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Antibiosis in soybean cultivars to *Heliothis virescens* (Lepidoptera: Noctuidae)

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Abstract

The tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), damages soybean crops by feeding on the leaves, pods, and terminal buds. The purpose of this study was to evaluate antibiosis resistance to *H. virescens* by different soybean cultivars. Soybean cultivars evaluated were P 98Y30 RR, NA 7337 RR, SYN 1163 RR, NK 7059 RR, ANTA 82 RR, M 7110 IPRO, BRS 8160 RR, BRSGO Jataí, and IAC 100. The variables analyzed were duration of the larval stage, percentage of larval survival, larval weight at 10 d, duration of the prepupal stage, percentage of prepupae survival, duration of the pupal stage, percentage of pupal survival, pupal weight at 24 h, total duration of the life cycle, overall survival, and adult longevity. The cultivars BRS 8160 RR, BRSGO Jataí, and P 98Y30 RR were highly susceptible to tobacco budworm, whereas IAC 100 and M 7110 IPRO showed antibiosis resistance to *H. virescens*.

Key Words: *Glycine max*; Heliothinae; tobacco budworm; host plant resistance

Resumo

A lagarta-da-maçã do algodoeiro *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) pode causar prejuízos na cultura da soja alimentando-se de folhas, vagens e brotos terminais. Objetiva-se com este trabalho avaliar a resistência do tipo antibiose em diferentes cultivares de soja a *H. virescens*. Foram utilizadas sementes de soja das cultivares P 98Y30 RR, NA 7337 RR, SYN 1163 RR, NK 7059 RR, ANTA 82 RR, M 7110 IPRO, BRS 8160 RR, BRSGO Jataí e IAC 100. As variáveis avaliadas foram: período, e % de sobrevivência do estágio larval e peso de lagartas aos dez dias de idade, período e sobrevivência pré-pupal, período, sobrevivência e peso pupal com 24 horas de idade, ciclo e sobrevivência total e longevidade de adultos. As cultivares BRS 8160 RR, BRSGO Jataí, P 98Y30 RR foram altamente suscetíveis e as cultivares IAC 100 e M 7110 IPRO apresentaram resistência do tipo antibiose à *H. virescens*.

Palavras Chave: *Glycine max*; Heliothinae; lagarta-da-maçã do algodoeiro; resistência de planta a insetos

Soybean is an important crop that suffers severe damage from insect pests (Silva et al. 2014). Defoliating lepidopterans are particularly important soybean pests in Brazil. Depending on the intensity of the infestation and the phenological stage of the plant, damage by lepidopteran larvae can hinder crop production (Lourenção et al. 2010; Bueno 2011; Hoffmann-Campo et al. 2012). The tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), is a particularly important lepidopteran that has become a plant health problem for soybean crops over the last few years, especially in the Cerrado region of Brazil (Tomquelski & Maruyama 2009). This pest causes damages to all stages of crop development, feeding on the leaves, the terminal buds, and the pods (Degrande & Vivan 2010).

Pest control in soybean crops is mostly performed by application of chemical insecticides. This approach carries risks to the environment and the natural enemies of pests, and favors the selection of insecticide-resistant individuals (Sosa-Gomez & Silva 2010). Therefore, seeking alternative pest control methods is important. Host plant resistance to insects is one such method, which is especially important for integrated pest management (Souza et al. 2014a,b,c). The use of resistant plant varieties can be considered an efficient alternative method to the use of insecticides for the control of agricultural pests. In addition to decreasing populations of pest insects without interfering with the environment, the effects of this method are cumulative and persistent, and the method is not a pollutant, does not increase production costs, and does not demand specialized knowledge by farmers for its use (Smith 2005; Seifi et al. 2013).

Soybean possesses defense mechanisms that include a series of morphological characteristics and a complex of chemical substances that may make it tolerant, repellent, or inadequate for the development of pest insects (Piubelli et al. 2005; Silva et al. 2013, Silva et al. 2014; Timbó et al. 2014). The most abundant defense substances in soybean are the flavonoid rutin and the isoflavonoid genistein (Hoffmann-Campo et al. 2001). These substances are present in higher concentrations in some soybean varieties, such as PI 227687 and IAC 100 (Piubelli et al. 2005).

Souza et al. (2014a) performed a study on the selection of genotypes resistant to *Spodoptera eridania* (Stoll) (Lepidoptera: Noctuidae) and observed antibiosis in the genotypes PI 227687 and PI 227682. The IAC 100 variety has been observed to exhibit resistance to *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) (Souza et al. 2014b) and

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Anticarsia gemmatalis (Hübner) (Lepidoptera: Noctuidae) (Piubelli et al. 2005). Due to the lack of information on the development of *H. virescens* on soybean crops and the limited knowledge regarding the resistance of soybean varieties to this pest, the purpose of this study was to evaluate the antibiosis to *H. virescens* in different soybean cultivars.

Materials and Methods

The experiment was performed at the Laboratory of Agricultural Entomology of the Instituto Federal Goiano, Urutaí Campus, located in Urutaí, Goiás, Brazil, under controlled conditions (25 ± 2 °C, 70 ± 10% relative humidity, and 12:12 h L:D photoperiod), using the following soybean cultivars: P 98Y30 RR, NA 7337 RR, SYN 1163 RR, NK 7059 RR, ANTA 82 RR, M 7110 IPRO, BRS 8160 RR, and BRSGO Jataí, which are the cultivars most often used by farmers in Southeast Goiás (Brazil), as well as IAC 100. M 7110 IPRO contains the gene for a toxin of *Bacillus thuringiensis* Berliner (*Bt*; Eubacteriales: Bacillaceae) and is known as *Bt* soybean. Leaves of the tested cultivars were obtained by sowing seeds in 5 L pots containing a soil:organic compost (3:1) mixture as the substrate.

REARING OF *HELIOTHIS VIRESCENS*

Caterpillars (F1 generation) were obtained from the Laboratory of Entomology of the Brazilian Agricultural Research Corporation for Rice and Beans (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA] Arroz e Feijão). Pupae of *H. virescens* were sexed and placed in polyvinyl chloride (PVC) cages (20 × 20 cm, height × diameter), where the adults emerged and mated. The adults were fed a 10% honey solution provided in soaked cotton balls placed in the lids, which were changed every 2 d. Eggs were removed from the cages daily, sterilized with 5% sodium hypochlorite solution, washed with distilled water, and placed in Petri dishes (9.0 × 1.5 cm) lined with moistened filter paper until the larvae hatched. Groups of 4 newly hatched caterpillars were placed in 150 mL plastic containers containing artificial diet (Greene et al.1976). When they reached the third instar, the caterpillars were placed singly in plastic containers with diet until they reached the pupal stage, beginning a new rearing cycle.

ASSESSMENT OF ANTIBIOSIS IN SOYBEAN GENOTYPES

Newly hatched caterpillars were placed singly in Petri dishes (9 cm in diameter, 2 cm high) containing moistened filter paper and sealed with polyethylene film. The caterpillars were fed soybean leaves, which were replaced daily or after consumption by the caterpillars. Caterpillars were kept in the Petri dishes until they reached the pupal stage. The leaf supply was terminated when the pupal stage was attained. Adults (moths) were not fed.

The following biological parameters were evaluated: (a) duration of the larval stage, % survival of larvae, larval weight at 10 d; (b) duration of the prepupal stage (shrinking of the larva), % survival of prepupae; (c) duration of the pupal stage, % survival of the pupal stage, pupal weight at 24 h; (d) total duration of the life cycle, overall survival; and (e) adult longevity. A completely randomized experimental design was used, with nine treatments (cultivars) and 50 replicates of each treatment.

STATISTICAL ANALYSES

The data were subjected to an analysis of variance (ANOVA), and the means were compared using the Scott–Knott test at 5% probability, with Sisvar 5.3 (Ferreira 2011). Also, a principal component analysis and a cluster analysis were performed, and dissimilarity was measured using Euclidean distances in Statistica 7.0 (StatSoft 2004) to identify groups of soybean cultivars with different degrees of resistance to *H. virescens*.

Results

Significant differences in the duration of the larval stage, survival of larvae, and duration of the pupal stage were observed among the *H. virescens* fed different soybean cultivars (Table 1). Duration and survival of the prepupal stage, and pupal survival, were not significantly affected by the soybean cultivars. The duration of the larval stage was highest for the caterpillars fed the cultivar IAC 100 (29.7 d) and lowest for caterpillars fed cultivar P 98Y30 RR (25.5 d). The biological parameters for caterpillars fed cultivar M 7110 IPRO could not be evaluated because all caterpillars died before the second instar.

Significant differences in larval and pupal weight, duration of the entire life cycle, and adult longevity were observed among the *H. virescens* fed different soybean cultivars (Table 2). No statistically significant differences in overall survival were observed. The highest mean larval weights were assocated with caterpillars fed the leaves of P98Y30 RR,

Table 1. Mean (± SE) duration of the larval, prepupal, and pupal stages, and survival of *Heliothis virescens* (Lepidoptera: Noctuidae) fed leaves of different soybean cultivars in Urutaí, Goiás, Brazil.

	Larval		Prepupal		Pupal	
Cultivar	Duration (d)	Survival (%)	Duration (d)	Survival (%)	Duration (d)	Survival (%)
P 98Y30 RR	25.5 ± 0.3 d	84 ± 5 a	1.8 ± 0.1	100 ± 0.00	11.6 ± 0.3 b	75 ± 10
NA 7337 RR	28.1 ± 0.3 b	76 ± 6 b	1.8 ± 0.1	100 ± 0.00	11.4 ± 0.3 b	75 ± 10
SYN 1163 RR	28.3 ± 0.5 b	68 ± 7 b	1.7 ± 0.1	100 ± 0.00	11.6 ± 0.3 b	70 ± 11
NK 7059 RR	28.3 ± 0.2 b	76 ± 6 b	1.7 ± 0.1	96 ± 3	11.5 ± 0.3 b	85 ± 8
ANTA 82 RR	28.4 ± 0.3 b	74 ± 6 b	1.7 ± 0.1	100 ± 0.00	11.4 ± 0.2 b	65 ± 11
BRS 8160 RR	26.3 ± 0.3 c	92 ± 4 a	1.6 ± 0.1	100 ± 0.00	10.7 ± 0.2 b	90 ± 7
BRSGO Jataí	27.1 ± 0.3 c	86 ± 5 a	1.6 ± 0.1	100 ± 0.00	11.2 ± 0.3 b	85 ± 8
IAC 100	29.7 ± 0.4 a	58 ± 7 b	1.6 ± 0.1	96 ± 3.0	12.9 ± 0.4 a	75 ± 10
M 7110 IPRO[a]	—	—	—	—	—	—
Statistics						
F	14.43	3.35	0.84	0.85	4.24	0.80
P	<0.0001	0.0017	0.5539	0.5412	0.0003	0.5825

Means in a column followed by the same letter are not significantly different according to the Scott–Knott test at 5% probability.
[a]Insufficient number of replications for statistical analysis.

Table 2. Mean (± SE) larval and pupal weight, duration of the total life cycle, overall survival, and adult longevity of *Heliothis virescens* (Lepidoptera: Noctuidae) fed the leaves of different soybean cultivars in Urutaí, Goiás, Brazil.

	Weight (mg)				
Cultivar	Larval	Pupal	Total life cycle (d)	Overal survival (%)	Adult longevity (d)
P 98Y30 RR	70.3 ± 3 a	172.7 ± 11 b	39.0 ± 0.5 c	78 ± 6	4.2 ± 0.3 b
NA 7337 RR	53.5 ± 3 b	160.2 ± 7 b	41.2 ± 0.6 b	66 ± 7	3.9 ± 0.2 b
SYN 1163 RR	51.8 ± 3 b	142.5 ± 5 c	41.0 ± 0.6 b	62 ± 7	3.2 ± 0.4 c
NK 7059 RR	50.6 ± 3 b	165.1 ± 8 b	41.6 ± 0.4 b	68 ± 7	3.9 ± 0.3 b
ANTA 82 RR	47.0 ± 3 b	172.7 ± 9 b	41.2 ± 0.4 b	62 ± 7	3.7 ± 0.3 b
BRS 8160 RR	77.8 ± 3 a	213.0 ± 10 a	39.0 ± 0.4 c	84 ± 5	5.1 ± 0.2 a
BRSGO Jataí	76.7 ± 4 a	181.4 ± 13 b	40.7 ± 0.3 b	72 ± 6	4.2 ± 0.2 b
IAC 100	34.9 ± 3 c	130.7 ± 4 c	44.4 ± 0.6 a	56 ± 7	2.5 ± 0.2 c
M 7110 IPRO[a]	—	—	—	—	—
Statistics					
F	24.42	7.50	11.19	1.98	7.92
P	<0.0001	<0.0001	<0.0001	0.0564	<0.0001

Means in a column followed by the same letter are not significantly different according to the Scott–Knott test at 5% probability.
[a]Insufficient number of replicates for statistical analysis.

BRS 8160 RR, and BRSGO Jataí, and the lowest mean larval weight was associated with IAC 100.

Pupal weight was highest for the caterpillars fed the cultivar BRS 8160 RR and lowest for those fed the cultivars SYN 1163 RR and IAC 100. Total life cycle duration was longest for the caterpillars fed the cultivar IAC 100 and shortest for those fed the cultivars P98Y30 RR and BRS 8160 RR. Adult longevity was greatest in the caterpillars fed the cultivar BRS 8160 RR and least in those fed the cultivars SYN 1163 RR and IAC 100.

The hierarchical cluster analysis revealed differences among the tested soybean cultivars, separating them into different groups based on the degree of similarity (Fig. 1). The cluster analysis dendrogram revealed 4 distinct groups at a Euclidean distance of 0.35. Four different groups with different levels of resistance to *H. virescens* could, therefore, be defined: susceptible soybean cultivars (P 98Y30 RR, BRSGO Jataí, and BRS 8160 RR), moderately resistant cultivars (NA 7337 RR, SYN 1163 RR, NK 7059 RR, and ANTA 82 RR), one cultivar classified as resistant (IAC 100), and one cultivar classified as highly resistant (M 7110 IPRO).

The principal component analysis revealed four groups with identical composition (Fig. 2). The cultivars P 98Y30 RR, BRSGO Jataí, and BRS 8160 RR formed the group with higher susceptibility to *H. virescens*; cultivars NA 7337 RR, SYN 1163 RR, NK 7059 RR, and ANTA 82 RR, formed the group with moderate resistance; cultivar IAC 100 was separated from the remaining cultivars and was classified as resistant; and cultivar M 7110 IPRO also was separated from the remaining cultivars and determined as having high resistance.

Discussion

Antibiosis is a type of plant resistance that can cause detrimental effects to insect herbivores such as failure to grow, higher rates of mortality, and prolongation of the life cycle (Lara 1991; Smith 2005). The

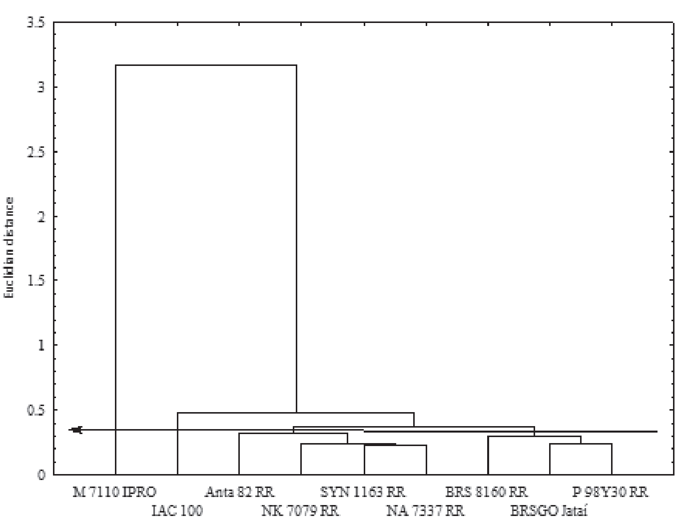

Fig. 1. Dendrogram based on the biological parameters of *Heliothis virescens* larvae fed different soybean cultivars in Urutaí, Goiás, Brazil. The hierarchical cluster analysis was performed using Ward's method with Euclidean distances as the measure of dissimilarity. The arrow indicates the distance used to separate the groups.

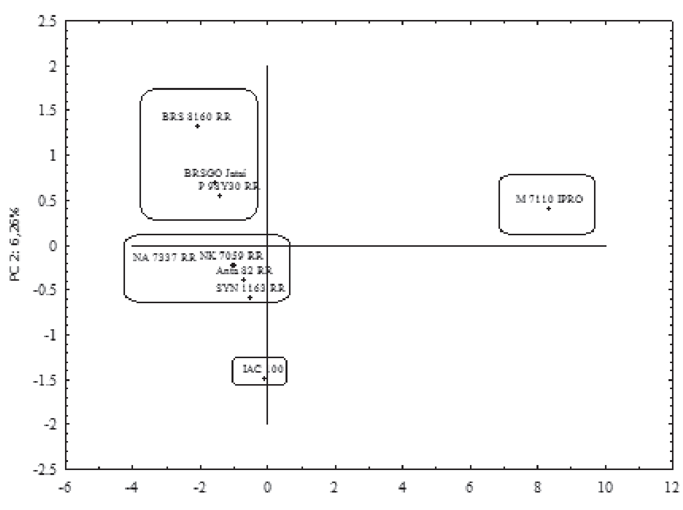

Fig. 2. Principal component analysis plot showing the distribution of different soybean cultivars fed to *Heliothis virescens* in Urutaí, Goiás, Brazil.

results of this study revealed different degrees of antibiosis when *H. virescens* were fed different soybean cultivars.

Cultivar M 7110 IPRO, which contains the gene that encodes the toxin Cry1Ac from the biopesticide *B. thuringiensis* (*Bt*), caused mortality of caterpillars in the first 2 instars, showing that *H. virescens* is *Bt*-sensitive. This result is consistent with the studies of Bernardi et al. (2014) and Bortolotto et al. (2014), who previously reported that *Bt* soybean cultivars were resistant to *H. virescens,* thus constituting an important tactic for the management of this pest. The antibiosis resistance observed for M 7110 IPRO is due to the presence of these polypeptide toxins, which are transformed to active endotoxins in the gut of insects following ingestion (Waquil et al. 2002). These endotoxins are toxic to insects, especially lepidopterans. The polypeptides bind to receptors in the microvilli of the intestinal cells, causing osmotic lysis and resulting in insect death (Schnepf et al. 1998; Bobrowski et al. 2003).

The longest developmental period was observed for caterpillars fed the cultivar IAC 100. This cultivar has previously been shown to prolong development in *A. gemmatalis* (Fugi et al. 2005), *S. eridania* (Souza et al. 2014a), and *Spodoptera cosmioides* Walker (Lepidoptera: Noctuidae) (Boiça Júnior et al. 2015). The extension of the larval stage has been attributed to lower adequacy of the food, possibly due to the presence of chemical compounds that confer resistance (Silveira et al. 1997).

Larval viability was higher with BRS 8160 RR, P 98Y30 RR, and BRSGO Jataí, and lower with NA 7337 RR, SYN 1163 RR, NK 7059 RR, ANTA 82 RR, and IAC 100. Similar to the observations made in the present study, Boiça Júnior et al. (2015) observed IAC 100 to cause higher mortality in *S. cosmioides* in the larval stage. The cultivar IAC 100 resulted in the lowest larval and pupal weights of *H. virescens*. This cultivar has been reported to be resistant to *C. includens* (Souza et al. 2014b), *A. gemmatalis* (Piubelli et al. 2005), and stink bugs (Hemiptera: Pentatomidae) (McPherson et al. 2007; Silva et al. 2013; Silva et al. 2014; Souza et al. 2014c).

The lower performance of *H. virescens* fed cultivar IAC 100 indicates indirect plant defense, which may be due to a higher production of secondary compounds in response to herbivory (Fischer et al. 1990; Piubelli et al. 2003; Li et al. 2004; Piubelli et al. 2005). The main herbivore-induced compounds in this cultivar are the flavonoid rutin and the isoflavonoid genistein, which have been observed to decrease preferential feeding by a lepidopteran (Hoffmann-Campo et al. 2001). In addition to the production of compounds that negatively affect insect biology, the cultivar IAC 100 exhibits herbivory induced expression of enzymes that activate metabolic pathways, showing defense responses directed at minimizing the stress caused by insect attacks (Timbó et al. 2014).

The *H. virescens* total life cycle was longer with cultivar IAC 100, which was 5 d longer than with the cultivars resulting in the shortest life cycles. Panizzi and Silva (2009) studied the feeding ecology of pentatomid stink bugs and reported that when fed on nutrient deficient food they attempted to store more lipids, thereby extending the total life cycle and directly affecting the performance of the adults. From the point of view of pest management and control, extending the total life cycle of pests is advantageous, as the longer the life cycle is, the lower the number of insect generations per crop cycle, therefore decreasing the pest population density and consequently the damages caused by pest incidence (Lara 1991). Also, adult longevity in the individuals fed cultivars IAC 100 and SYN 1163 RR were shortest. The adults with the lowest longevity exhibited the lowest weights in the juvenile stages, and that longevity is directly linked to the feed-conversion efficiency in the previous stages (Luginbill 1928).

Both the cluster and principal component analysis efficiently separated the different groups of cultivars, identifying the different levels of resistance, and may be used as a complement univariate analysis for the selection of insect-resistant genotypes (Pitta et al. 2010). Cultivars BRS 8160 RR, BRSGO Jataí, and P 98Y30 RR were highly susceptible to *H. virescens*, whereas the cultivars IAC 100 and M 7110 IPRO exhibited antibiosis resistance to the pest. These latter cultivars can be used by soybean producers or plant breeders as donors of resistance genes in plant improvement programs for resistance to *H. virescens*.

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