## Antixenosis and antibiosis characterization on soybean cultivars of *Anticarsia* gemmatalis (Lepidoptera: Erebidae)

Fabrício de Lima<sup>1</sup><sup>®</sup> Otacílio Divino Rezende Neto<sup>1</sup><sup>®</sup> Frederico Landim Teixeira<sup>2</sup><sup>®</sup> Márcio da Silva Araújo<sup>2</sup><sup>®</sup> André Cirilo de Sousa Almeida<sup>1</sup><sup>®</sup> Edson Hirose<sup>3</sup><sup>®</sup> Flávio Gonçalves de Jesus<sup>1\*</sup><sup>®</sup>

<sup>1</sup>Instituto Federal Goiano (IFGoiano), 75790-000, Urutaí, GO, Brasil. E-mail: flavio.jesus@ifgoiano.edu.br.\*Corresponding author. <sup>2</sup>Universidade Estadual de Goiás (UEG), Ipameri, GO, Brasil. <sup>3</sup>Embrapa Soja, Londrina, PR, Brasil.

ABSTRACT: Anticarsia gemmatalis (Lepidoptera: Erebidae) is distributed in tropical and subtropical regions of America, and is an important pest of Fabaceae, such as: soybean, peanut, common bean, cowpea, pea, chickpea and kudzu. In soybean, the velvet bean caterpillar is important due to the plant defoliation in their larval stage. This study evaluated soybean cultivars as source of resistance to *A. gemmatalis* by antixenosis (attractiveness and non-preference for feeding) and antibiosis (biological parameters of the insect). The parameters evaluated were: antixenosis: attractiveness and non-preference, dry mass consumed and attractiveness index and antibiosis: duration of larval and pupal stages, adult longevity, total cycle, larval and pupal weights and larval, pupal and total viability. Considering antixenosis and antibiosis the least suitable cultivars for *A. gemmatalis* were found to be BRS 8383 IPRO, BRS 1074 IPRO, BRS 1061 IPRO, BRS 7180 IPRO, BRS 9383 IPRO, BRS 8980 IPRO and BRS 1003 IPRO due to high mortality in the larval phase. The cultivars BRS 523 and BRS 543 RR "block technology" suggest displays antixenosis and or antibiosis to *A. gemmatalis*. These cultivars can be used by soybean producers in combination with other control tactics in soybean IPM.

Key words: Glycine max, Erebidae, Host plant resistance, block technology, IPM.

## Caracterização da Antixenose e antibiose em cultivares de soja a Anticarsia gemmatalis (Lepidoptera: Erebidae)

**RESUMO**: *Anticarsia gemmatalis* (Lepidoptera: Erebidae) está distribuída nas regiões tropicais e subtropicais da América, trata-se de importante praga de Fabaceae, como: soja, amendoim, feijão, feijão-caupi, ervilha, grão de bico e kudzu. Na soja, a lagarta-da-soja é importante devido à desfolha da planta em sua fase larval. O objetivo deste trabalho foi avaliar cultivares de soja como fonte de resistência a *A. gemmatalis* por antixenose (atratividade e não-preferência para alimentação) e antibiose (parâmetros biológicos do inseto). Os parâmetros avaliados foram: antixenose: atratividade e não-preferência, massa seca consumida e índice de atratividade e antibiose: duração dos estágios larval e pupal, longevidade de adultos, ciclo total, peso larval e pupal e viabilidade larval, pupal e total. Na antixenose e antibiose os cultivares menos favoráveis a *A. gemmatalis* foram: BRS 8383 IPRO, BRS 1074 IPRO, BRS 1061 IPRO, BRS 7180 IPRO, BRS 9383 IPRO, BRS 8980 IPRO e BRS 1003 IPRO devido à alta mortalidade larval. Os cultivares BRS 523 e BRS 543 RR "Tecnologia block" apresentaram antixenose e/ou antibiose a *A. gemmatalis*. Essas cultivares podem serem utilizadas por produtores de soja em combinação com outras táticas de controle no MIP.

Palavras-chave: Glycine max, Erebidae, Resistência da planta a insetos, tecnologia block, MIP.

### **INTRODUCTION**

The Lepidoptera are considered the most important defoliator group of pests in soybean, causing total or partial defoliation in all phenological stages of the plant, affecting grain yield (BUENO et al., 2011; FORMENTINI et al., 2015). The velvet bean caterpillar *Anticarsia gemmatalis* (Lepidoptera: Erebidae) stands out among the main soybean pests (QUEIROZ et al., 2020a; QUEIROZ et al., 2020b; ONGARATTO et al., 2021).

Damages by first instars *A. gemmatalis* has been observed by defoliation in the upper third

of the plants on young leaves. In the third instar, the caterpillars cause small perforations in the leaf and keep the veins intact. It is only from the fourth instar onwards that the caterpillar is able to completely feed on the leaf, and defoliation can reach up to 100%, causing high reductions in grain yield (BUENO et al., 2011).

The main control strategy for this pest has been the use of chemical insecticides. However, due to the large cultivated areas and the high frequency of applications, negative effects on beneficial insects, risk to applicators and the environment, selection of resistant insects and outbreaks of secondary pests are potentiated. Others strategies as the use of virus based on

Received 09.26.22 Approved 06.20.23 Returned by the author 08.16.23 CR-2022-0534.R1 Editors: Leandro Souza da Silva o Orcial Bortolotto entomopathogenic insecticides (*Baculovirus anticarsia*) and transgenic plants expressing the insecticidal gene of the *Bacillus thuringiensis* (Cry1Ac) has been widely adopted in agriculture in the soybean IPM (MURÚA et al., 2018; FIGUEIREDO et al., 2020). Although, resistance to Bt technology may arise due to excessive selection pressure, as already identified for *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize (FARIAS et al., 2014; OMOTO et al., 2015).

IPM programs require strategies that can reduce insecticide use (SOSA-GOMEZ & SILVA, 2010; BERNARDI et al., 2014; BORTOLOTTO et al., 2016). In this sense, Plant resistance to insects (PRI) is an important tactic in integrated pest management - IPM that is usually simple and inexpensive for farmers to adopt. The effects of plant resistance are often constant and cumulative, negatively affect in the biological parameters of pests, are not harmful to the environment and are usually compatible with other control tactics such as chemical and biological controls (BOIÇA JÚNIOR et al., 2015; BUENO et al., 2020).

PRI is expressed by different mechanisms: antixenosis (non-preference), antibiosis and tolerance. Antixenosis is associated with the presence of trichomes, leaf color or the presence of volatile compounds in the plant (SMITH, 2005; SEIFI et al., 2013; ALMEIDA et al., 2017a; QUEIROZ et al., 2020a). Antibiosis is manifested mainly by chemical constituents in the plant and affects the biology and/ or physiology of the insect, such as: reduction in larval and pupal weight, prolongation of the life cycle, adult deformation, alteration in sex ratio (SOUZA et al., 2014; BOIÇA JÚNIOR et al., 2015; ALMEIDA et al., 2017b; QUEIROZ et al., 2020b) and tolerance, which is the ability of the plant to resist or recover from insect damage due to the production of new vegetative or reproductive structures or improvement in the plant physiology system (SMITH, 2005; SEIFI et al., 2013; ALMEIDA et al., 2021).

Soybean genotypes have been studied as a source of resistance to different lepidopteran defoliator. SCHILIK-SOUZA et al. (2018) classified the genotypes IAC 19, IAC 18, IAC 23, L1-1-01, PI 274453, PI 229358, PI 171451, IAC 100, IAC 24, IAC 17 and IAC 74-2832 as repellent to *Chrysodeixis includens* (Lepidoptera: Noctuidae). Also, PI 171451, PI 274453, IAC 18, L1-1-01 and IAC 23 were less oviposited by *C. includens*. ONGARATTO et al. (2021) found sources of antixenosis and antibiosis to *A. gemmatalis* in genotypes: TMG 133 RR, TMG 1179 RR, IAC 19, IAC 17, IAC 100, D75-10169 and IAC 78-2318. BOIÇA JÚNIOR et al. (2015) observed susceptibility to *Spodoptera cosmioides* (Lepidoptera: Noctuidae) in genotypes Dowling, PI 274454, IGRA RA 626 RR, BRSGO 8360, IGRA RA 516 RR, P98Y11 RR, PI 227682, PI 227687, IAC 100 and BR 16. The genotypes IAC 100, PI 227682 and PI 227687 showed antixenosis to *Spodoptera cosmioides* (Lepidoptera: Noctuidae)(QUEIROZ et al., 2020a; BOIÇA JÚNIOR et al. 2015) and the genotypes PI 227687, PI 227682, IAC 100 and BRS 7270 IPRO showed significant levels of antibiosis against *S. cosmioides* (QUEIROZ et al., 2020b). The soybeans cultivars IAC 100 and M 7110 IPRO showed antixenosis and antibiosis to *Chloridea virescens* (Lepidoptera: Noctuidae) (ALMEIDA et al., 2017a; ALMEIDA et al., 2017b).

Therefore, in this study we evaluated the effect of soybean cultivars on larval attractiveness and consumption and larval performance of *A. gemmatalis* by antixenosis and antibiosis characterization.

## MATERIALS AND METHODS

Experiments were carried out at the laboratory of Integrated Pest Management of Federal Goiano Institute, Campus Urutaí, Goiás, Brazil. Insect rearing bioassays were conducted under controlled conditions of temperature (25±2 °C), relative humidity (70±10%) and photoperiod (12h).

### Anticarsia gemmatalis rearing

A insect colony was established from eggs of *A. gemmatalis*. After larvae hatching, the insects were kept in Petri dishes (15 cm in diameter and 2 cm high) in groups of 20 caterpillars and were fed on artificial diet, based on combination containing common bean, soybean protein, wheat germ, casein, yeast beer and others ingredients and raised until the pupal stage. The pupae were sexed and placed in PVC tubes (20 cm high  $\times$  20 cm in diameter) for emergence and mating.

The adults were fed on 10% honey solution that was soaked in cotton, fixed to the lid and changed every two days. The eggs were removed daily and placed in plastic containers (500 mL) until egg eclosion.

#### Plant material

The assays were performed with 20 soybean cultivars (Table 1). The seeds were sown in 5 liter pots containing a substrate (3:1:1 soil, sand, and organic compost) that was corrected and fertilized according to soybean recommendation (SOUSA & LOBATO, 2004). The plants were kept in a greenhouse free from insect infestation, under natural light and temperature conditions, and irrigation was performed daily as needed. Leaves of soybean plants at V4/V5 developmental stages were used in the experiments.

Cultivars	Maturity group	Growth habitat	Transgenic event	Plant resistance
BRS 6680	6.6	indeterminate	-	
BRS 523	6.7	indeterminate	-	ST
BRS 7980	7.0	determinate	-	
BRS 391	7.0	determinate	-	ST
BRS 7481	7.4	indeterminate	-	
BRS 511	8.5	indeterminate	-	
BRS 543 RR	6.0	indeterminate	HT	ST
BRS 7380 RR	7.3	indeterminate	HT	
BRS 7880 RR	7.8	indeterminate	HT	
BRSGO 7858 RR	7.8	indeterminate	HT	
BRS 8280 RR	8.0	determinate	HT	
BRS 8781 RR	8.7	determinate	HT	
BRS 9280 RR	9.2	determinate	HT	
BRS 1061 IPRO	6.1	indeterminate	HT + IR	
BRS 1074 IPRO	6.9	indeterminate	HT + IR	
BRS 1003 IPRO	7.0	indeterminate	HT + IR	ST
BRS 7180 IPRO	7.1	indeterminate	HT + IR	
BRS 8383 IPRO	8.0	indeterminate	HT + IR	
BRS 8980 IPRO	8.9	determinate	HT + IR	
BRS 9383 IPRO	9.3	determinate	HT + IR	

Table 1 - Name and agronomic characteristics of soybean cultivars. Urutaí, GO, Brazil.

HT – Glyphosate herbicide tolerance. IR – Bt (Cry1Ac) lepidopteran resistance. ST – Stinkbug tolerance.

Antixenosis - feeding attractiveness and nonpreference for feeding assays

In the non-choice test, one leaf disk (2.5 cm diameter) was offered to a one of 3rd instar larvae in Petri dishes (9 cm in diameter and 2 cm high). After releasing, the number of larvae on disk leaf was evaluated at 1, 3, 5, 10, 15, 30, 60, 120, 360, 720 and 1440 min. The bioassay was laid out in a completely randomized design with 20 treatments (cultivars) and 20 replications.

In the free-choice test, a leaf disc (2.5 cm diameter) of each cultivars was distributed equidistantly in a circular arena (14 cm in diameter and 2 cm high) on moistened filter paper. Then, one 3rd instar larvae per disk was released and the attractiveness was evaluated in the same time described previously. The test was laid out in a randomized block design with 20 treatments (cultivars) and ten replications (arenas).

Two symmetrically positioned leaf disks were cut from the leaves collected from each cultivar (non-choice and free-choice test), one disk was offered to a larvae and the other (aliquot), was dried in an oven at 60 °C for 48 h. Subsequently, the dry weight consumed by the larva was determined by the weight difference between the dried aliquot and the dried leftovers from the offered leaf disk. Also, the preference index (AI) was calculated according to the following formula: AI = 2C/(C+S), where C = the number of insects attracted to a given cultivar and S = the number of insects attracted to the standard susceptible cultivar (BRS 6680 – free choice test and BRS 511 non-choice test). The standard susceptible cultivar was obtained from the average number of larvae attracted to the cultivar at all assessment times.

#### Antibiosis – Biological parameters

First instar larvae were individualized into Petri dishes (9.0 cm diameter) lined with moistened filter paper and sealed with polyethylene film. Leaves from the median region of each cultivar were placed and replaced in the dishes as they were consumed (usually every 2 days). The insects were kept in these containers until adult emergence. After emergence, the adults were transferred to plastic containers (150 mL) where they remained until the end of the cycle. The following biological parameters were evaluated: (a) larval phase: period and viability of the larval stage and larval weight at 10 days old; (b) pre-pupal phase: period; (c) pupal phase: period, weight at 24 h

old and viability; (d) total cycle: period and viability; and (e) adult stage: longevity (without feed). The experiment used a completely randomized design with 20 treatments (cultivars) and 20 repetitions.

## Statistical analysis

The results were submitted to multivariate analysis of variance (MANOVA). There was a significant effect (P < 0.05) of cultivars when the means were compared by the Scott Knott test at 5% probability (R DEVELOPMENT CORE TEAM, 2017, Scott Knott Package). A Canonical Discriminant Analysis – CVA was performed to study the distance relationship between genotypes, as well as their relationship with the resistance variables (R DEVELOPMENT CORE TEAM, 2017, Candisc package) to determine the degree of resistance between the soybean cultivars to *A. gemmatalis*. A cluster analysis was performed using the Hierarchical Cluster Analysis – UPGMA method based on the Euclidian distance to group cultivars by their level of resistance (R DEVELOPMENT CORE TEAM, 2017, Biotools package).

## RESULTS

The free-choice test showed significant differences among the soybean cultivars regarding nonpreference to *A. gemmatalis* (F = 2.918; df = 19; P = 0.0004). The cultivars BRS 9280 RR, BRS 511, BRS 7880 RR, BRS 8280 RR, BRS 7980 and BRS 7481 were the most preferred and the other cultivars showed the less attractiveness to *A. gemmatalis*. The soybean cultivars BRS 511, BRS 7880 RR, BRS 7481, and BRSGO 7858 RR showed the highest dry mass consumed by *A. gemmatalis* (F = 4.52; df = 19; P = < 0.0001) and according to the preference indexes, the genotype BRS 511 (1.34; P = 0.0791) was the most preferred and BRS 1003 IPRO (0.11; P = 0.0014), BRS 8980 IPRO (0.24 p = 0.0393) and BRS 391 (0.29 P = 0.0670) were the less preferred by *A. gemmatalis* (Table 2).

 Table 2 - Attractiveness (mean±standard error), dry mass consumed (mg) and attractiveness index in free-choice test of Anticarsia gemmatalis (Lepidoptera: Erebidae) in soybean cultivars. Urutaí, GO, Brazil.

Cultivars	Attractiveness	Dry mass consumed	Atractiveness Index (P value)
BRS 511	0.76±0.09 a	14.56±0.16 a	1.34 (0.0791)
BRS 9280 RR	1.05±0.04 a	6.58±0.44 b	1.34 (0.1146)
BRS 7880 RR	0.76±0.06 a	12.28±0.71 a	1.28 (0.2940)
BRS 7980	0.60±0.13 a	7.50±0.02 b	1.26 (0.1616)
BRS 8280 RR	0.67±0.18 a	7.08±3.04 b	1.26 (0.2780)
BRS 7481	0.60±0.09 a	11.64±0.44 a	1.18 (0.4730)
BRS 543 RR	0.51±0.17 b	7.24±2.00 b	1.12 (0.6338)
BRSGO 7858 RR	0.40±0.07 b	10.02±1.06 a	1.07 (0.5923)
BRS 523	0.40±0.06 b	1.52±0.44 b	1.05 (0.5602)
BRS 8383 IPRO	0.36±0.06 b	4.52±1.80 b	1.03 (0.8638)
BRS 1074 IPRO	0.34±0.04 b	1.60±0.72 b	1.01 (0.9450)
BRS 6680	0.42±0.06 b	6.00±1.80 b	1.00
BRS 7380 RR	0.34±0.02 b	2.95±0.96 b	0.96 (0.6423)
BRS 1061 IPRO	0.34±0.03 b	4.02±1.32 b	0.95 (0.6307)
BRS 7180 IPRO	0.31±0.03 b	2.00±0.63 b	0.92 (0.5355)
BRS 9383 IPRO	0.27±0.04 b	6.10±0.91 b	0.87 (0.3739)
BRS 8781 IPRO	0.22±0.03 b	2.92±1.60 b	0.65 (0.2934)
BRS 391	0.18±0.03 b	4.32±1.03 b	0.29 (0.0670)
BRS 8980 IPRO	0.11±0.02 b	3.50±1.09 b	0.24 (0.0393)
BRS 1003 IPRO	0.04±0.01 b	1.20±0.86 b	0.11 (0.0014)
F treatments	2.918	4.52	-
P value	0.0004	< 0.0001	-

\*Means followed by the same letter do not differ statistically according to the Scott-Knott test at 5% probability.

The soybean cultivars did not differ statistically regarding attractiveness in the non-choice test (F = 1.662; df = 19; P = 0.0611). The cultivars differed statistically regarding the dry mass consumed in the non-choice test (F = 10.618; df = 19; P = < 0.0001). BRS 8280 RR and BRS 391 presented the highest dry mass consumed by *A. gemmatalis* and regarding to the preference indexes, the genotype BRS 7858 RR (0.12; P = 0.0059), BRS 523 (0.02; P = < 0.001), BRS 7380 RR (0.25; P = 0.0576), BRS 1061 IPRO (0.40 P = 0.0808) and BRS 7180 IPRO (0.23 P = 0.0426) were the less preferred by *A. gemmatalis* (Table 3).

The development of *A. gemmatalis* was influenced by the soybean cultivars. The larval period was higher (F = 18.98; df = 19; P = < 0.0001) on BRS 7980, BRS 523, BRS 9280 RR, BRS 8280 RR, BRS 511 and BRS 7880 RR and shorter on BRS 6680 (Table 4). The pre-pupal period (F = 19.95; df = 19; P = < 0.0001) of *A. gemmatalis* fed on BRS 7880 RR,

BRSGO 7858 RR, BRS 543 RR, BRS 9280 RR and BRS 523 was higher. The pupal period (F = 1.23, df = 19, P = 0.2957) of *A. gemmatalis* was not influenced by the soybean cultivars. The adult longevity (F = 5.32, df = 19, P = 0.0006) was highest in all soybean cultivars, except in BRS 511, BRS 391 and BRS 8280 RR that presented the lowest value. Also, the insect from BRS 9280 RR, BRS 523, BRS 7880 RR, BRS 543 RR, BRS 7481 and BRS 511 presented the highest total cycle (F = 5.53, df = 19, P = < 0.0001).

Anticarsia gemmatalis fed on soybean cultivars BRS 511, BRS 9280 RR, BRSGO 7858 RR, BRS 523 and BRS 6680 presented the lowest larval weight (F = 8.99, df = 19, P = <0.0001) (Table 5). Also, pupae from soybean cultivar BRS 511, BRS 8280 RR and BRS 523 showed the lowest pupal weight (F = 7.03, df = 19, P = <0.0001). Larval viability (F = 1.07, df = 19, P = 0.3830), pupal viability (F = 0.99, df = 19, P = 0.4505) and total viability (F = 0.96, df

 Table 3 - Attractiveness (mean±standard error), dry mass consumed and attractiveness index in non-choice test of Anticarsia gemmatalis (Lepidoptera: Erebidae) in soybean cultivars. Urutaí, GO, Brazil.

Cultivars	Attractiveness	Dry mass consumed	Atractiveness Index (P value)
BRS 511	0.22±0.03	8.90±0.42 b	1.00
BRS 9280 RR	0.28±0.02	6.36±1.13 b	0.96 (0.9107)
BRS 7880 RR	0.24±0.02	10.22±0.68 b	0.78 (0.4747)
BRS 7980	0.14±0.06	11.68±0.28 b	0.63 (0.3895)
BRS 8280 RR	0.30±0.09	16.70±0.32 a	0.95 (0.7854)
BRS 7481	0.26±0.04	8.12±0.34 b	1.04 (0.8811)
BRS 543 RR	0.12±0.04	5.90±0.20 b	0.54 (0.1158)
BRSGO 7858 RR	0.28±0.04	12.70±0.88 b	0.87 (0.7612)
BRS 523	$0.06 \pm 0.02$	6.80±0.25 b	0.12 (0.0059)
BRS 8383 IPRO	0.01±0.01	2.52±0.69 d	0.01 (<0.001)
BRS 1074 IPRO	0.18±0.02	2.40±0.59 d	0.73 (0.5750)
BRS 6680	$0.44{\pm}0.02$	5.08±1.02 c	1.14 (0.6352)
BRS 7380 RR	0.36±0.09	6.06±1.38 b	1.23 (0.2727)
BRS 1061 IPRO	$0.04{\pm}0.02$	4.92±1.43 c	0.25 (0.0576)
BRS 7180 IPRO	0.12±0.04	5.76±1.40 c	0.39 (0.0808)
BRS 9383 IPRO	0.10±0.03	0.93±0.36 d	0.73 (0.5152)
BRS 8781 IPRO	0.16±0.04	1.18±1.99 d	0.22 (0.0426)
BRS 391	0.46±0.06	16.14±1.32 a	1.45 (0.1523)
BRS 8980 IPRO	0.30±0.06	2.22±0.90 d	1.06 (0.7889)
BRS 1003 IPRO	0.08±0.02	1.70±0.28 d	0.54 (0.2891)
F treatments	1.662	10.618	-
P value	0.0611	< 0.0001	-

\*Means followed by the same letter do not differ statistically according to the Scott-Knott test at 5% probability.

a. k *	× 1		<b>D</b> 1	¥	<b>T</b> . 1 1
Cultivars*	Larval	Pre-pupal	Pupal	Longevity	Total cycle
BRS 391	12.6±1.01 b	1.5±0.23 b	5.0±0.42	3,0±0.32 b	22.0±0.48 c
BRS 6680	11.3±0.90 c	1.7±0.20 b	5.0±0.34	4.0±0.58 a	22.0±0.58 c
BRS 8781 RR	11.7±0.90 b	1.7±0.21 b	5.7±1.04	3.0±0.33 a	22.1±1.42 c
BRS 7380 RR	11.8±0.72 b	1.7±0.17 b	5.0±0.41	3.7±0.42 a	22.2±0.48 c
BRS 8280 RR	14.3±0.30 a	1.3±0.19 b	6.0±1.28	2.9±0.38 b	24.5±1.12 b
BRS 7980	15.3±0.30 a	1.2±0.18 b	5.3±1.17	3.4±0.38 a	24.8±1.82 b
BRSGO 7858 RR	13.1±0.30 b	2.6±0.21 a	5.8±0.87	3.5±0.29 a	25.0±0.68 b
BRS 511	14.2±0.95 a	1.2±0.28 b	7.5±1.16	3.0±0.38 b	25.6±1.40 a
BRS 7481	13.0±1.23 b	1.1±0.16 b	7.2±0.68	4.3±0.28 a	25.7±0.42 a
BRS 543 RR	11.7±0.30 b	2.5±0.28 a	8.0±0.38	3.6±0.38 a	25.8±0.74 a
BRS 7880 RR	13.9±1.23 a	2.9±0.26 a	5.0±0.86	4.0±0.46 a	25.8±1.28 a
BRS 523	14.8±0.39 a	2.1±0.22 a	6.2±0.78	3.5±0.42 a	26.6±0.62 a
BRS 9280 RR	14.7±0.30 a	2.1±0.18 a	7.4±0.52	3.2±0.28 a	27.4±1.36 a
BRS 1061 IPRO	-	-	-	-	-
BRS 1074 IPRO	-	-	-	-	-
BRS 1003 IPRO	-	-	-	-	-
BRS 7180 IPRO	-	-	-	-	-
BRS 8383 IPRO	-	-	-	-	-
BRS 8980 IPRO	-	-	-	-	-
BRS 9383 IPRO	-	-	-	-	-
F treatments	18.98	19.95	1.23	5.32	5.53
P value	< 0.0001	< 0.0001	0.2957	0.0007	< 0.0001

Table 4 - Duration (mean±standard error) of the larval, pupal, longevity and total cycle (days) of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in soybean cultivars. Urutaí, GO, Brazil.

\*Means followed by the same letter do not differ statistically according to the Scott-Knott test at 5% probability.

= 19, P = 0.4888) were not statistically influenced by soybean cultivars.

By hierarchical grouping analysis -UPGMA (Euclidian distance) was observed the influence of soybean cultivars in the biological parameters of *A. gemmatalis* (Figure 1). Four groups were established according to the level of resistance. Groups I (BRS 391) and group III (BRS 6680, BRS 7380 RR, BRS 8781 RR, BRS 8280 RR, BRS 523 and BRSGO 7858 RR) presented high susceptibility to *A. gemmatalis*. Group IV (BRS 7481 and BRS 7980) and group II (BRS 511, BRS 7880 RR, BRS 543 RR and BRS 9280 RR) were moderated resistance to *A. gemmatalis*.

This was supported by the canonical variable analysis - CVA (Figure 2). Both multivariate methods provided similar results regarding the resistance grouping. The cultivars BRS 543 RR and BRS 7880 RR that appears isolated in the CVA analysis, also appears in group II in the UPGMA,

influencing the *A. gemmatalis* total cycle. The susceptible cultivar BRS 523, which appears isolated in the CVA analysis, also appears in group III in the UPGMA analysis, composed by the susceptible soybeans cultivars. The susceptible cultivars BRS 391, BRS 6680 and BRS 8781 RR appears grouped in group III in the UPGMA analysis showed the same distribution in CVA analysis.

The first canonical variable explained 54.8% of the total parameters evaluated for the characteristics described in the soybean cultivars and this component was influenced by larval period, pupal weight and larval weight. The second component explained 25.3% of the variability and was influenced by pupal period and total cycle.

#### DISCUSSION

Antixenosis is a category of resistance in which a plant is relatively less exploited by an insect

Cultivars*	Larval weight	Pupal weight	Larval viability	-Pupal viability-	Total viability
BRS 6680	0.13±0.02 c	0.17±0.02 b	48.45±2.86	38.75±2.92	15.60±2.86
BRS 523	0.13±0.02 c	0.12±0.01 c	46.80±2.80	36.67±2.82	16.67±2.80
BRS 7380 RR	0.23±0.01 a	0.15±0.01 b	42.22±3.54	32.22±1.78	16.67±2.80
BRS 511	0.13±0.02 c	0.14±0.01 c	59.41±2.05	39.41±3.08	20.60±3.64
BRS 391	0.16±0.02 b	0.17±0.02 b	43.50±3.50	36.61±2.12	20.80±3.82
BRS 543 RR	0.18±0.01 a	0.22±0.01 a	42.22±3.85	32.22±3.12	22.22±3.04
BRS 8280 RR	0.15±0.02 b	0.13±0.01 c	33.33±3.75	32.22±1.98	22.22±3.04
BRS 7880 RR	0.18±0.01 a	0.16±0.01 b	54.44±3.38	37.80±2.88	22.78±2.78
BRS 7980	0.17±0.03 b	0.16±0.01 b	60.33±4.02	37.78±2.92	24.78±3.64
BRS 8781 RR	0.17±0.03 b	0.16±0.01 b	43.33±3.52	36.38±3.12	24.78±3.64
BRS 7481	0.16±0.02 b	0.16±0.01 b	44.41±4.31	44.33±4.12	33.33±3.72
BRSGO 7858 RR	0.14±0.01 c	0.15±0.01 b	48.42±2,80	38.42±2.39	38.89±4.04
BRS 9280 RR	0.13±0.02 c	0.16±0.01 b	55.56±3.39	55.56±4.04	55.60±4.78
BRS 1061 IPRO	-	-	-	-	-
BRS 1074 IPRO	-	-	-	-	-
BRS 1003 IPRO	-	-	-	-	-
BRS 7180 IPRO	-	-	-	-	-
BRS 8383 IPRO	-	-	-	-	-
BRS 8980 IPRO	-	-	-	-	-
BRS 9383 IPRO	-	-	-	-	-
F treatments	8.99	7.03	1.07	0.99	0.96
P value	< 0.0001	< 0.0001	0.3830	0.4505	0.4888

Table 5 - Larval and pupal weight (mg) (mean±standard error) and larval, pupal and total viability (%) of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in soybean cultivars. Urutaí, GO, Brazil.

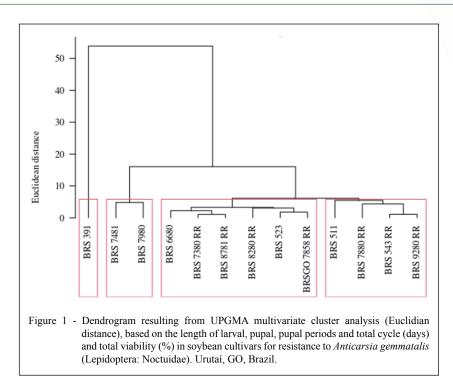
\*Means followed by the same letter do not differ statistically according to the Scott-Knott test at 5% probability.

for feeding, oviposition or shelter. This cause of resistance results from plant morphology, chemical constituents and physical characteristics (ALMEIDA et al., 2017a; QUEIROZ et al., 2020a). In general, the cultivars BRS 8980 IPRO and BRS 1003 IPRO (free-choice test) and BRSGO 7858 RR, BRS 523 and BRS 7380 RR (non-choice test) were less attractiveness, consumed and presented the less attractiveness index to *A. gemmatalis*.

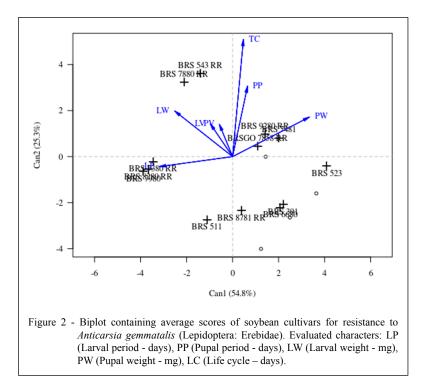
Attractiveness and non-preference for feeding are mechanism of antixenosis and are associated with physics, morphological or chemical defense in plants. The physics defenses are characterized by substrate color and affecting the feeding and oviposition insect behavior (QUEIROZ et al., 2017a; SILVA et al., 2021). The morphological defense understands the structural and morphological characteristics of the plant that confer negatively effect to insect. This attributes often are associated with epidermis surface such as: hairiness - trichome densities, waxiness, thickness, hardness and texture (SCHLICK-SOUZA et al., 2018). Also, the plant chemical constituents can be associated with repellent or deterrence to insects (HOFFMANN-CAMPO et al., 2006).

Research have been contributed to select soybean cultivars with antixenosis to soybean pests. The soybean genotypes IAC 100, PI 227682 and PI 227687 showed antixenosis to *Spodoptera cosmioides* (Lepidoptera: Noctuidae) mediated by leaf color and trichome density (QUEIROZ et al., 2017a). The cultivars IAC 100 and M 7110 IPRO (Bt) were less consumed and showed antixenosis to *C. virescens* (ALMEIDA et al., 2017b). The flavonoids genistein and rutin appear to play a role in the resistance of PI 274453, PI 274454, and IAC 100 genotypes against *Piezodozus guildinii* (Hemiptera: Pentatomidae) (BENTIVENHA et al., 2018).

Recently, the soybean cultivars with resistance (suggested tolerance) to stink bugs 'block technology' was released by the Embrapa Soybean



Research Center, Londrina, Paraná, Brazil (LUCINI et al., 2021). Results from our study demonstrated that the resistant soybean cultivars with 'block technology' BRS 543 RR and BRS 1003 IPRO suggest displays antixenosis to *A. gemmatallis*. LUCINI et al. (2021) studding the feeding behavior of *Euschistus heros* (Hemiptera: Pentatomidae) by electropenetrography (EPG), observed that adults spent significantly less



Ciência Rural, v.54, n.4, 2024.

time in feeding on resistant plants (cvs. BRS 391, BRS 543 RR and BRS 1003 IPRO – block technology) compared to the susceptible cultivar (BRS 5601 RR), showing that cultivar with block technology is not a feeding stimulant host for this stink bug. Thus, soybean cultivar with 'block technology' become an important tool in soybean IPM.

Antibiosis is the resistance that occurs when the insect feeds on the plant and negatively affecting in the insect's biology are observed. The cause of antibiosis can be chemical and/or morphological plant defensive factors (ALMEIDA et al., 2017b; QUEIROZ et al., 2020b).

The results showed that soybean cultivars influenced the biological parameters and presented different degrees of resistance to *A. gemmatalis*. Cultivars BRS 7980, BRS 523, BRS 9280 RR, BRS 8280 RR, BRS 511 and BRS 7880 RR extended the larval period of *A. gemmatalis* in relation to BRS 6680. Also, the pre-pupal period was extended in BRS 7880 RR, BRSGO 7858 RR, BRS 543 RR, BRS 9280 RR and BRS 523.

The prolonged periods of development in the larval and pupal period and total cycle and reduction of body weight are characteristics of antibiosis (SMITH, 2005; SEIFI et al., 2013). The prolongation of the larval and pre-pupal period may be associated with the presence of chemical compounds which confers antibiosis and/or antixenosis (BOIÇA JÚNIOR et al., 2015). Defenses in soybean resistant to insect that confer antibiosis results mainly from the presence of allelochemicals such as alkaloids, ketones, glucosinolates, isoflavonoids, terpenoids and organic acids (WAR et al., 2012).

The effects of antibiosis often cause high larval and pupal mortality (SMITH, 2005). A. gemmatalis fed on BRS 8383 IPRO, BRS 1074 IPRO, BRS 1061 IPRO, BRS 7180 IPRO, BRS 9383 IPRO, BRS 8980 IPRO and BRS 1003 IPRO did not reach the pupal stage. The fact that IPRO cultivars had better antibiosis results was expected due to the presence of Bt gene insertion (Bacillus thuringiensis - Bacillacea) in the plant. This gene that encodes the toxin Cry1Ac from the biopesticide B. thuringiensis (Bt), caused mortality of caterpillars in the first 2 instars, showing that A. gemmatalis is Bt-sensitive (ALMEIDA et al., 2017b). This finding characterizes antibiosis to A. gemmatalis and shown that this Bt cultivars remains an efficient tools in IPM of this primary Lepidopteran pest of soybean (MURÚA et al., 2018).

The lowest larval weight of *A. gemmatalis* was observed on BRS 511, BRS 9280 RR, BRSGO 7858 RR, BRS 523 and BRS 6680. This lower weight

may be due the presence of secondary metabolites in these cultivars. The flavonoids (rutin and genistin) were identified in soybean genotypes PI 274454, PI 227687, and IAC-100, characterizing defense in soybean by deterrence, consequently conferring resistance to insects (PIUBELLI et al., 2005). Also, the flavonoid rutin prolonged the larval period, reduced the larval and pupal weight and decreased the pupal viability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (SILVA et al., 2016).

Plant that cause the pest life cycle prolongation is desirable in plant resistance to insects, since the insect will have fewer generations, reducing population density generating a consequent reduction in the damage to agricultural crops (Smith 2005). In this sense, BRS 9280 RR, BRS 523, BRS 7880 RR, BRS 543 RR, BRS 7481 and BRS 511 prolonged the *A. gemmatalis* life cycle.

Considering antixenosis and antibiosis the least suitable cultivars for *A. gemmatalis* were found to be BRS 8383 IPRO, BRS 1074 IPRO, BRS 1061 IPRO, BRS 7180 IPRO, BRS 9383 IPRO, BRS 8980 IPRO and BRS 1003 IPRO due to high mortality in the larval phase. The cultivars BRS 523 and BRS 543 RR "block technology" suggest displays antixenosis and or antibiosis to *A. gemmatalis* and can be used by soybean producers in combination with other control tactics in soybean IPM.

#### ACKNOWLEDGMENTS

The authors recognize the Federal Goiano Institute for support and Embrapa Soja (Londrina, Paraná, Brazil) for providing the soybean cultivars. This study was partially supported by the National Council of Research and Technology of Brazil (CNPq), grant 307029/2019-0. We also thank Jeffrey Lee Wangen for English revision and valuable insights into this manuscript.

# DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

### **AUTHORS' CONTRIBUTIONS**

This research was carried out in collaboration between the authors. FGJ and FL designed and performed the trials with the help of ODR, FLT, MSA, ACSA and EH. FGJ, ACSA and EH analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

#### REFERENCES

ALMEIDA, A. C. S. A. et al. Attractiveness and non-preference of soybean cultivars to *Heliothis virescens* (Lepidoptera: Noctuidae) feeding. **Australian Journal of Crop Science**. v.11, p.453-458, 2017a. Available from: <a href="http://dx.doi.org/10.21475/">http://dx.doi.org/10.21475/</a>

ajcs.17.11.04.355>. Accessed: Feb. 15, 2022. doi: 10.21475/ ajcs.17.11.04.355.

ALMEIDA, A. C. S. A. et al. Antibiosis in soybean cultivars to *Heliothis virescens* (Lepidoptera: Noctuidae) feeding. Florida Entomologist, v.100, p.334-338. 2017b. Available from: https: <http://doi.org/10.1653/024.100.0231>. Accessed: Jan. 20, 2022. doi: 10.1653/024.100.0231.

ALMEIDA, A. C. S. et al. Evidence for rice tolerance to *Tibraca limbativentris* (Hemiptera: Pentatomidae). **Pest Management** Science, v.77, p.4181-4191, 2021. Available from: <a href="https://doi.org/10.1002/ps.6455">https://doi.org/10.1002/ps.6455</a>. Accessed: Feb. 15, 2022. doi: 10.1002/ps.6455.

BENTIVENHA, J. P. F. et al. Role of the rutin and genistein flavonoids in soybean resistance to *Piezodorus guildinii* (Hemiptera: Pentatomidae). **Arthropod-Plant Interactions**, v.12, p.311-320, 2018. Available from: <a href="https://doi.org/10.1007/s11829-017-9578-5">https://doi.org/10.1007/s11829-017-9578-5</a>. Accessed: Feb. 18, 2022. doi: 10.1007/s11829-017-9578-5.

BERNARDI, O. et al. High levels of biological activity of Cry1Ac protein expressed on MON 87701 x MON 89788 soybean against *Heliothis virescens* (Lepidoptera: Noctuidae). **Pest Management Science**, v.70, p.588-594, 2014. Available from: <a href="https://doi.org/10.1002/ps.3581">https://doi.org/10.1002/ps.3581</a>. Accessed: Feb. 22, 2022. doi: 10.1002/ps.3581.

BOIÇA JÚNIOR, A. L. et al. Determination of the resistance types to *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae) in soybean genotypes. **Semina: Ciências Agrárias**, v.36, p.607-618, 2015. Available from: <dx.doi.org/10.5433/1679-0359.2015v36n2p607>. Accessed: Feb. 22, 2022. doi: 10.5433/1679-0359.2015v36n2p607.

BORTOLOTTO, O. C. et al. Larval development of *Spodoptera* eridania and *Spodoptera frugiperda* fed on fresh ear of field corn expressing the Bt proteins (Cry1F and Cry1F + Cry1A.105 + Cry2Ab2). **Ciência Rural**, v.46, p.1898-1901, 2016. Available from: <a href="http://dx.doi.org/10.1590/0103-8478cr20151461">http://dx.doi.org/10.1590/0103-8478cr20151461</a>. Accessed: Jan. 18, 2022. doi: 10.1590/0103-8478cr20151461.

BUENO, A. F. et al. Challenges for adoption of integrated pest management (IPM): the Soybean Example. **Neotropical Entomology**, v.50, p.5-20, 2020. Available from: <a href="https://doi.org.ez369.periodicos.capes.gov.br/10.1007/s13744-020-00792-9">https://doi.org.ez369.periodicos.capes.gov.br/10.1007/s13744-020-00792-9</a>. Accessed: Apr. 28, 2022. doi: 10.1007/s13744-020-00792-9.

BUENO, R. C. O. F. et al. Lepidopteran larva consumption of soybean foliage: Basis for developing multiple- species economic thresholds for pest management decisions. **Pest Management Scince**, v.67, p.170-174, 2011. Available from: <a href="https://doi:10.1002/ps.2047">https://doi:10.1002/ps.2047</a>. Accessed: Apr. 28, 2022. doi: 10.1002/ps.2047.

FARIAS, J. R. et al. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. **Crop Protection**, v.64, p.150-158, 2014. Available from: <a href="https://doi.org/10.1016/j.cropro.2014.06.019">https://doi.org/10.1016/j.cropro.2014.06.019</a>>. Accessed: Feb. 12, 2022. doi: 10.1016/j.cropro.2014.06.019.

FIGUEIREDO, C. A. et al. Effect of the Cry1, Cry2 and Vip3 protein combinations on the control of *Anticarsia gemmatalis* (Erebidae) and *Chrysodeixis includens* (Noctuidae) Lepidoptera, **International Journal of Pest Management**, v.68, p.175-183, 2020. Available from: <a href="https://doi.org/10.1080/09670874.2020.1817617">https://doi.org/10.1080/09670874.2020.1817617</a>. Accessed: May, 28, 2022. doi: 10.1080/09670874.2020.1817617.

FORMENTINI, A. C. et al. Lepidoptera (Insecta) associated with soybean in Argentina, Brazil, Chile and Uruguay. **Ciência Rural**, v.45, p.2113-2120, 2015. Available from: <a href="http://dx.doi.org/10.1590/0103-8478cr20141258">http://dx.doi.org/10.1590/0103-8478cr20141258</a>. Accessed: Jul. 18, 2022. doi: 10.1590/0103-8478cr20141258.

HOFFMANN-CAMPO, C. B. et al. Detrimental effect of rutin on *Anticarsia gemmatalis*. **Pesquisa Agropecuária Brasileira**, v.41, p.1453-1459, 2006. Available from: <a href="https://doi.org/10.1590/S0100-204X2006001000001">https://doi.org/10.1590/S0100-204X2006001000001</a>). Accessed: May, 28, 2022. doi: 10.1590/S0100-204X2006001000001.

LUCINI, T. et al. Evaluating resistance of the soybean block technology cultivars to the Neotropical brown stink bug, *Euschistus heros* (F.). **Journal of Insect Physiology**, v.131, 104228. 2021. Available from: <a href="https://doi.org/10.1016/j.jinsphys.2021.104228">https://doi.org/10.1016/j.jinsphys.2021.104228</a>. Accessed: Jun. 18, 2022. doi: 10.1016/j.jinsphys.2021.104228.

MURÚA, M. G. et al. Defoliation of soybean expressing crylac by lepidopteran pests. **Insects**. v.9, p.93, 2018. Available from: <a href="http://dx.doi.org/doi:10.3390/insects9030093">http://dx.doi.org/doi:10.3390/insects9030093</a>. Accessed: Jun. 18, 2022. doi: 10.3390/insects9030093.

OMOTO, C. et al. Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. **Pest Management Science**, v.72, p.1727-1736, 2015. Available from: <a href="https://doi.org/10.1002/">https://doi.org/10.1002/</a> ps.4201>. Accessed: May, 18, 2022. doi: 10.1002/ps.4201.

ONGARATTO, S. Resistance of soybean genotypes to *Anticarsia gemmatalis* (Lepidoptera: Erebidae): antixenosis and antibiosis characterization. **Journal of Economic Entomology**, v.14, p.2571-2580, 2021. Available from: <a href="https://doi-org.ez369.periodicos">https://doi-org.ez369.periodicos</a>. capes.gov.br/10.1093/jee/toab197>. Accessed: May, 28, 2022. doi: 10.1093/jee/toab197.

PIUBELLI, G. C. et al. Are chemical compounds important for soybean resistance to *Anticarsia gemmatalis*? **Journal of Chemical Ecology**, v.31, p.1509-1525, 2005. Available from: <a href="http://dx.doi.org/10.1007/s10886-005-5794-z">http://dx.doi.org/10.1007/s10886-005-5794-z</a>. Accessed: Jun. 12, 2022. doi: 10.1007/s10886-005-5794-z.

QUEIROZ, E. B. et al. Antixenosis in soybean to *Spodoptera cosmioides* (Lepidoptera: Noctuidae) mediated by leaf color and trichome density. **Phytoparasitica**, v.48, p.813-821, 2020a. Available from: <<u>https://doi.org/10.1007/s12600-020-00840-5></u>. Accessed: May, 28, 2022. doi: 10.1007/s12600-020-00840-5.

QUEIROZ, E. B. et al. Antibiosis in soybean to *Spodoptera* cosmioides (Lepidoptera: Noctuidae). **Revista Brasileira de Entomologia**, v.64, e20200010, 2020b. Available from: <a href="https://doi.org/10.1590/1806-9665-RBENT-2020-0010">https://doi.org/10.1590/1806-9665-RBENT-2020-0010</a>. Accessed: May, 28, 2022. doi: 10.1590/1806-9665-RBENT-2020-0010.

R CORE TEAM. **R: the R project for statistical computing.** Version 3.3.3. R Foundation for Statistical Computing, Vienna, Austria, 2017.

SCHLICK-SOUZA, E. C. et al. Antixenosis to *Chrysodeixis includens* (Lepidoptera: Noctuidae) among soybean. **Bragantia**, v.77, p.124-133, 2018. Available from: <a href="https://doi.org/10.1590/1678-4499.2016449">https://doi.org/10.1590/1678-4499.2016449</a>. Accessed: Jan. 23, 2022. doi: 10.1590/1678-4499.2016449.

SEIFI, A. et al. How to effectively deploy plant resistances to pests and pathogens in crop breeding. **Euphytica**, v.190, p.321-334, 2013. Available from: <a href="http://dx.doi.org/10.1007/s10681-012-0823-9">http://dx.doi.org/10.1007/s10681-012-0823-9</a>. Accessed: May, 28, 2022. doi: 10.1007/s10681-012-0823-9.

SILVA, C. L. T. et al. Resistance of rice genotypes to fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). **Revista Brasileira de Entomologia**, v.65, e20210020. 2021. Available from: <a href="https://doi.org/10.1590/1806-9665-RBENT-2021-0020">https://doi.org/10.1590/1806-9665-RBENT-2021-0020</a>). Accessed: Aug. 18, 2022. doi: 10.1590/1806-9665-RBENT-2021-0020.

SILVA, T. R. F. B. et al. Effect of the flavonoid rutin on the biology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Acta Scientiarum Agronomy. v.38, n.2, p.165-170. 2016. Available from: <a href="https://doi.org/10.4025/actasciagron.v38i2.27956">https://doi.org/10.4025/actasciagron.v38i2.27956</a>>. Accessed: Feb. 18, 2022. doi: 10.4025/actasciagron.v38i2.27956.

SMITH, C. M. Plant resistance to arthropods: molecular and conventional approaches. Springer, Berlin, 423pp. 2005. Available from: <a href="http://dx.doi.org/10.1007/1-4020-3702-3">http://dx.doi.org/10.1007/1-4020-3702-3</a>. Accessed: May. 28, 2022. doi: 10.1007/1-4020-3702-3.

SOSA-GÓMEZ, D. R. SILVA, J. J. Neotropical brown stink bug (*Euschistus heros*) resistance to methamidophos in Paraná. **Pesquisa**  Agropecuária Brasileira, v.45, n.7, p.767-769. 2010. Available from: <a href="http://dx.doi.org/10.1590/S0100-204X2010000700019">http://dx.doi.org/10.1590/S0100-204X2010000700019</a>. Accessed: Jul. 12, 2022. doi: 10.1590/S0100-204X2010000700019.

SOUSA, D. M. G. LOBATO, E. Calagem e adubação para culturas anuais e semiperenes. In: SOUSA, D. M. G., LOBATO, E. (Eds), **Cerrado: correção do solo e adubação**. 2 ed. Embrapa Informação Tecnológica, Brasília, pp. 283-316. 2004.

SOUZA, B. H. S. et al. Antibiosis in soybean genotypes and the resistance levels to *Spodoptera eridania* (Cramer) (Lepidoptera: noctuidae). **Neotropical Entomology**, v.43, p.582-587, 2014. Available from: <a href="http://dx.doi.org/10.1007/s13744-014-0241-x">http://dx.doi.org/10.1007/s13744-014-0241-x</a>. Accessed: May, 28, 2022. doi: 10.1007/s13744-014-0241-x.

WAR, A. R. et al. Mechanisms of plant defense against insect herbivores. **Plant Signal Behavior**, v.7, p.1306-1320, 2012. Available from: <a href="http://dx.doi.org/10.4161/psb.21663">http://dx.doi.org/10.4161/psb.21663</a>>. Accessed: Jun. 18, 2022. doi: 10.4161/psb.21663.