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Attractiveness and non-preference of soybean cultivars to *Heliothis virescens* (Lepidoptera: Noctuidae) feeding

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Abstract

The tobacco budworm (*Heliothis virescens*) is a polyphagous species that damages soybean crops. In the search for sustainable pest control methods, the objective of this study was to evaluate *H. virescens* non-preference (antixenosis) of soybean cultivars by examining larval attraction and feeding responses to the nine cultivars. At 45 days after planting, the attraction and feeding tests were performed with third-instar larvae, and the number of larvae attracted to leaf disks, their leaf consumption, and an attractiveness index were determined in free-choice and no-choice tests. For the free-choice attractiveness test, arranged in a randomized block design with 10 replications were performed. For the no-choice attractiveness test, a completely randomized design with 20 replications was adopted. The cultivar NK 7059 RR was the most attractive and consumed, showing susceptibility to *H. virescens*. The cultivars IAC 100 and M 7110 IPRO (*Bt*) showed non-preference-type resistance 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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Keywords: Antixenosis, Insecta, arthropod-plant interaction, tobacco budworm, plant resistance to insects.

Introduction

The soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), is one of the main economically important crops in the world, and the largest soybean producers are the United States, Brazil, and Argentina, in that order (Castanheira and Freire, 2013; Souza et al., 2014a). One of the main factors causing production losses is pest damage (Bueno et al., 2011b; Hoffnann-Campo et al., 2012), including damage by defoliating caterpillars (Lourenção et al., 2010; Bueno et al., 2012). The tobacco budworm, *Heliothis virescens* (Fabricius 1781), is a defoliating caterpillar that damages soybean crops at all stages of crop development, feeding on pods and flower buds, promoting defoliation, and reducing the plant photosynthetic rate and consequently the crop yield (Tomquelski and Maruyama, 2009; Bueno et al. 2013; Owen et al., 2013). The main host of this pest is cotton (*Gossypium hirsutum* L.), but because of its polyphagous feeding behavior, this pest also feeds on tomato, sunflower, bean, and tobacco (Fitt, 1989; Pratissoli et al., 2006; Karpinski et al., 2014). This insect is a facultative migratory pest that can fly long distances in search of its host plants (Farrow and Daly, 1987). Inappropriate insecticide applications and their undesirable effects as well as the selection of individuals resistant not only to chemical insecticides but also to transgenic plants (Blanco et al., 2009; Sosa-Gomez and Silva, 2010; Bernardi et al., 2014; Bortolotto et al., 2014) lead to the need to investigate alternative pest control methods and strengthen integrated pest management (IPM) (Bueno et al., 2011a). Among the alternative methods, the exploitation of plant resistance to insects is considered ideal because this approach does not affect the environment, may be concomitantly used with other control methods, and does not require producers to deal with sophisticated technology (Smith, 2005; Seifi et al., 2013; Boiça Junior et al., 2015).

Several studies report the resistance of soybean genotypes to different insect pest species (Silva et al., 2012; 2013; 2014; Souza et al., 2014a; 2014b). Souza et al. (2012) concluded that the IAC 100 cultivar was highly resistant to *Spodoptera eridania* (Lepidoptera: Noctuidae). Silva et al. (2014) observed antixenosis-type resistance in soybean genotypes IAC 100 and IAC 19 to the stink bug *Piezodorus guildinii* (Hemiptera: Pentatomidae). Additionally, Souza et al. (2014a, b) observed antixenosis-type resistance in genotypes IAC 100 and IAC 17 to *Euschistus heros* (Hemiptera: Pentatomidae) and antibiosis in IAC 100 to *Chrysodeixis includens* (Lepidoptera: Noctuidae). Factors such as the presence of trichomes, food substrate color, and the release of volatile compounds cause plants to express antixenosis-type resistance (Smith, 2005). Souza et al. (2014b) and Silva et al. (2014) observed that soybean varieties with a greater number of trichomes showed less consumption by stink bugs. In view of the importance of *H. virescens* in soybean crops and the scarcity of information on not only the pest but also pest resistant varieties, the objective of this study was to evaluate antixenosis-type resistance in soybean cultivars to *H. virescens*.

Results

In the free-choice test, significant differences in attractiveness were observed among the soybean cultivars at 6, 12, and 24 h (Table 1). At 6 h, the most attractive cultivars ($p = 0.0066$) were NA 7337 RR, SYN 1163 RR, and NK

Table 1. Mean number (± SEM) of *Heliothis virescens* larvae (Lepidoptera: Noctuidae) on soybean leaf disks and dry disk weight consumed (mg) in the free-choice test of different soybean cultivars. Urutaí, Goias, Brazil.

Cultivars	Time in minutes [1]					
	1	3	5	10	15	30
P 98Y30 RR	0.1±0.10	0.2±0.13	0.1±0.10	0.1±0.10	0.2±0.13	0.1±0.10
NA 7337 RR	0.4±0.22	0.0±0.00	0.2±0.13	0.7±0.30	0.2±0.13	0.3±0.15
SYN 1163 RR	0.4±0.16	0.2±0.13	0.3±0.21	0.1±0.10	0.1±0.10	0.0±0.00
NK 7059 RR	0.2±0.13	1.0±1.00	0.1±0.10	0.3±0.21	0.3±0.21	0.5±0.22
ANTA 82 RR	0.2±0.13	0.2±0.13	0.2±0.13	0.2±0.13	0.3±0.21	0.5±0.22
M 7110 IPRO	0.1±0.10	0.3±0.21	0.3±0.15	0.3±0.21	0.1±0.10	0.6±0.22
BRS 8160 RR	0.0±0.00	0.2±0.13	0.1±0.10	0.4±0.16	0.4±0.22	0.3±0.15
BRSGO Jataí	0.0±0.00	0.3±0.15	0.0±0.00	0.3±0.15	0.2±0.13	0.1±0.10
IAC 100	0.2±0.13	0.5±0.16	0.2±0.13	0.1±0.10	0.5±0.22	0.1±0.10
$F_{8,9}$	1.31	0.62	0.60	1.22	0.59	1.74
P	0.2504	0.7574	0.7693	0.297	0.7828	0.1024
Cultivars	Time in hours					Weight consumed
	1	2	6	12	24	
P 98Y30 RR	0.1±0.10	0.2±0.13	0.1±0.10b	0.3±0.16b	0.1±0.10b	3.35±0.48b
NA 7337 RR	0.1±0.10	0.1±0.10	0.7±0.15a	0.6±0.15b	0.7±0.21a	4.16±0.86b
SYN 1163 RR	0.1±0.10	0.2±0.13	0.6±0.16a	0.6±0.22b	0.5±0.22a	5.09±1.04b
NK 7059 RR	0.2±0.13	0.2±0.13	0.9±0.24a	1.2±0.22a	0.7±0.15a	10.87±1.26a
ANTA 82 RR	0.2±0.20	0.4±0.16	0.4±0.10b	0.5±0.16b	0.5±0.16a	4.69±0.86b
M 7110 IPRO	0.4±0.16	0.7±0.26	0.4±0.26b	0.5±0.16b	0.2±0.13b	3.32±0.66b
BRS 8160 RR	0.2±0.13	0.2±0.13	0.2±0.13b	0.2±0.10b	0.1±0.10b	2.58±0.35b
BRSGO Jataí	0.0±0.00	0.3±0.21	0.1±0.10b	0.2±0.10b	0.2±0.13b	3.23±0.74b
IAC 100	0.3±0.15	0.2±0.13	0.0±0.00b	0.0±0.00b	0.1±0.00b	1.82±0.43b
$F_{8,9}$	0.85	1.21	2.94	4.47	2.97	11.67
P	0.559	0.3053	0.0066	<0.0001	0.0061	<0.0001

[1] Means followed by the same letter do not differ significantly according to the Scott-Knott test at the 5% probability level. SEM = standard error of the mean.

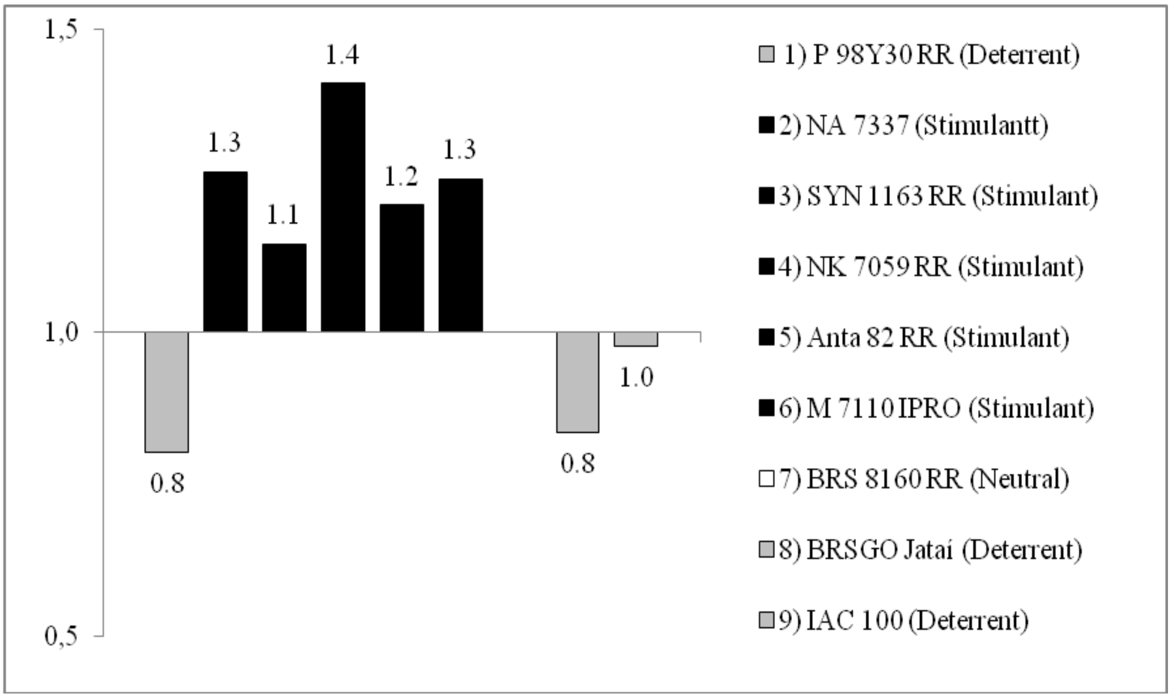

Fig 1. Attractiveness index (AI) of *Heliothis virescens* larvae (Lepidoptera: Noctuidae) for leaf disks of soybean cultivars in the free-choice test. Urutaí, Goias, Brazil.

Table 2. Mean number (± SEM) of *Heliothis virescens* larvae (Lepidoptera: Noctuidae) on soybean leaf disks and dry disk weight consumed (mg) in the no-choice test of different soybean cultivars. Urutaí, Goias, Brazil.

Cultivars	Time in minutes [1]					
	1	3	5	10	15	30
P 98Y30 RR	0.05±0.05	0.05±0.05	0.00±0.00b	0.10±0.06b	0.10±0.06b	0.00±0.00b
NA 7337 RR	0.00±0.00	0.05±0.05	0.05±0.05b	0.00±0.00b	0.20±0.09b	0.10±0.06b
SYN 1163 RR	0.05±0.05	0.05±0.05	0.00±0.00b	0.00±0.00b	0.10±0.06b	0.00±0.00b
NK 7059 RR	0.15±0.08	0.30±0.10	0.35±0.10a	0.35±0.10a	0.45±0.11a	0.40±0.11a
ANTA 82 RR	0.10±0.06	0.10±0.06	0.00±0.00b	0.05±0.05b	0.10±0.06b	0.15±0.08b
M 7110 IPRO	0.55±0.49	0.10±0.06	0.05±0.05b	0.05±0.05b	0.25±0.09a	0.30±0.10a
BRS 8160 RR	0.30±0.10	0.20±0.09	0.25±0.09a	0.20±0.09a	0.30±0.10a	0.15±0.08b
BRSGO Jataí	0.10±0.08	0.05±0.05	0.05±0.05b	0.05±0.05b	0.00±0.00b	0.05±0.05b
IAC 100	0.10±0.06	0.10±0.06	0.10±0.05b	0.05±0.05b	0.10±0.06b	0.05±0.05b
$F_{8,19}$	0.91	1.51	4.03	3.27	2.76	3.60
P	0.5089	0.1546	0.0002	0.0017	0.0068	0.0007
Cultivars	Time in hours					Weight consumed
	1	2	6	12	24	
P 98Y30 RR	0.20±0.09b	0.10±0.00b	0.45±0.11b	0.55±0.11b	0.80±0.09a	3.62±0.91a
NA 7337 RR	0.30±0.10a	0.30±0.10a	0.55±0.11a	0.55±0.11b	0.70±0.10a	3.95±0.66a
SYN 1163 RR	0.00±0.00b	0.05±0.05b	0.35±0.10b	0.30±0.10c	0.65±0.10a	2.59±0.59b
NK 7059 RR	0.40±0.11a	0.40±0.11a	0.80±0.09a	1.00±0.00a	0.90±0.06a	3.50±0.41a
ANTA 82 RR	0.35±0.10a	0.20±0.09a	0.60±0.11a	0.65±0.10b	0.60±0.11a	1.97±0.34b
M 7110 IPRO	0.40±0.11a	0.35±0.10a	0.25±0.09b	0.20±0.09c	0.10±0.06b	0.58±0.17b
BRS 8160 RR	0.30±0.10a	0.30±0.10a	0.65±0.10a	0.60±0.11b	0.35±0.10b	2.66±0.60b
BRSGO Jataí	0.15±0.08b	0.00±0.00b	0.60±0.11a	0.50±0.11b	0.25±0.09b	2.07±0.54b
IAC 100	0.10±0.06b	0.00±0.00b	0.15±0.08b	0.15±0.08c	0.50±0.11a	1.85±0.50b
$F_{8,19}$	2.23	3.49	3.85	6.75	7.03	3.49
P	0.0273	0.0009	0.0003	<0.0001	<0.0001	0.0019

[1] Means followed by the same letter do not differ significantly according to the Scott-Knott test at the 5% probability level. SEM = standard error of the mean.

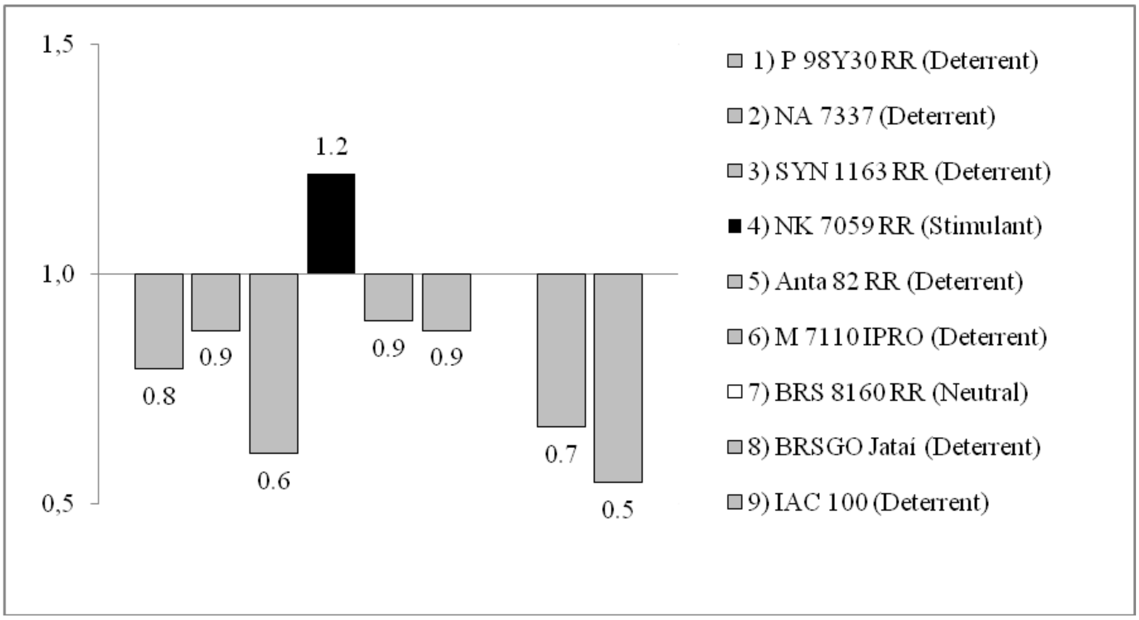

Fig 2. Attractiveness index (AI) of *Heliothis virescens* larvae (Lepidoptera: Noctuidae) for leaf disks of soybean cultivars in the no-choice test. Urutaí, Goias, Brazil.

7059 RR, with mean larval counts of 0.7, 0.6, and 0.9, respectively. These means were higher than those of the other cultivars, which ranged from 0.0 (IAC 100) to 0.4 (ANTA 82 and M 7110 IPRO). After 12 h of evaluation, the cultivar that presented the highest mean attractiveness ($p < 0.0001$) was NK 7059 RR (1.2 larvae), differing from the others, which ranged from 0.0 (IAC 100) to 0.6 (NA 7337 RR and SYN 1163 RR). At 24 h, the cultivars with the highest attractiveness ($p = 0.0061$) were NA 7337 RR and NK 7059 RR (0.7 larvae) as well as SYN 1163 RR and ANTA 82 RR (0.5 larvae). Regarding the dry weight consumed in the free-choice test, the most heavily consumed cultivar ($p < 0.0001$) was NK 7059 RR (10.87 mg), the consumption of which differed from that of the more lightly consumed cultivars, except that of IAC 100 (1.82 mg).

Based on these AIs, the cultivars NA 7337 RR, SYN 1163 RR, NK 7059 RR, ANTA 82 RR, and M 7110 IPRO stimulated *H. virescens* in the free-choice test. By contrast, the cultivars P 98Y30 RR, BRSGO Jataí, and IAC 100 acted as deterrents (Figure 1).

In the no-choice test, significant differences in the mean number of larvae that fed on the leaf disks occurred at all the observation times, except at 1 and 3 min (Table 2). In this test, there was a significant difference ($p = 0.0019$) in the dry weight consumed, and the most heavily consumed cultivars were NA 7337 RR (3.95 mg), P 98Y 30 RR (3.62 mg), and NK 7059 RR (3.50 mg).

At 5 and 10 min ($p = 0.0002$ and 0.0017), the greatest mean attractiveness was observed for the NK 7059 RR and BRS 8160 RR cultivars, which differed significantly from the other cultivars at both times. At 15 min, in addition to the aforementioned two most attractive cultivars ($p = 0.0068$), M 7110 IPRO was also among the most attractive cultivars.

At 30 min, the NK 7059 RR and M 7110 IPRO cultivars were the most attractive ($p = 0.0007$), with 0.4 and 0.3 larvae, respectively, per leaf disk. At 1 and 2 h after larval release, the NK 7059 RR, ANTA 82 RR, M 7110 IPRO, and BRS 8160 RR cultivars showed the largest means ($p = 0.0273$ and 0.0009, respectively), differing from the others.

At 6 h, the NK 7059 RR, BRS 8160 RR, ANTA 82 RR, BRSGO Jataí, and NA 7337 RR cultivars were the most attractive. At 12 h, NK 7059 RR was the most attractive ($p < 0.0001$), obtaining a mean of one larva per leaf disk. The treatments with the smallest means were SYN 1163 RR, M 7110 IPRO, and IAC 100 (0.30, 0.20 and 0.15, respectively).

At 24 h, the most attractive cultivars were P 98Y30 RR, NA 7337 RR, SYN 1163 RR, NK 7059 RR, ANTA 82 RR, and IAC 100. The least attractive cultivars were M 7110 IPRO, BRS 8160, and BRSGO Jataí. Using their AIs, the NK 7059 RR cultivar was considered stimulatory and the others were considered deterrent to the feeding of *H. virescens* (Figure 2).

Discussion

In the free-choice test, a significant difference was observed after the 6-h evaluation, whereas the cultivars exhibited no differences at the first evaluation times. These results can be explained by the fact that the larvae fed on the artificial diet until the completion of the experiment, without undergoing a period of feeding interruption. Boiça Junior et al. (2015) assessed the types of resistance to *Spodoptera cosmioides* (Lepidoptera: Noctuidae). Souza et al. (2012) examined the feeding nonpreference of *Spodoptera eridania* (Lepidoptera: Noctuidae) in a free-choice test with soybean genotypes and also found significant differences only in the last times assessed.

Starting at 6 h, the NK 7059 RR cultivar was the most attractive to *H. virescens*, obtaining the largest mean number of larvae per leaf disk until the end of the experiment and suffering the heaviest consumption. The cultivar IAC 100 was one of the least attractive cultivars, presenting relatively few larvae per disk leaf and being the least heavily consumed.

The feeding preference of an herbivorous insect depends on plant stimuli, which can be positive or negative as well as chemical (Hoffmann-Campo et al., 2001), physical (Coelho et al., 2009), and/or morphological (Silva et al. 2012; Smith et al., 2014) in nature. Therefore, the *H. virescens* larvae received positive stimuli from the NK 7059 RR cultivar, which obtained the highest AI in the free-choice test.

In the no-choice test, differences were observed among the cultivars starting at 5 min. NK 7059 RR was the cultivar most attractive to the *H. virescens* larvae, obtaining the most larvae per leaf disk, on average, and a relatively high AI as well as suffering relatively high consumption.

Cultivar IAC 100 presented relatively small attractiveness means in the no-choice test, being repellent and appearing deterrent to *H. virescens*. According to Souza et al. (2012), Valle et al. (2012), Silva et al. (2013), Souza et al. (2014a), and Souza et al. (2014b), the IAC 100 cultivar was resistant to several species of phytophagous insects, serving as the basis for soybean genetic improvement programs aimed at incorporating insect resistance genes (McPherson and Buss, 2007).

The M 7110 IPRO (*Bt*) cultivar experienced the least consumption in the no-choice test and attained the lowest AI, being repellent to *H. virescens*. Bernardi et al. (2014) and Bortolotto et al. (2014) assessed the larval biology of *H. virescens* in genetically modified soybean plants (*Bt*) and concluded that the pest is highly susceptible, thereby suffering larval mortality. The lower consumption indexes in this cultivar are due to the presence of toxins from *Bacillus thuringiensis* (Eubacteriales: Bacillaceae), which induce the formation of endotoxins (Waquil et al., 2002) poisonous to insects, especially lepidopterans. These polypeptides bind to receptors on the microvilli of insect intestinal cells, causing osmotic lysis, which results in insect death (Schnepf et al., 1998; Bobrowski et al., 2003).

These soybean cultivars resistant to *H. virescens* can be used directly by soybean producers or in association with other control strategies for the management of this defoliating caterpillar.

Materials and Methods

Experiment site

The study was conducted at the Laboratory of Agricultural Entomology of the Federal Institute of Goiás (Instituto Federal Goiano), Urutaí Campus, Urutaí, Goiás State, Brazil, under controlled conditions (25 ± 2 °C, 70 ± 10% relative humidity, and a 12-h photoperiod) in heated room.

Rearing of *H. virescens*

H. virescens pupae, which developed from larvae donated by the Laboratory of Entomology EMBRAPA Rice and Beans, were sexed and placed in PVC cages (20-cm height x 20-cm diameter), where adult emergence and mating occurred. The adults were fed a 10% honey solution placed in polyethylene terephthalate (PET) plates on a wad of cotton, which was replaced every two days. Eggs were removed daily and disinfected with 5% sodium hypochlorite solution for five seconds, rinsed with distilled water, and placed in Petri dishes (9.0 x 1.5 cm) with moistened filter paper until larval hatching. The hatched larvae were placed in groups of four within 150-mL plastic pots containing artificial diet (Greene et al, 1976). On becoming third instars, the larvae were placed individually in plastic pots until the pupal stage to provide insects for the next cohort.

Acquisition of plants and performance of tests

Seeds of the soybean cultivars P 98Y30 RR, NA 7337 RR, SYN 1163 RR, NK 7059 RR, ANTA 82 RR, M 7110 IPRO (*Bt*), BRS 8160 RR, BRSGO Jataí, and IAC 100 were sown in 5-L pots with a substrate composed of a 3:1 soil:organic compost mixture to obtain the leaves used in attractiveness and nonpreference tests.

Attractiveness and non-preference for feeding

Two laboratory tests with third-instar *H. virescens* larvae were started 45 days after the soybean seeds were sown. A free-choice attractiveness test was conducted by offering leaf disks of the different genotypes to the third-instar larvae. Leaves were collected from the plants and cut to provide 2.5-cm-diameter disks that were distributed circularly in test arenas (14-cm diameter x 2-cm height) on moistened filter paper. Two symmetrically positioned leaf disks were cut from the leaves collected from each genotype; one disk was offered to a larva and the other, designated an 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.

A no-choice attractiveness test was performed by offering the same genotypes individually. The leaves were collected and prepared as described for the previous test. One leaf disk per 6-cm-diameter Petri dish was placed on moistened filter paper. The dry weight consumed was determined using the previously described aliquot method.

In both tests, the attractiveness was evaluated at 1, 3, 5, 10, 15, and 30 min as well as 1, 2, 6, and 24 h after larval release by counting the number of insects attracted to the leaf disk of each genotype. For the free-choice attractiveness test, arranged in a randomized block design with 10 replications were performed. For the no-choice attractiveness test, a completely randomized design with 20 replications was adopted.

At the end of the tests, an attractiveness index (AI) was calculated according to Kogan and Goeden (1970) with the following formula: $AI = 2C/(C + S)$, where C = the number of insects attracted to the evaluated genotype and S = the number of insects attracted to the standard susceptible genotype (BRS 8160 RR according to Souza et al., 2012). The AI values can vary between zero and two: $AI = 1$, < 1, and > 1 indicate similar, lesser and greater attractiveness, respectively, of the evaluated genotype compared with the standard susceptible genotype.

Statistical analysis

The data obtained in the tests were subjected to analysis of variance (ANOVA) and F-test, and the means were compared using the Scott-Knott test in Sisvar 5.3 (Ferreira, 2011). A probably level of 5% or less was considered statistically significant.

Conclusion

Cultivar NK 7059 RR was the most susceptible to *H. virescens* in the feeding nonpreference tests. Cultivars IAC 100 and M 7110 IPRO (*Bt*) showed nonpreference-type resistance to *H. virescens*. 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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