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Characterization of antixenosis to *Dichelops melacanthus* (Hemiptera: Pentatomidae) in soybean genotypes

Vinícius Fernandes Canassa ^a, Edson Luiz Lopes Baldin^a, José Paulo Franco Bentivenha^a,
Luiz Eduardo da Rocha Pannuti^a and André Luiz Lourenção^b

^aDepartment of Crop Protection, College of Agronomic Science, Sao Paulo State University, Botucatu, Brazil; ^bAgronomic Institute of Campinas, Campinas, Brazil

ABSTRACT

Soybeans are of great importance in the world agricultural landscape, and their productive potential is significantly reduced by attacks from insect pests. Factors such as the expansion of national agricultural regions, together with no-tillage management and “off-season” maize cultivation, have favored the increase of secondary species such as *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae), intensifying the damage caused by the soybean stink bug complex. The use of resistant genotypes may be a valuable strategy as an alternative to the excessive use of chemical control in crops. This study evaluated the attractiveness and feeding preferences of the green-belly stink bug in 17 soybean genotypes in different maturity groups (early, semiearly, and late) to characterize the expression of antixenosis resistance. To this end, free-choice tests of attractiveness and food preference were performed under laboratory conditions. The early genotypes PI 171451 and D 75-10169, the semiearly genotypes IAC 78-2318, “IAC 100”, IAC 74-2832, PI 227687, and “IAC 24” and the late genotypes PI 274454, PI 274453, and L 1-1-01 expressed significant levels of antixenosis against adult *D. melacanthus*. These results will be useful for soybean breeding programs focusing on the stink bug resistance complex.

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Antixenosis; *Glycine max* (L.) Merrill; host plant resistance; green-belly stink bug

1. Introduction

The pentatomids stink bugs play an important role in soybean [*Glycine max* (L.) Merrill] cultivation, potentially causing significant damage to plants. The predominant species from this group found in crop fields are the green stink bug, *Nezara viridula* (L.); the brown stink bug, *Euschistus heros* (Fabr.); and the red-banded stink bug, *Piezodorus guildinii* (West.). These species receive the most attention from farmers, and their prevalence varies depending on the year and the area of cultivation (Panizzi 2007). However, other species, such as the green-belly stink bug, *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae), that were previously considered secondary pests, are becoming increasingly prevalent on crops, resulting in greater damage from the soybean stink bug complex (Blasioli-Moraes et al. 2014).

In recent decades, Brazil has undergone significant changes in grain production systems, especially concerning the expansion of the agricultural region, the intensification of the tillage system, and the cultivation of “off-season” maize (Smaniotto & Panizzi 2015). These factors have contributed to changes in the composition of the insect fauna associated with soybeans and changes in the “status” of pest species that are usually associated with agriculture. Regarding the green-

belly stink bug, straw and crop residues left by no-tillage management and rotations involving corn and soybeans provide shelter and plentiful food, favoring the survival of nymphs and adult insects (Chocorosqui & Panizzi 2004; Smaniotto & Panizzi 2015).

The stink bug, *D. melacanthus*, feeds throughout the reproductive period of soybean plants (Smaniotto & Panizzi 2015). As the plant cycle comes to an end, the plants become less attractive and provide inadequate food and other resources for insect breeding, encouraging dispersion toward late genotypes and/or alternative hosts such as dayflowers [*Commelina benghalensis* (L.)] or *Indigofera* spp. (Silva, Ventura et al. 2013; Smaniotto & Panizzi 2015). The bugs feed directly from the seeds, causing damage to soybeans from the beginning of pod formation until the end of grain filling. Nymphs and adults insert their stylets into the pods, sucking out nutrients necessary for their development and forming small dark spots on the seeds (Corrêa-Ferreira 2005; Sosa-Gómez et al. 2010). The attacked grains become smaller, wrinkled, hollow, and darker, and they suffer a reduction of the oil content and an increased in the protein content (Corrêa-Ferreira 2005). This damage also facilitates the development of fungi such as *Nematospora coryli* (Peglion), which causes stigma to mycosis, affecting the quality of

soybeans and hindering their commercialization (Panizzi 1997). In addition, feeding and injection of toxins by stink bugs often lead to physiological disorders in soybean. The affected plants exhibit abnormal retention of leaves, irregularly ripening pods and the presence of green rods, forcing farmers to use desiccants to facilitate harvesting (Sosa-Gómez and Moscardi 1995).

Among the control tactics recommended for managing populations of stink bugs in soybean crops, chemical methods are still the most commonly employed (Brown et al. 2012). However, the excessive use of synthetic broad spectrum insecticides can cause a major imbalance in insect fauna, increasing the population levels of major pests and changing the “status” of insects of secondary importance (EMBRAPA 2010). The misuse of chemical insecticides can also generate environmental pollution, eliminate beneficial insects, poison animals and humans, and accelerate the selection of individuals that are resistant to the ingredients of the most commonly applied products (Prabhaker et al. 1985; Baur et al. 2010).

In this context, the use of resistant genotypes stands out as an important tool for pest control in crops (Painter 1951; Smith 2005). Among the main advantages of this method, we highlight the absence of toxic residues in food and the environment, the performance achieved against any level of pest infestation, and the reduction of populations of insect pests to levels that do not cause economic damage (Smith 2005). According to Painter (1951), such resistance can be divided into three categories: antixenosis, antibiosis, and tolerance. Antixenosis causes changes in insect behavior during the selection of the host due to the presence of allelochemicals or the morphological characteristics of the plant, making the plant less favorable for feeding, shelter and/or oviposition by insects. In antibiosis, the plant is typically used for food, but it negatively affects the insect’s biology, interfering with its development, reproduction and/or survival. Tolerance is defined as

the ability of the plant to resist or recover from an injury caused by an insect without affecting its biology or behavior (Panda and Khush 1995; Smith 2005).

Faced with the growing potential for *D. melacanthus* damage in soybean crops and the need to develop less aggressive methods of control, this study evaluated the attractiveness and food preferences of the green-belly stink bug in 17 soybean genotypes to characterize the expression of resistance.

2. Materials and methods

The attractiveness and feeding preferences of *D. melacanthus* were evaluated through free-choice tests under laboratory conditions ($T = 26 \pm 2^\circ\text{C}$, $\text{RH} = 65\% \pm 10\%$, and photoperiod = 14 h), using green pods (R5/R6) from 17 soybean genotypes (Table 1), belonging to different maturity groups (early, semiearly, and late).

The rearing of the green-belly stink bug stock began with eggs from the DuPont Experimental Station in Paulinia, São Paulo, Brazil. The insects were placed in plastic containers ($40 \times 22 \times 14$ cm), covered with organdy fabric to allow adequate ventilation. The inner bottom surface of the containers was completely covered with filter paper, facilitating the absorption of droppings. To prevent escape and movement of the insects at the top of the containers, a layer (2 cm) of solid Vaseline was applied with a number 18 brush.

The nymphs and adults of *D. melacanthus* were kept in separate containers based on their development stage, and were provided a natural diet. Each container was provided with separately wrapped fresh green bean pods [*Phaseolus vulgaris* (L.)] (five in total), sunflower seeds [*Helianthus annuus* (L.)], and raw shelled peanuts [*Arachis hypogaea* (L.)], placed in Petri dishes ($\varnothing = 4$ cm). The food was replaced every seven days to prevent the growth of micro-organisms. Water was provided in cotton packed inside a Petri dish ($\varnothing = 4$ cm), which was moistened with distilled water every day,

Table 1. Soybean genotypes used, grouped as phenology, their genealogies and resistance history.

Circle	Genotype	Genealogy	Resistance historic
Early	“IAC 17”	D72-9601-1 × “IAC 8”	Antixenosis to <i>B. tabaci</i> biotype B (Valle & Lourenção 2002; Silva et al. 2012)
	“IAC 23”	BR-6 × IAC 83-23	Resistant to insects (Miranda et al. 2003)
	PI 171451	Japan	Resistant to stinkbugs (Rossetto et al. 1986; Silva, Baldin et al. 2013)
	PI 229358	Tokyo, Japan	Resistant to stinkbugs (Rossetto et al. 1986; Silva, Baldin et al. 2013)
	D 75-10169	“Govan” × (F4 “Bragg” × PI 229358)	Multiple insect resistance in its genealogy (Silva, Baldin et al. 2013); resistant to <i>B. tabaci</i> biotype B (Valle & Lourenção 2002)
	“Coodetec 208”	OC-4 × Williams 20	Commercial susceptible (Silva, Baldin et al. 2013)
Semiearly	“IAC 18”	D72-9601 × “IAC 8”	Antibiosis to <i>P. guildinii</i> (Silva, Baldin et al. 2013)
	“IAC 24”	IAC80-1177 × IAC 83-288	Antixenosis to <i>B. tabaci</i> biotype B (Valle & Lourenção 2002; Silva et al. 2012)
	“IAC 100”	“IAC-12” × IAC 78-2318	Antibiosis to <i>P. guildinii</i> (Silva, Baldin et al. 2013)
	IAC 74-2832	“Hill” × PI 274454	Antibiosis to <i>P. guildinii</i> (Silva, Baldin et al. 2013)
	IAC 78-2318	D72-96-1 × IAC 73-227	Resistant to stinkbugs (Rossetto et al. 1986; Silva, Baldin et al. 2013)
Late	PI 227687	Okinawa, Japan	Resistant to stinkbugs (Rossetto et al. 1986; Silva, Baldin et al. 2013)
	“IAC 19”	D72-9601-1 × “IAC 8”	Antixenosis to <i>B. tabaci</i> biotype B (Valle & Lourenção 2002); antibiosis to <i>P. guildinii</i> (Silva, Baldin et al. 2013)
	PI 274453	Okinawa, Japan	Resistant to stinkbugs (Rossetto et al. 1986; Silva, Baldin et al. 2013)
	PI 274454	Okinawa, Japan	Resistant to stinkbugs (Rossetto et al. 1986; Silva, Baldin et al. 2013)
	L 1-1-01	BR-6 × “IAC 100”	Antibiosis to <i>P. guildinii</i> (Silva, Baldin et al. 2013)
	“Conquista”	Lo76-44842 × Numbaira	Commercial susceptible (Silva, Baldin et al. 2013)

and the water dish was replaced once per week to prevent the growth of micro-organisms.

Additionally, cotton pads were placed in the corners of the containers to serve as a substrate for oviposition by the bugs. The oviposited eggs were collected daily, to avoid the consumption of eggs by the stink bugs. The eggs (up to 24 h) were collected and placed in Petri dishes ($\varnothing = 8.5$ cm), which were lined with filter paper on the bottom surface.

The tests were conducted in spherical PVC arenas (20 cm $\varnothing \times 6$ cm height) with coated base paper placed on a filter with a Styrofoam plate of 2 cm. Within each arena, two pods per genotype were placed with a random, equidistant distribution. Two pairs of adult insects per genotype (up to 48 h old) were subsequently released into the center of each arena. The insects were fasted for 24 h before the initiation of testing. Evaluations were performed by noting the number of insects from each genotype present on the pods at 15, 30, 45, 60, and 90 min and 2, 3, 12, and 24 h after release.

Along with the recording of the attractiveness ratings, the consumption of the pods of the different genotypes by the insects was assessed for a period of 90 min, evaluating the number of insect punctures per genotype, the total feeding time on the material and the alimentation time per puncture, with the aid of a chronometer. At the end of the test, the attractiveness index was calculated as follows: $IA = (2 \times G)/(G + P)$, where G is the number of insects observed on the evaluated genotype, and P is the number of insects observed on a standard genotype (more susceptible commercial material within each phenological group). The genotypes “Coodetec 208” (early), “IAC 24” (semi-early), and “Conquista” (late) were adopted as standards. The index ranges from 2 (attractive) to 0 (repellent), and a value of 1 indicates neutrality (Silva et al. 2014).

The pods were obtained from plants grown in 3 L pots inside a greenhouse, were free of insect infestation, and presented a phenological stage of R5/R6, as described by Fehr and Caviness (1977). The pots were sown in stages to provide pods (R5/R6) throughout the test period. As a substrate, we used fertilized soil with the pH adjusted to be suitable for soybean culture (Mascarenhas & Tanaka 1997).

Determination of the number of punctures on the pods was conducted using visual inspection, which is one of the methods utilized by Rossetto et al. (1981). The feeding time was determined with the aid of a digital timer, which was activated at the beginning of feeding by each insects and stopped at the time when the bug withdrew its stylet from the pod. At the end of the observation period, the insects used in each experiment were discarded.

The trials followed a randomized block design with 10 repetitions for each evaluated soybean phenological group (early, semiearly, and late).

The data were subjected to analysis of variance using the *F*-test. Normality was verified with the Shapiro–Wilk test and homogeneity through the Levene test. To check for significance in the effects of the treatments, we used the Fisher LSD test ($P \leq 0.05$) for the comparison of means. For the statistical analyses, we employed the statistical package PROC MIXED-SAS 9.2 (SAS Institute 2001).

3. Results and discussion

Considering the general average results of the attractiveness test, the pods of the assessed early genotypes showed no differences in attractiveness to adult *D. melananthus* (Figure 1). Thus, all of the materials were classified as neutral compared with the standard “Coodetec 208” (Table 2). Regarding the feeding behavior of the stink bugs, genotypes PI 171451 and D 75-10169 stood out due to presenting the lowest mean number of stink bug punctures (1.40 and 1.30, respectively), the lowest mean total feeding time (24.20 and 28.40), and the lowest mean feeding time per puncture (6.47 and 9.35), indicating the presence of antixenosis (Table 3). In contrast to the results obtained in the present work, a recent study that evaluated the feeding of *N. viridula* on different soybean genotypes noted low consumption of “IAC 17” pods and suggested the presence of inhibitory compounds deterring feeding by stink bugs (Souza et al. 2013). The fact that this genotype was observed to be very attractive to *D. melananthus* (Figure 1) indicates that the same genotype may have different susceptibility patterns in relation to different species of insect (Smith 2005).

When evaluating the behavior of the whitefly *Bemisia tabaci* (Gennadius) biotype B in relation to different soybean genotypes, other authors (Valle & Lourenção 2002; Valle et al. 2012) have also reported the expression of antixenosis resistance (low attractiveness and oviposition) in D 75-10169. The expression of multiple resistances (against two or more insect species) is highly desirable and can be related to the genealogy of the material and the possibility of inheriting genes that confer resistance (Hoffmann-Campo et al. 1994). Compared with other soybean cultivars, the PI 171451 genotype has also been characterized as resistant to the red-banded stink bug *P. guildinii*, (Silva et al. 2014), similar to the data obtained in this work. According to these authors, the pods of this material are less attractive and less frequently consumed by adult insects.

Antixenosis in resistant soybean plants can derive from morphological factors, such as hardness of the plant tissue (preventing the penetration of the stink bug stylet) and the length and density of trichomes on pods (Silva et al. 2014), chemical compounds, such as saponins and protease inhibitors, which can reduce or

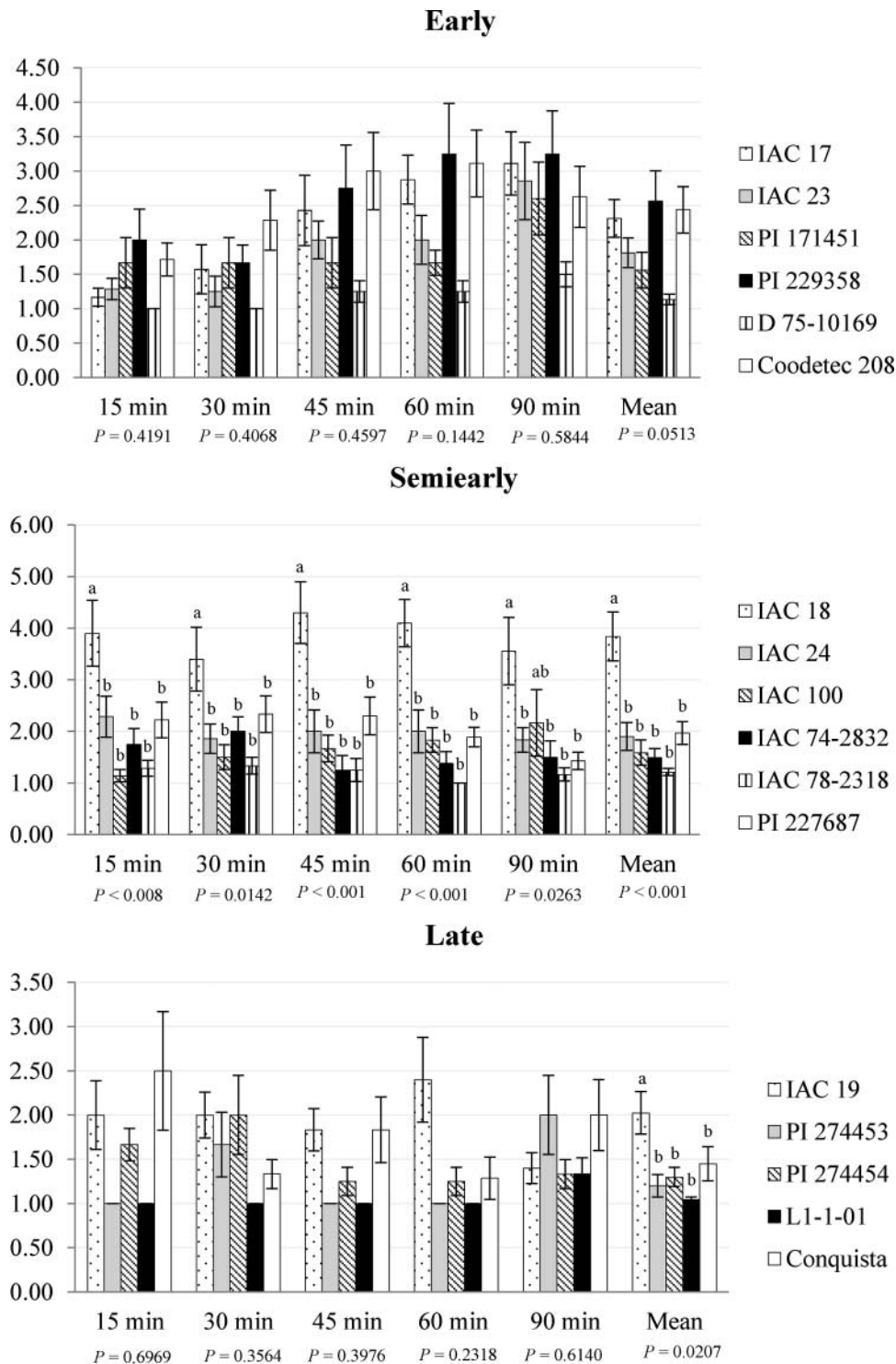


Figure 1. Mean number (\pm SE) adult *D. melacanthus* attracted by pods R5/R6 in early, semiearly, and late maturing soybean genotypes obtained in different periods of observation.

inhibit insect attacks on plants (Smith 2005) and nutritional deficiency (Panda & Khush 1995).

In experiments involving *P. guildinii*, Silva et al. (2014) observed differences in trichome density among 17 genotypes of soybean with different maturation periods. In early genotypes, these authors found a positive correlation of the presence of these structures, which can interfere with the mobility of insects and act as a barrier to feeding, with insect attraction to the materials, observing that the higher the trichome density, the

lower the infestation by stink bugs. Testing of multiple choices using *Anticarsia gemmatilis* (Hübner) (Lepidoptera: Noctuidae) revealed that leaf disc materials from PI 227687, PI 229358, and PI 274454 were eaten less frequently indicating antixenosis resistance (Hoffmann-Campo et al. 1994). According to the authors, the effect was caused by volatile compounds that inhibited or repelled feeding lepidopterans.

Regarding the attractiveness of genotypes with a semiearly cycle, significant differences were found for

Table 2. Index of attractiveness to stink bug *D. melananthus* in the respective groups and maturing soybean genotypes rating after 24 h of evaluation.

Circle	Genotype	IA ^a	Classification
Early	"IAC 17"	0.91 ± 0.035	Neutral
	"Coodetec 208"	1.00 ± 0.035	Standard
	PI 229358	1.00 ± 0.035	Neutral
	"IAC 23"	0.83 ± 0.035	Neutral
	D 75-10169	0.64 ± 0.035	Neutral
Semiearly	PI 171451	0.84 ± 0.035	Neutral
	"IAC 18"	1.00 ± 0.020	Standard
	"IAC 24"	0.68 ± 0.020	Repellent
	PI 227687	0.68 ± 0.020	Repellent
	IAC 74-2832	0.57 ± 0.020	Repellent
	"IAC 100"	0.59 ± 0.020	Repellent
	IAC 78-2318	0.47 ± 0.020	Repellent
Late	"IAC 19"	1.04 ± 0.057	Attractive
	"Conquista"	1.00 ± 0.057	Standard
	PI 274453	0.85 ± 0.057	Neutral
	PI 274454	0.92 ± 0.057	Neutral
	L 1-1-01	0.76 ± 0.057	Neutral

^a IA – Index of attractiveness: $IA = (2 \times G)/(G + P)$, where G = treatment and P = standard.

all the evaluation periods (Figure 1). At the first four observation times (15–90 min), all of the other genotypes (except "IAC 100" at 90 min) were less attractive than "IAC 18". Considering the general averages of the evaluations, genotypes "IAC 18" (3.84) were the most attractive, and IAC 78-2318 (1.21), IAC 74-2832 (1.49), "IAC 100" (1.58), "IAC 24" (1.90), and PI 227687 (1.96) were the least attractive (Figure 1), indicating the expression of antixenosis. Silva et al. (2014) also assessed the attractiveness of semiearly soybean genotypes to *P. guildinii* and highlighted "IAC 100", IAC 78-2318, and PI 227687 as being less attractive, similar to the findings for *D. melananthus*. Additionally, according to those authors, the genotype "IAC 18" was the most attractive to the red-banded stink bug. As noted above, the resistance of the early genotypes "IAC 100" and IAC 78-2318 verified in this work is probably related to the genealogy of the materials as well as the presence of substances such as saponins

and protease inhibitors within the grains, which may inhibit insect feeding (Silva et al. 2014).

Regarding the genotype "IAC 100", in addition to being a descendant of IAC 78-2318, its genealogy includes PIs (Plant Introductions) 229358 and 274454, which are considered to be sources of resistance to the stink bug complex. "IAC 100" has been used in several studies as a standard genotype with resistance against defoliators and sucking insects. Exploration of the "IAC 100" genotype as a germplasm source of multiple resistance to insect pests is common, because, this genotype also exhibits low leaf retention, few spots on the seeds in the presence of a heavy stink bug infestation and good grain productivity (McPherson et al. 2007).

Building on the attractiveness index calculated for semiearly genotypes (Table 2), all of the genotypes were classified as repellents compared with the standard "IAC 18" genotype. These results are similar to those obtained by Silva et al. (2014), who also classified the green pods of the genotype "IAC 100" and PI 227687 as repellent to *P. guildinii*, even when "IAC 24" was adopted as the standard genotype. Regarding the food preferences of the stink bugs in relation to semiearly genotypes (Table 3), the "IAC 18" genotype stood out negatively, showing the highest mean number of punctures (9.70) and total consumption (261.30 min), indicating that it was more susceptible than the other genotypes. No differences were found regarding the feeding time per puncture (Table 3). Although there are no previous studies that have evaluated the feeding behavior of *D. melananthus* in relation to this group of soybean genotypes, PI 227687, "IAC 100", IAC 74-2832, and IAC 78-2318 have been reported to exhibit antixenosis (feeding) against *P. guildinii* (Silva et al. 2014), similar to the findings of this study.

Table 3. Means (± SE) number of punctures, total feeding time, and feeding time per puncture of adult *D. melananthus* in pods R5/R6 soybean genotypes early, semiearly, and late.

Circle	Genotype	No. of punctures ^a	Total time (min) ^a	Feeding/puncture (min) ^a
Early	"IAC 17"	4.80 ± 0.93 a	103.00 ± 21.13 a	26.97 ± 6.98 a
	PI 229358	4.30 ± 1.05 a	147.00 ± 43.54 a	28.69 ± 7.00 a
	"Coodetec 208"	4.40 ± 0.79 a	142.30 ± 27.10 a	30.92 ± 5.93 a
	"IAC 23"	3.40 ± 0.92 ab	95.90 ± 22.64 ab	29.17 ± 5.10 a
	"PI 171451"	1.40 ± 0.65 b	24.40 ± 12.61 b	6.47 ± 3.09 b
	D 75-10169	1.30 ± 0.58 b	28.40 ± 14.00 b	9.35 ± 3.61 b
	P	0.009	0.002	0.004
Semiearly	"IAC 18"	9.70 ± 1.21 a	261.30 ± 33.06 a	27.54 ± 1.78
	"IAC 24"	3.10 ± 0.74 b	109.60 ± 32.18 b	25.51 ± 5.92
	PI 227687	4.00 ± 0.52 b	124.40 ± 22.71 b	33.55 ± 5.64
	IAC 78-2318	2.80 ± 0.65 b	63.10 ± 10.85 b	25.34 ± 5.54
	IAC 74-2832	2.50 ± 0.27 b	70.60 ± 13.83 b	27.98 ± 4.17
	"IAC 100"	2.40 ± 0.37 b	72.50 ± 15.72 b	34.56 ± 8.44
	P	<0.001	<0.001	0.791
Late	"IAC 19"	3.10 ± 0.96 a	47.30 ± 17.81	11.81 ± 3.63
	"Conquista"	2.30 ± 0.47 ab	34.30 ± 11.11	14.28 ± 4.56
	PI 274454	1.40 ± 0.48 bc	27.30 ± 12.09	9.82 ± 4.42
	PI 274453	0.60 ± 0.22 c	3.80 ± 2.84	2.35 ± 1.44
	L 1-1-01	0.90 ± 0.38 bc	15.09 ± 6.87	12.30 ± 6.57
	P	0.016	0.086	0.376

^a Mean followed by the same lower case letter per column do not differ by LSD test ($P \leq 0.05$).

In the attractiveness assays with late maturing soybean (Figure 1), the general averages identified L 1-1-01 (1.04), PI 274453 (1.20), PI 274454 (1.30), and “Conquista” (1.45) as less attractive to the stink bugs, indicating the expression of antixenosis and differing from “IAC 19” (2.02), which was more attractive (Figure 1). Building on the attractiveness index calculated for late genotypes (Table 2), the genotypes L 1-1-01 (0.76), PI 274453 (0.85), and PI 274454 (0.92) were classified as neutral compared with the standard “Conquista” genotype and the genotype “IAC 19” (1.04) was classified as attractive. Silva et al. (2014) reported the same feeding behavior of *P. guildinii* on those same genotypes.

Regarding the feeding behavior of the stink bugs, genotypes PI 274454 (1.4), PI 274 453 (0.60), and L 1-1-01 (0.90) stood out because they presented the lowest mean numbers of stink bug punctures, indicating the expression of antixenosis. Although there were no significant differences between these materials regarding the total feeding time and the feeding time per puncture, there was a lower tendency of the insects to feed on the pods of PI 274 453 (3.80 and 2.35 min/puncture), suggesting a higher level of resistance compared with PI 274454 (27.30 and 9.82 min/puncture) and L 01-01-01 (15.09 and 12.30 min/puncture). In a study in which the feeding preferences of *N. viridula* were evaluated among 10 soybean genotypes, Souza et al. (2013) also reported a high mean number of individuals attracted to and number of punctures on “IAC 19” and “Conquista”, confirming the sensitivity of these materials to feeding by the members of the stink bug complex. The occurrence of antixenosis (feeding) in genotypes PI 274454 and PI 274453 has also been observed in feeding preferences studies using *P. guildinii* (Silva et al. 2014), confirming the results obtained with the green-belly stink bug.

In this work, the proportion of stink bugs by genotypes, time assessment, as well as the arenas used was adequate to characterize antixenosis (feeding) in genotypes (Silva et al. 2014). Future studies may be conducted using fewer insects and/or genotypes per arena to check the observations obtained in this study. Here, significant levels of antixenosis were observed in genotypes PI 171451 and D 75-10169 (early); IAC 78-2318, “IAC 100”, IAC 74-2832, PI 227687, and “IAC 24” (semiearly); and PI 274454, PI 274453, and L 1-1-01 (late). These results are unprecedented for the species *D. melacanthus* and may be useful for future soybean breeding programs focusing on the development of resistant genotypes against the complex of pentatomid pests in crops.

4. Conclusions

The early genotypes PI 171451 and D 75-10169, the semiearly genotypes IAC 78-2318, “IAC 100”, IAC 74-

2832, PI 227687, and “IAC 24”, and the late genotypes PI 274454, PI 274453, and L 1-1-01 were shown to express antixenosis against adults of the green-belly stink bug, *D. melacanthus*.

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ORCID

Vinícius Fernandes Canassa  <http://orcid.org/0000-0002-6978-9551>

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