

Feeding preference of *Nezara viridula* (Hemiptera: Pentatomidae) and attractiveness of soybean genotypes

Efrain de Santana Souza^{1*}, Edson Luiz Lopes Baldin¹, José Paulo Gonçalves Franco da Silva¹, and André Luiz Lourenção²

Nezara viridula (L.) (Hemiptera: Pentatomidae) is a cosmopolitan insect that causes economic damages to several cultures, in particular soybeans (*Glycine max* [L.] Merr.) Among the techniques that involve Integrated Pest Management, the resistance of plants is pointed as a tool of great value and can contribute to the reduction of populations of insects. The feeding preferences of adults of southern green stink bug (*N. viridula*), and the attractiveness of soybean genotypes were evaluated under laboratory conditions to detect the most resistant material against attack from this insect. A choice test, using mature grains and green pods of the genotypes was carried out, in which the number of individuals attracted in different periods was counted. Feeding preference was evaluated in the choice tests using green pods and the number of pricks and the average time spent feeding by pricks were evaluated. In addition, texture and trichome density in the green pods were evaluated. The mature grains of 'TMG 117RR' and 'TMG 121RR' were less attractive to the adults of *N. viridula*. Regarding the green pods, 'IAC 17' and PI 227687 were less attractive; 'IAC 17' and 'IAC PL1' were less consumed, indicating the feeding non-preference as a resistance mechanism. 'IAC 17', 'TMG-117RR' and PI 227687 presented high levels of trichome density, and in 'IAC 17' this morphological characteristic was considered to be the main resistance factor against *N. viridula*. These results may be useful for breeding programs that focus on the resistance of soybeans to insects.

Key words: Antixenosis, *Glycine max*, host plant resistance, Southern green stink bug.

INTRODUCTION

Currently, soybeans (*Glycine max* [L.] Merr.) stand out as both a healthy food source and a renewable energy source, known as biodiesel. According to forecasts by the United States Department of Agriculture, in 2014/2015, Brazil will produce 71 million tons of soybeans from the cultivation of 25 million hectares (USDA, 2009).

Southern green stink bug (*Nezara viridula* [L.]), redbanded stink bug (*Piezodorus guildinii* [West.]), and neotropical brown stink bug (*Euschistus heros* [Fabr.]) (Hemiptera: Pentatomidae) are stink bugs considered as important pests of soybeans, causing serious damage to crops (Chocorosqui and Panizzi, 2004; Temple et al., 2013). During feeding, the bugs, both at a young age and as adults, constantly suck the grains inside the green pods, causing punctures, mottles and a reduction in the number and quality of seeds, an increase in the protein content, a

reduction of the oil and a reduction in seed germination, in addition to changes in the physiology of the plant, causing a disorder known as "crazy soybean" (Panizzi and Slansky, 1985).

Nezara viridula has been reported throughout all of South America, especially in Brazil (Panizzi and Slanski, 1985; Knight and Gurr, 2007). This hemipterous bug starts its attack on crops either after the vegetative phase or during flowering, increasing its incidence when the green pods sprout and the grains expand, the phase in which the greatest losses take place in crops (Gore et al., 2006; Olson et al., 2011). The occurrence of *N. viridula* has been reported more frequently than other Pentatomidae of soybeans in tropical and subtropical regions, being found throughout all of America (Panizzi and Slansky, 1985), as well as in Africa and Europe (Čokl and Millar, 2009). In Brazil, this insect has been reported in the states of Goiás, São Paulo, Rio Grande do Sul, Paraná, and other states where soybean is produced (Panizzi and Slansky, 1985).

Insecticides are generally used as a control method of *N. viridula*. Even though they are often recommended, these products generally cause environmental disequilibrium and intoxication to mankind when used in excess or incorrectly (Byrne et al., 2003). In addition, some authors (Hart and Pimentel, 2002; Sosa-Gomez and Silva 2010) have reported that successive applications of the same chemical product help the insect to quickly develop

¹Universidade Estadual Paulista UNESP, Faculdade de Ciências Agrônomicas, Departamento Proteção Vegetal, Botucatu, São Paulo, Brasil.

*Corresponding author (efrainsantana2@yahoo.com.br).

²Instituto Agronômico de Campinas, Centro de Fitossanidade, Entomologia, Campinas, São Paulo, Brasil.

Received: 7 May 2013.

Accepted: 22 October 2013.

doi:10.4067/S0718-58392013000400004

resistance to its action due to great selection pressure.

The use of resistant genotypes against pests may be a reductive alternative or even substitutive to insecticides in crops, and it may also present other advantages such as easy access, compatibility with microbial control, the non-requirement of previous knowledge, a cost reduction in cultivation and a decrease in the pest population to levels that do not cause economic damage and do not interfere with the ecosystem, making the crop more profitable for the producer (Smith, 2005).

Considering the damages and significant losses both in productivity and quality in the cultivation of soybeans, and also the need for the development of more effective and less aggressive control methods both to the environment and to humankind, this research evaluated the response of different genotypes of soybeans to attack by *N. viridula*. Assays of attractiveness and feeding preferences under laboratory conditions were performed. When the occurrence of resistance was detected, some morphological aspects were also sought by evaluating the texture and density of the trichomes in the green pods.

MATERIALS AND METHODS

Soybean genotypes evaluated

Ten soybean genotypes were evaluated (Table 1), of which 'IAC 17', 'IAC 19', 'IAC 24' and PI 227687 were selected for research purposes since they were previously shown to be less frequently attacked by Pentatomidae in other studies (Lourenção et al., 1999; 2000; Miranda et al., 2003; Piubelli et al., 2003). The 'IAC PL1' is a susceptible genotype, according to previous studies (Lourenção et al., 1997). The 'TMG 103RR', 'TMG 117RR', 'TMG 121RR' and 'BRS 242RR' are transgenic currently being launched. 'Conquista' is a standard genotype that is highly cultivated in Brazil and resistant to various diseases of this crop (Embrapa, 2010).

In order to obtain grains and pods, the genotypes were sown (4 plants per pot) in 20 L pots containing a mixture of autoclaved soil, sand and Plantmax-HT® (DDL Agro, Paulínia, São Paulo, Brazil) substrate in a ratio of 4:1:1, respectively. The plants were kept in greenhouses, lined with aphid-proof netting to protect against attack from other pests, once they did not receive any type of

pulverization with insecticides. They also received mulch, as normally recommended for this crop (Raij et al., 1997), and they were irrigated periodically according to the need.

Stock rearing of *N. viridula*

To begin rearing *N. viridula* in the laboratory (RH = 70 ± 10%; T = 25 ± 2 °C and photoperiod 12:12 h), masses of eggs were supplied by Dr Daniel R. Sosa-Gomez, from EMBRAPA-Centro Nacional de Pesquisa de Soja (CNPSo), Londrina, Paraná, Brazil. These eggs were placed in Petri dishes inside wooden cages (30 × 30 × 45 cm) open at the front (for insect manipulation) and lined with aphid-proof netting, according to the methodology described by Corrêa-Ferreira (1985). Simultaneously, adult green stink bugs were also collected in soybean areas (different from the genotypes studied). The collections were always carried out in the morning, when the temperature was mild since the insects are more exposed in this situation. The adults were reared in glass cages (50 × 50 × 70 cm) with the upper part covered by voile cloth. Eggs were collected daily and kept in wooden cages made for the nymphs, in order to avoid the eggs being consumed by the insects themselves, which, according to Panizzi (1991), frequently occurs in the rearing of *N. viridula*. Both green and ripe fruits of privet (*Ligustrum* sp.) and peanut, which were periodically replaced, were used to feed the green stink bugs.

Food attractiveness and feeding preference

The assays of attractiveness and choice preference were carried out simultaneously in the laboratory under controlled conditions (RH = 70 ± 10%; T = 25 ± 2 °C and photoperiod 12:12 h). Green pods were collected in their phenological stage, starting from V5, in accordance with the scale proposed by Fehr and Caviness (1977). In both assays, 20 adult insects were used per repetition (48 h lifespan) with a 24 h fast before infestation.

For the attractiveness test with mature grains, 20 g grains of each genotype was used, which were placed equidistantly in small bags made with voile cloth (spaced out in ± 20 cm) laterally (5 cm height) of identical cages to the ones used for the rearing of adults. Wooden stems (25 cm length) were placed inside, all coming out from the same central beam (which the insects were released

Table 1. Soybean genotypes evaluated for resistance to *Nezara viridula* with their genealogies and origins.

Genotype	Genealogy	Origin
'IAC 17'	D72-9601-1 × IAC-8	IAC-Campinas, SP
'IAC 19'	D72-9601-1 × IAC-8	IAC-Campinas, SP
'IAC 24'	IAC80-1177 × IAC83-288	IAC-Campinas, SP
'IAC PL1'	Mutation of Japanese material for a later cycle	IAC-Campinas, SP
PI 227687	Okinawa, Japan	IAC-Campinas, SP
'Conquista'	Lo76-4484 ² × Numbaíra	FMT-Rondonópolis, MT
'TMG 103RR'	TMGLM-3295 × TMGLM-3324	FMT-Rondonópolis, MT
'TMG 117RR'	(FT Corvina × M soybeans 8888RR) × MG/BR 46	FMT-Rondonópolis, MT
'TMG 121RR'	(FMT Cachara × M soybeans 8080RR) × FMT Tucunaré	FMT-Rondonópolis, MT
'BRS 242RR'	Embrapa 58*5' × (E96-246 × Embrapa 59)	Embrapa, Londrina, PR

IAC: Instituto Agronômico de Campinas; SP: São Paulo; FMT: Fundação MT; MT: Mato Grosso; PR: Paraná.

onto) and connected to each bag of grains. The cages were covered with voile cloth in order to prevent the insects from escaping. The wooden stems served as “bridges” to the grains, offering a free choice of all genotypes.

Regarding the attraction of green pods, round aluminum arenas (60 cm diameter × 10 cm height) covered with a glass plate and lined with damp filter paper at the bottom were used. Three green pods of each genotype were placed in each arena, which were set up in a randomized block design equal distances (approximately 15 cm apart), where the bugs were later released into the centre.

The attractiveness was observed in both assays. The number of insects present at each genotype was registered 15, 30, 45, 60, 120, and 180 min after their release.

At the same time as the evaluation of attractiveness of green pods, the consumption of the insects was observed for 180 min, where the number of pricks by insect per genotype and the time the insects spent feeding from the material were evaluated. At the end of these observations, the time of feeding/pricking of each insect was determined. The number of stings on the green pods was counted with the naked eye, as described by Rossetto et al. (1981). The length of time of pricks, however, was determined with the aid of digital chronometers, which were activated at the beginning of the feeding of each insect and deactivated when the bug removed the stylus from the green pod. Four evaluators were working, each one was in charge of the observation of the feeding of five insects in each repetition.

At the end of the evaluations the attractiveness index (AI) of each genotype was determined, referring to ‘Conquista’ as the standard. This index was obtained from the equation proposed by Lin et al. (1990):

$$AI = 2G/G + S$$

where G is the number of insects at the evaluated genotype and S is the number of insects at the standard genotype. ‘Conquista’ was used as the standard because it is widely cultivated throughout Brazil, and also because its susceptibility has been observed under field conditions (Belorte et al., 2003).

Determining trichome density in the green pods

The number of trichomes found in 0.25 cm² was counted (Paron and Lara, 2005) in order to correlate the density of trichomes in the green pods with the feeding preference of *N. viridula*. For this, a stereomicroscope and graph paper (0.25 cm²) were used, the middle of the pods (on the second grain) was standardized and then the total number of trichomes (hooked and acicular) found in the delimited area in 10 pods per genotype was recorded. Each observation represented one repetition, set up in a randomized complete block design.

Determining the texture of the green pods

In order to correlate the texture of the pods of each genotype to the consumption of pods by adults of *N.*

viridula, a texturometer (Stevens – Lfra Texture Analyzer, Brookfield, Middleboro, Massachusetts, USA) was used, calibrated for a 10 mm penetration and a 2.0 mm s⁻¹ speed, using a probe tip TA 9/1000. The results of the measurements are shown in gram-force per square centimeter (gf cm⁻²) and represent the maximum strength required for a part of the probe tip to penetrate the pulp of the pod, simulating a real situation in which the insect is found inserting its stylus into the pod.

This assay was standardized so that the green pod plants to be evaluated were in phenological phase V5, where the penetration resistance in the second grains of the pod would be tested. Ten green pods of each genotype were evaluated, where each one represented one repetition, in a randomized design.

Data analysis

In multi-choice tests, data for the number of adults of *N. viridula*, the number of pricks, feeding time per prick, the number of trichomes and the texture of the green pods were analyzed by a one-way ANOVA; the original data (x) were transformed $(x + 0.5)^{1/2}$ for analysis. Tukey’s HSD test was used for mean comparisons (Winer et al., 1991). The statistical analyses were performed using the PROC GLM procedure of SAS (SAS Institute, 2001). The means were separated by using the LSD test at a 5% level of significance.

RESULTS AND DISCUSSION

Attractiveness of mature grains

Fifteen minutes after releasing the insects (Table 2), it was observed that the mature grains of ‘IAC 24’ were more attractive to *N. viridula* than PI 227687, ‘TMG 103RR’, ‘TMG 117RR’ and ‘TMG 121RR’, which were found to be less attractive. At 30, 60, and 180 min after release, a preference of *N. viridula* to any of the genotypes being analyzed was not found. At 45 min after release, ‘TMG 117RR’ was the least attractive compared to ‘IAC PL1’, with no insects attracted to it. After 120 min evaluation, ‘IAC 19’ and ‘TMG 121RR’ was the least attractive compared to ‘IAC 24’. In general, ‘TMG 117RR’, ‘TMG 121RR’, ‘Conquista’, ‘TMG 103RR’ and PI 227687 stood out as being the least attractive. Conversely, it was found that the grains in ‘IAC 24’ and ‘IAC PL1’ were the most attractive to *N. viridula* adults.

The AI of the grains (Figure 1) showed that, ‘TMG 117RR’ and ‘TMG 121RR’ were considered repellent when compared to the commercial standard ‘Conquista’. PI 227687, ‘IAC 17’, ‘IAC 19’, ‘BRS 242RR’, ‘IAC PL1’ and ‘IAC 24’ were attractive. ‘TMG 103RR’ was classified as neutral, since its index did not differ from the one presented by the standard.

According to the mean number of adults at the mature grains, ‘TMG 117RR’, ‘TMG 121RR’, ‘Conquista’, ‘TMG 103RR’, and PI 227687 stood out as being the least

Table 2. Mean (\pm SE) number of *Nezara viridula* adults attracted to mature grains of different soybean genotypes at 15, 30, 45, 60, 120, and 180 min after the release.

Genotype	Number of adults						Mean
	15 min	30 min	45 min	60 min	120 min	180 min	
'TMG 117RR'	0.1 \pm 0.10b	0.2 \pm 0.13	0.0 \pm 0.0b	0.2 \pm 0.13	0.1 \pm 0.10ab	0.1 \pm 0.10	0.11 \pm 0.02e
'TMG 121RR'	0.1 \pm 0.10b	0.1 \pm 0.10	0.2 \pm 0.13ab	0.2 \pm 0.13	0.3 \pm 0.15b	0.4 \pm 0.22	0.21 \pm 0.02cde
'Conquista'	0.3 \pm 0.21ab	0.2 \pm 0.13	0.4 \pm 0.16ab	0.3 \pm 0.15	0.4 \pm 0.16ab	0.4 \pm 0.16	0.33 \pm 0.01cde
'TMG 103RR'	0.1 \pm 0.10b	0.3 \pm 0.15	0.5 \pm 0.17ab	0.7 \pm 0.15	0.5 \pm 0.22ab	0.5 \pm 0.22	0.43 \pm 0.02cde
PI 227687	0.1 \pm 0.10b	0.4 \pm 0.16	0.3 \pm 0.15ab	0.9 \pm 0.31	0.7 \pm 0.26ab	0.5 \pm 0.16	0.48 \pm 0.03cde
'IAC 17'	0.3 \pm 0.15ab	0.4 \pm 0.22	0.5 \pm 0.22ab	0.7 \pm 0.26	0.7 \pm 0.30ab	0.7 \pm 0.33	0.55 \pm 0.03cd
'IAC 19'	0.4 \pm 0.16ab	0.7 \pm 0.15	0.9 \pm 0.35ab	0.6 \pm 0.27	0.3 \pm 0.15b	0.6 \pm 0.31	0.58 \pm 0.04cd
'BRS 242RR'	0.2 \pm 0.13ab	0.8 \pm 0.25	0.6 \pm 0.22ab	0.7 \pm 0.33	0.8 \pm 0.36ab	0.9 \pm 0.48	0.66 \pm 0.05bc
'IAC PL1'	0.1 \pm 0.42ab	1.1 \pm 0.35	1.1 \pm 0.31a	1.0 \pm 0.30	0.7 \pm 0.30ab	0.9 \pm 0.38	0.96 \pm 0.02ab
'IAC 24'	1.1 \pm 0.38a	1.1 \pm 0.43	1.0 \pm 0.45ab	1.3 \pm 0.45	1.8 \pm 0.53a	1.4 \pm 0.58	1.28 \pm 0.03a
CV, %	33.03	34.52	34.91	36.21	35.94	41.79	8.15

Means followed by the same lower case letter in each column did not differ according to the Tukey test ($P \leq 0.05$). The original data were transformed to $(x + 0.5)^{1/2}$ for analysis. CV: coefficient of variation.

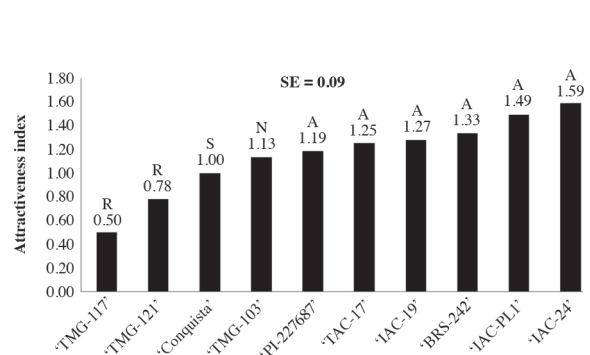


Figure 1. Index of mean attractiveness of *Nezara viridula* to the grains of the different soybean genotypes and classification of the materials; attractiveness index AI: $AI = (2 \times G)/(G + S)$, where G = standard treatment and A = attractive, N = neutral, R = repellent, S = standard.

attractive genotypes, suggesting the presence of volatile compounds that are undesirable to the insects or even provide a physical resistance to feeding. Conversely, 'IAC 24' and 'IAC PL1' may be considered attractive to the adults of *N. viridula*, which corroborated the classification of these genotypes by the AI.

Table 3. Mean (\pm SE) number of *Nezara viridula* adults attracted to the green pods of the different soybean genotypes at 15, 30, 45, 60, 120, and 180 min after release.

Genotype	Number of adults						Mean
	15 min	30 min	45 min	60 min	120 min	180 min	
'IAC 17'	0.2 \pm 0.13	0.2 \pm 0.13	0.4 \pm 0.27	0.1 \pm 0.10b	0.1 \pm 0.10	0.1 \pm 0.10	0.18 \pm 0.05f
PI 227687	0.4 \pm 0.16	0.7 \pm 0.21	0.8 \pm 0.25	0.6 \pm 0.22ab	0.7 \pm 0.30	0.2 \pm 0.13	0.57 \pm 0.09ef
'IAC PL1'	0.9 \pm 0.43	0.9 \pm 0.50	0.9 \pm 0.28	1.0 \pm 0.42ab	0.2 \pm 0.13	0.0 \pm 0.00	0.65 \pm 0.18de
'BRS 242RR'	0.5 \pm 0.22	0.8 \pm 0.29	0.6 \pm 0.22	0.6 \pm 0.22ab	1.4 \pm 0.34	0.7 \pm 0.26	0.77 \pm 0.13cde
'TMG 121RR'	1.1 \pm 0.35	1.1 \pm 0.38	0.8 \pm 0.36	1.1 \pm 0.31ab	0.5 \pm 0.17	0.3 \pm 0.15	0.82 \pm 0.14cde
'IAC 24'	1.0 \pm 0.30	1.2 \pm 0.42	1.5 \pm 0.37	1.3 \pm 0.33ab	1.1 \pm 0.31	0.5 \pm 0.22	1.10 \pm 0.14bcd
'TMG 103RR'	1.1 \pm 0.35	1.4 \pm 0.40	1.2 \pm 0.36	0.8 \pm 0.25ab	1.1 \pm 0.31	1.0 \pm 0.33	1.10 \pm 0.08bcd
'Conquista'	0.9 \pm 0.35	1.2 \pm 0.36	1.4 \pm 0.48	1.3 \pm 0.33ab	1.2 \pm 0.29	1.0 \pm 0.33	1.17 \pm 0.08bc
'TMG 117RR'	1.8 \pm 0.59	1.7 \pm 0.58	2.0 \pm 0.49	1.6 \pm 0.50ab	1.3 \pm 0.58	0.8 \pm 0.33	1.53 \pm 0.17ab
'IAC 19'	1.8 \pm 1.07	2.3 \pm 1.01	2.1 \pm 0.66	2.6 \pm 0.75a	1.6 \pm 0.65	0.8 \pm 0.25	1.87 \pm 0.26a
CV, %	44.72	41.51	38.18	37.90	36.79	33.26	9.16

Means followed by the same lower case letter in each column did not differ according to the Tukey test ($P \leq 0.05$). The original data were transformed to $(x + 0.5)^{1/2}$ for analysis. CV: coefficient of variation.

Attractiveness and consumption of the green pods

Differences between the means of the soybeans genotypes concerning attractiveness of the green pods at 15, 30, 45, 120, and 180 min after release of the insects were not found (Table 3).

Only after 60 min 'IAC 17' was the least attractive, differing from 'IAC 19', which was the most attractive to adults of the bugs.

By analyzing all of the observation (Table 3), 'IAC 17' and PI 227687 were found to be less attractive to adults of *N. viridula*. A higher level of attraction was observed in pods from 'IAC 19' and 'TMG 117RR', whereas all of the others showed intermediate levels of attractiveness.

According to the AI of the green pods (Figure 2), 'IAC 17', PI 227687, 'TMG 121RR', 'BRS 242RR' and 'IAC PL1' were considered repellents. 'TMG 103RR' and 'IAC 24' were classified as neutral, and 'IAC 19' and 'TMG 117RR', attractive, with AI higher than those of the standard 'Conquista'.

The highest level of preference by adults of *N. viridula* for pods 'TMG 117RR' (Table 3) is in contrast with the results obtained for these genotypes in the attractiveness tests for mature grains, where they showed little attractiveness. Such a fact shows that the feeding stimulus

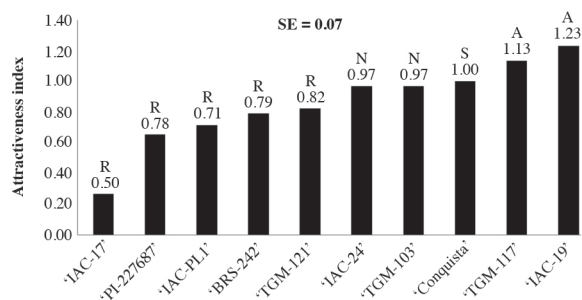


Figure 2. Mean index of attractiveness of *Nezara viridula* to the green pods of the different soybean genotypes and classification of materials; attractiveness index AI: $AI = (2 \times G)/(G + S)$, where G = standard treatment and A = attractive, N = neutral, R = repellent, S = standard.

may vary according to the structure of the plant. Corrêa-Ferreira and Panizzi (1999) reported a higher occurrence and feeding preference of southern green stink bugs for green pods and during the period of grain filling. The preference of *N. viridula* for green pods and mature grains of the same genotype may not be maintained, showing that this preference may increase in certain phenological phases of the plant, as occurred with 'IAC 17', PI 227687, 'BRS 242RR' and 'IAC PL1'.

Authors such as McMillian and Wiseman (1972), Lordello and Lara (1980) and Moscardi et al. (1981) reported the influence of the phenological phase of the plants on the expression of resistance of non-preferred types, which may explain the behavior of soybean genotypes previously mentioned. Panizzi (1991) also highlighted the hydric influence in different phenological phases of soybean plants on seed sucking insects in general, showing a higher level of preference for these pods.

The green pods of 'IAC 17' presented the lowest means per number of pricks and feeding time (Table 4). The mean number of pricks in 'IAC PL1' was intermediate; however, the mean feeding time per prick was the second lowest. 'Conquista', 'IAC 19', 'IAC 24', 'BRS 242RR',

Table 4. Mean (\pm SE) number of pricks and mean (\pm SE) length of feeding per prick by adults of *Nezara viridula* in green pods of the different soybean genotypes.

Genotype	Number mean of prick	Mean time prick
'IAC 17'	0.7 \pm 0.37b	6.90 \pm 4.37c
'IAC PL1'	2.6 \pm 0.87ab	11.30 \pm 3.40bc
PI 227687	2.7 \pm 0.52ab	21.41 \pm 6.32ab
'TMG 121RR'	3.9 \pm 0.90ab	22.27 \pm 4.44ab
'TMG 103RR'	4.3 \pm 0.92ab	28.19 \pm 8.55ab
'IAC 24'	4.7 \pm 0.92a	28.01 \pm 6.65ab
'Conquista'	5.7 \pm 0.97a	22.91 \pm 4.01ab
'BRS 242RR'	5.9 \pm 1.73a	18.30 \pm 4.16ab
'TMG 117RR'	6.5 \pm 1.34a	21.48 \pm 5.05ab
'IAC 19'	7.1 \pm 2.41a	33.39 \pm 4.56a
CV, %	38.73	37.16

Means followed by the same lower case letter in each column did not differ according to the Tukey test ($P \leq 0.05$). The original data were transformed to $(x + 0.5)^{1/2}$ for analysis. CV: coefficient of variation.

and 'TMG 117RR' presented the highest means per number of pricks.

The low consumption by the insects of 'IAC 17' could have been due to the presence of few stimuli for the start of feeding (low number of pricks) and/or the presence of unpalatable compounds (short feeding period) to *N. viridula* adults, which inhibits feeding behavior (Lara, 1991), indicating the expression of feeding non-preference as a mechanism of resistance. 'IAC PL1' could contain feeding inhibitors, but at lower concentrations. These inhibitors, according to Vendramim and Guzzo (2009), are called fagodeterrents, which may reduce the consumption but not prevent it, as was observed in the current research.

'Conquista', 'IAC 19', 'IAC 24', 'BRS 242RR' and 'TMG 117RR' were found to be more stimulating at the beginning of the feeding process. PI 227687 presented a relatively low number of pricks in comparison to the others, indicating the expression of feeding non-preference. However, the time spent feeding was long, suggesting that the insects were not stimulated to start feeding on this genotype, but that they were stimulated by the first prick to taste.

Density of trichomes in the green pods

The green pods of 'IAC 17' presented a higher density of trichomes (hooked and acicular), not just differing from 'TMG 117RR' and PI 227687 (Table 5). 'TMG 103RR', 'Conquista', 'TMG 121RR', and 'BRS 242RR' presented the lowest density levels, whereas all of the others showed intermediate means.

The initial idea of a negative correlation between a high number of trichomes in the green pods and a low feeding preference applied to 'IAC 17', which showed a high level of non-preference. Conversely, 'TMG 103RR', 'TMG 121RR', 'Conquista' and 'BRS 242RR', which stood out as being the most frequently consumed, but which presented a lower density of trichomes, corroborated the influence of this structure on the

Table 5. Mean density (\pm SE) of trichomes in 0.25 cm² and mean texture (\pm SE) of the green pods of the different soybean genotypes.

Genotype	Density of trichomes	Texture (gf cm ⁻²)
'TMG 103RR'	135.0 \pm 3.54e	99.2 \pm 3.07b
'Conquista'	166.3 \pm 3.01d	67.2 \pm 2.48c
'TMG 121RR'	173.4 \pm 3.65d	116.2 \pm 9.27b
'BRS 242RR'	175.0 \pm 3.18cd	70.4 \pm 4.25c
'IAC 24'	191.1 \pm 3.90bc	74.4 \pm 4.85c
'IAC PL1'	192.4 \pm 3.65b	59.6 \pm 2.26c
'IAC 19'	192.5 \pm 3.97b	69.7 \pm 1.99c
PI 227687	199.9 \pm 4.56ab	216.6 \pm 7.92a
'TMG 117RR'	206.8 \pm 3.48ab	74.9 \pm 3.23c
'IAC 17'	212.2 \pm 3.94a	75.7 \pm 3.24c
CV, %	3.19	16.62

Means followed by the same lower case letter in each column did not differ according to the Tukey test ($P \leq 0.05$). The original data were transformed to $(x + 0.5)^{1/2}$ for analysis. CV: coefficient of variation.

expression of resistance of soybean genotypes to the bugs. This relationship between the number of trichomes and the level of consumption of green pods did not apply to 'TMG 117RR' or 'IAC 19', suggesting a suppression of the action of this physical barrier by a greater stimulus, for instance, a volatile stimulus.

When observing the behavior of various soybean genotypes against attack by *N. viridula*, Panizzi et al. (1981) reported a low percentage of damaged seeds in PI 227687. It is possible to infer that its resistance is connected to the morphology of the green pods, which inhibited pricking, when taking into account the high density of trichomes, as observed for this genotype in this research, and the low number of pricks. Lourenção et al. (1997) reported an influence of the density of trichomes in green pods on field infestation, especially at young stages of this pentatomid, since, due to its small size, they are more influenced by trichomes and other physical barriers of the plant.

In the present study, since the preference tests for the green pods were only carried out for adults, it is possible that the trichomes may have greater influence on the feeding of younger stages. The index of correlation (Figure 3) between the number of insects attracted 60 min after their release and the density of trichomes was negative and significant for 'IAC 17', indicating that trichomes are the main resistance factor of this genotype to this insect.

Texture of the green pods

The textural analysis of the pods (Table 5) showed that PI 227687 has greater resistance to penetration than all of the other genotypes. 'TMG 121RR' and 'TMG 103RR' remained in an intermediate position; whereas all of the other genotypes presented lower means.

As the texture of pods decreased, the insects performed a greater number of pricks in 'Conquista', 'IAC 19', 'IAC 24', 'TMG 117RR', and 'BRS 242RR', indicating a lower preference of the insects to feed from plants with a more rigid epidermis. According to Vendramim and Guzzo

(2009), the difference in texture is directly related to deposits of silica and lignin, which work as a resistance factor to insects in many cultivated plants. As for 'IAC 17', the opposite behavior was observed, showing that the trichomes have a greater influence on host selection than texture itself. This may explain the fact that this material presented a low texture and also a low number of pricks.

In a general way, it was observed that the mature grains of 'TMG 117RR' and 'TMG 121RR' showed a low attractiveness to *N. viridula* adults. As for the green pods, 'IAC 17' and PI 227687 showed a non-feeding preference. In 'IAC 17', the non-feeding preference was related to the high density of trichomes that were present in the green pods.

CONCLUSIONS

Considering the parameters evaluated, 'IAC 17' and PI 227687 expressed resistance for non-preference against *N. viridula*, being 'IAC 17' less consumed. The green pods of 'IAC 17', 'TMG 117RR', and PI 227687 showed high density of trichomes and this morphological characteristic seems to be the main factor of resistant against adults of the southern green stink bug in 'IAC 17', indicating non-preference for feeding. Due to the great importance of *N. viridula* for the soybean crop worldwide, these results may be useful for soybean breeding programs aiming to develop genotypes that are resistant to this stink bug. In addition, 'IAC-17' is a genotype already commercially available and can be recommended in places where there is a history of incidence of *N. viridula*, aiming the population management of these species.

ACKNOWLEDGEMENTS

The authors thank Dr. Daniel R. Sosa-Gomez for supplying *N. viridula* eggs and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for financing this research.

LITERATURE CITED

- Belorte, L.C., Z.A. Ramiro, A.M. Faria, e C.A.B. Marino. 2003. Danos causados por percevejos (Hemiptera: Pentatomidae) em cinco cultivares de soja (*Glycine max* (L.) Merrill, 1917) no Município de Araçatuba, SP. Arquivos do Instituto Biológico 70:169-175.
- Byrne, F.J., S. Castle, N. Prabhaker, and N.C. Toscano. 2003. Biochemical study of resistance to imidacloprid in B biotype *Bemisia tabaci* from Guatemala. Pest Management Science 59:347-352.
- Chocorosqui, V.R., and A.R. Panizzi. 2004. Impact of cultivation systems on *Dichelops melacanthus* (Dallas) (Heteroptera: Pentatomidae) population and damage and its chemical control on wheat. Neotropical Entomology 33:487-492.
- Çokl, A., and J.G. Millar. 2009. Manipulation of insect signaling for monitoring and control of pest insects. Biorational Control of Arthropod Pests 279-316.

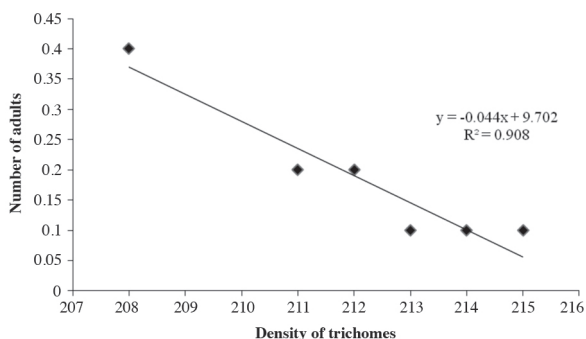


Figure 3. Correlation between attractiveness of adult *Nezara viridula* to green pods and trichome density of green pods of genotype 'IAC 17'.

- Corrêa-Ferreira, B.S. 1985. Criação massal do percevejo verde *Nezara viridula* (L.) Circular Técnica 11. 19 p. Embrapa-CNPSo, Londrina, Paraná, Brasil.
- Corrêa-Ferreira, B.S., e A.R. Panizzi. 1999. Percevejos da soja e seu manejo. Circular Técnica 24. 45 p. Embrapa-CNPSo, Londrina, Paraná, Brasil.
- EMBRAPA. 2010. Soja Conquista resiste ao cancro da haste. Empresa Brasileira de Pesquisa Agropecuária. 2010. Available at <http://www.embrapa.br/imprensa/noticias/1996/julho/bn.2004-11-25.6692269003/> (accessed October 2010).
- Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. 11 p. Iowa State University, Ames, Iowa, USA.
- Gore, J., A.C. Abel, J.J. Adamczyk, and G. Snodgrass. 2006. Influence of soybean planting date and maturity group on stink Bug (Heteroptera: Pentatomidae) populations. *Environmental Entomology* 35:531-536.
- Hart, K.A., and D. Pimentel. 2002. Environmental and economic costs of pesticide use. p. 237-239. In Pimentel, D. (ed.) *Encyclopedia of pest management*. Marcel Dekker, New York, USA.
- Knight, K.M.M., and G.M. Gurr. 2007. Review of *Nezara viridula* (L.) management strategies and potential for IPM in field crops with emphasis on Australia. *Crop Protection* 26:1-10.
- Lara, F.M. 1991. Princípios de resistência de plantas a insetos. 136 p. Icone, São Paulo, Brasil.
- Lin, H., M. Kogan, and D. Fischer. 1990. Induced resistance in soybean to the Mexican bean beetle (Coleoptera: Coccinellidae): comparisons of inducing factors. *Environmental Entomology* 19:1852-1857.
- Lordello, A.I.L., and F.M. Lara. 1980. Oviposition preference of *Spodoptera frugiperda* (J.E. Smith, 1797) to sorghum genotypes in laboratory conditions. *Anais da Sociedade Entomológica do Brasil* 9:11-21.
- Lourenção, A.L., M.A.C. Miranda, J.C.V.N.A. Pereira, e G.M.B. Ambrosano. 1997. Resistência de soja a insetos. X: Comportamento de cultivares e linhagens em relação a percevejos e desfolhadores. *Anais da Sociedade Entomológica do Brasil* 26:543-550.
- Lourenção, A.L., J.C.V.N.A. Pereira, M.A.C. Miranda, e G.M.B. Ambrosano. 1999. Danos de percevejos e de lagartas em cultivares e linhagens de soja de ciclos médio e semi-tardio. *Anais da Sociedade Entomológica do Brasil* 28:157-167.
- Lourenção, A.L., J.C.V.N.A. Pereira, M.A.C. Miranda, e G.M.B. Ambrosano. 2000. Avaliação de danos causados por percevejos e por lagartas em genótipos de soja de ciclo precoce e semiprecoce. *Pesquisa Agropecuária Brasileira* 35:879-886.
- McMillian, W.W., and B.R. Wiseman. 1972. Host plant resistance: A twentieth century look at the relationship between *Zea mays* L. and *Heliothis zea* (Boddie). 131 p. University of Florida, Gainesville, Florida, USA.
- Miranda, M.A.C., N.R. Braga, A.L. Lourenção, F.T.S. Miranda, S.H. Unêda, e M.F. Ito. 2003. Descrição, produtividade e estabilidade da cultivar de soja 'IAC 24', resistente a insetos. *Bragantia* 62:19-27.
- Moscardi, F., C.S. Barfield, and G.E. Allen. 1981. Effects of temperature on adult velvet bean caterpillar oviposition, egg hatch and longevity. *Annals of the Entomological Society of America* 74:167-171.
- Olson, D.M., J.R. Ruberson, A.R. Zeilinger, and D.A. Andow. 2011. Colonization preference of *Euschistus servus* and *Nezara viridula* in transgenic cotton varieties, peanut, and soybean. *Entomologia Experimentalis et Applicata* 139:161-169.
- Panizzi, A.R. 1991. Ecologia nutricional de insetos sugadores de sementes. p. 253-289. In Panizzi, A.R., and R.P. Parra (eds.) *Ecologia nutricional de insetos e suas implicações no manejo de pragas*. Manole, São Paulo, Brasil.
- Panizzi, M.C.C., I.A. Bays, R.A.S. Kiihl, e M.P. Porto. 1981. Identificação de genótipos fonte de resistência a percevejos-pragas da soja. *Pesquisa Agropecuária Brasileira* 6:33-37.
- Panizzi, A.R., and F.Jr. Slanski. 1985. Review of phytophagous pentatomids (Hemiptera: Pentatomidae) associated with soybean in the Americas. *Florida Entomologist* 68:184-214.
- Paron, M.J.F.O., e F. M. Lara. 2005. Relação entre tricomas foliares de genótipos de feijoeiro comum, *Phaseolus vulgaris* L. e resistência a *Diabrotica speciosa* Germar, 1824 (Coleoptera: Chrysomelidae). *Ciência e Agrotecnologia* 29:894-898.
- Piubelli, G.C., C.B. Hoffmann-Campo, I.C. Arruda, and F.M. Lara. 2003. Nymphal development, lipid content, growth and weight gain of *Nezara viridula* (L.) (Heteroptera: Pentatomidae) fed on soybean genotypes. *Neotropical Entomology* 32:127-132.
- Raij, B., H. Cantarella, J.Á. Quaggio, e A.M.C. Furlan. 1997. Recomendações de adubação e calagem para o Estado de São Paulo. 285 p. Instituto Agronômico, Fundação IAC, Campinas, São Paulo, Brasil.
- Rossetto, C.J., A.L. Lourenção, T. Igue, e M.A.C. Miranda. 1981. Picadas de alimentação de *Nezara viridula* em cultivares e linhagens de soja de diferentes graus de suscetibilidade. *Bragantia* 40:109-114.
- SAS Institute. 2001. SAS/STAT: Users guide. 502 p. SAS Institute, Cary, North Carolina, USA.
- Smith, C.M. 2005. Plant resistance to arthropods molecular and conventional approaches. Springer, Dordrecht, The Netherlands.
- Sosa-Gomez, D.R., J.J. Silva. 2010. Neotropical brown stink bug (*Euschistus heros*) resistance to methamidophos in Paraná, Brazil. *Pesquisa Agropecuária Brasileira* 45:767-769.
- Temple, J.H., J.A. Davis, S. Micinski, J.T. Hardke, P. Price, and B.R. Leonard. 2013. Species composition and seasonal abundance of stink bugs (Hemiptera: Pentatomidae) in Louisiana soybean. *Environmental Entomology* 42:648-657.
- USDA. 2009. Oilseeds: World markets and trade. Circular series FOP 06-09. 35 p. United States Department of Agriculture (USDA), Washington D.C., USA.
- Vendramim, D.J., e E.C. Guzzo. 2009. Resistência de plantas e a bioecologia e nutrição dos insetos. p. 1055-1105. In Panizzi, A.R., and J.R.P. Parra (eds.) *Bioecologia e nutrição de insetos: base para o manejo integrado de pragas*. Embrapa Informação Tecnológica, Brasília, Brasil.
- Winer, B.J., D.R. Brown, and K.M. Michels. 1991. *Statistical principles in experimental design*. 3rd ed. 1057 p. McGraw-Hill, New York, USA.