honey solution, were kept in the cages to feed the moths. These pads were changed every two days. The eggs were removed daily and placed in 100 mL plastic containers containing 5g of an artificial diet (Greene et al, 1976). Upon reaching the 3rd instar, the caterpillars were individually separated into plastic pots containing an artificial diet, and remained there until reaching the pupa stage, giving rise to a new generation.

Antixenosis

The free choice attractiveness test was carried out on 3rd instar caterpillars and when the sunflowers were at 30 days post emergence. Leaves were removed from the plants and cut into discs (2.5 cm diameter). One disk from each cultivar was place on moistened filter paper and distributed in a circular pattern within arenas (14 cm diameter). The experiment was set up in randomized blocks, with 21 treatments (cultivars) and 10 replications. Forty-two caterpillars were released (2 caterpillars per genotype) in the center of each arena and then the arenas were sealed with plastic film. The number of caterpillars feeding on each cultivar was recorded at 3, 5, 10, 15, 30 minutes and 1, 2, 3, 5, 12 and 24 hours after the release of the caterpillars.

The no-choice attractiveness test was carried out by offering the same genotypes individually. The leaves for this test were collected and processed in the same way as in the free choice test, and then placed individually on moistened filter paper in separate petri dishes (6 cm diameter). This trial was set up in a completely randomized design with 10 repetitions. The evaluation times were the same as those used in the previous test.

At the end of the trials, a Preference Index (PI) for *S. frugiperda* was calculated according to Kogan and Goeden (1970). The SYN 045 (no choice test) and Aguara 06 (no choice test) genotypes were used as reference standards since they are commercially available cultivars. The index was calculated as: PI = 2G/G+P, where G is the number of insects in a given genotype and P is the number of insects in the reference standard genotype. According to the methodology, PI = 1 indicates similar attraction between the evaluated genotype and the susceptible standard (neutral), PI< 1 indicates less attraction for the evaluated genotype (deterrent) and IP > 1 indicates greater attraction for the evaluated genotype (stimulant).

Antibiosis

First-instar caterpillars were placed in closed plastic containers (100 mL) (27.5 cm x 20 cm) (CM & CM Comercio de Plásticos, Pinheiros, SP, Brazil) containing moistened filter paper. The *S. frugiperda* larvae were fed leaf sections from each sunflower genotype. The leaves were replaced every two days until the end of the larval stage. The emerged adults were separated individually into cages without food to measure longevity.

The following biological parameters were evaluated: a) larval stage: period and viability of the larval stage and weight of caterpillars at ten days of age; b) pre-pupa stage: period and viability; c) pupal stage: period, weight at 24 hours of age and viability; d) complete cycle: period and viability; e) adult stage: daily longevity assessments and sex ratio. A completely randomized design was used with 21 treatments (genotypes) and 30 repetitions.

Nutritional Parameters

The *S. frugiperda* caterpillars were fed an artificial diet until the 3rd larval instar. Upon reaching the 3rd instar, 10 insects were weighed on an analytical scale (Marter, Santa Rita do Sapucaí, MG, Brazil) to obtain initial weights. Afterwards, the larvae were individualized in Petri dishes and kept in an acclimatized room (temperature 25±2°C, relative humidity 60±10% and photo phase 14 hours) for each respective treatment.

The food provided was weighed daily and any remaining food and feces were removed and stored at – 20ºC. After 5 days, the caterpillars were weighed, euthanized by freezing and then dried in an oven (Nova Etica, Vargem Grande Paulista, SP, Brazil), with the remaining food, at 70ºC for 48h, until reaching constant weight. The feces were kept at room temperature and weighed after 15 days.

The following variables were evaluated: initial weight of 3rd instar caterpillar (g), final caterpillar weight (g), weight of food provided (g), fecal weight (g) and feeding time (days). The fresh and dry weights of an aliquot of five caterpillars were recorded to obtain a correction factor for the initial dry weight, which was calculated as the average dry weight divided by the average fresh weight and then multiplied by all initial fresh weights of the caterpillars used in the test (PARRA, 1991).

The methodology proposed by Waldbauer (1968) and modified by Scriber and Slansky Junior (1981) was adopted to determine the quantitative nutrition indices of the larval stage. The following parameters were used to calculate these indices: T: duration of the feeding period (days); Af: weight of food supplied to the insect (g); Ar: weight of the leftover food supplied to the insect (g), after T; F: weight of feces produced (g) during T; B: larval weight gain (g) during T; B : average larval weight (g) during T; I: ingested food weight (g) during T; I - F: assimilated food (g) during T; M = (I - F) - B: food metabolized during the feeding period.

Indices of food consumption were determined by the following formulas: Relative consumption rate (RCR = I/ $\overline{B}$ x T), relative metabolic rate (RMR = M/ $\overline{B}$ x T), relative growth rate (RGR = B/ $\overline{B}$ x T), approximate digestibility (AD = ((I - F) / I) x 100), ingested food conversion efficiency (ICE = (B/ I) x 100), digested food conversion efficiency (DCE = (B/ (I - F)) x 100), metabolic cost (MC = 100 – DCE), and consumption index (CI = I / $\overline{B}$). A completely randomized design was used with 21 treatments and 30 repetitions, in which each caterpillar was considered a repetition.

Statistical analysis

Residual normality and homoscedasticity were checked using the Shapiro-Wilk and Bartlett tests. When the assumptions were not met, the means were transformed by $(X + 0.5)^{1/2}$. Analysis of variance (ANOVA) was performed using the F test, and the means were compared using the Scott-Knott test ($\alpha = 0.05$). Cluster analysis (Hierarchical Cluster Analysis – UPGMA), based on the Mahalanobis distance (R Core Team, 2017 – Biotools package), was used to determine the degree of resistance of the genotypes. All statistical analysis was performed using R software, version 3.6.0 (R Core Team-www.r-project.org).

Conclusions

The BRS 55 and BRS 64 genotypes showed antixenosis and were classified as repellent in the free-choice and no-choice tests while BRS 323, BRS 56, BRS 65 showed antibiosis and were unfavorable to the development of *S. frugiperda*. These genotypes showed moderate resistance and can be used by sunflower growers as a control strategy for *S. frugiperda*.

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