

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/317077197>

# Characterization of Antixenosis in Soybean Genotypes to *Bemisia tabaci* (Hemiptera: Aleyrodidae) Biotype B

Article in *Journal of Economic Entomology* · May 2017

DOI: 10.1093/jee/tox143

CITATIONS

2

READS

219

7 authors, including:



**Edson L. L. Baldin**

São Paulo State University

116 PUBLICATIONS 701 CITATIONS

[SEE PROFILE](#)



**Ivana FERNANDES DA Silva**

São Paulo State University

17 PUBLICATIONS 38 CITATIONS

[SEE PROFILE](#)



**José Paulo Franco Bentivenha**

Ihara

25 PUBLICATIONS 111 CITATIONS

[SEE PROFILE](#)



**Luiz Ricardo Tozin**

Federal Rural University of Rio de Janeiro

11 PUBLICATIONS 34 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Screening of essential oils extracted from cultivable plant species for their antimicrobial, antiparasitic, and insecticidal activities [View project](#)



Biological activities and spectrometric and spectroscopic studies of synthetic and natural products [View project](#)

## Characterization of Antixenosis in Soybean Genotypes to *Bemisia tabaci* (Hemiptera: Aleyrodidae) Biotype B

E.L.L. Baldin,<sup>1</sup> P. L. Cruz,<sup>2</sup> R. Morando,<sup>1</sup> I.F. Silva,<sup>1</sup> J.P.F. Bentivenha,<sup>3,4</sup> L.R.S. Tozin,<sup>5</sup> and T. M. Rodrigues<sup>5</sup>

<sup>1</sup>Department of Crop Protection, College of Agronomic Sciences, São Paulo State University, Botucatu, São Paulo 18610-307, Brazil (elbaldin@fca.unesp.br; rafaela\_morando@hotmail.com; ivanaf.silva@hotmail.com), <sup>2</sup>Federal Institute of Education, Science and Technology North of Minas Gerais, Arinos, Minas Gerais 38680-000, Brazil (patricia.agronomia@gmail.com), <sup>3</sup>Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo 13418-900, Brazil (jpbentivenha@gmail.com), <sup>4</sup>Corresponding author, e-mail: jpbentivenha@gmail.com, and <sup>5</sup>Department of Botanic, Institute of Biosciences of Botucatu, São Paulo State University, Botucatu 18618-000, São Paulo, Brazil (ricardo.tozin@gmail.com; tatiane@ibb.unesp.br)

Subject Editor: John Trumble

Received 27 February 2017; Editorial decision 26 April 2017

### Abstract

*Bemisia tabaci* biotype B (Gennadius) is one of the most important soybean pest worldwide. Herein, 15 soybean genotypes were evaluated, to characterize the occurrence of antixenosis to *B. tabaci* biotype B. Initially, a multiple-choice test with all genotypes was carried out, evaluating the settling and oviposition preference at 3 d after infestation, and the colonization by nymphs after 48 d of infestation. Subsequently, a no-choice test, using 14 genotypes, was conducted with infested plants individually, and the number of eggs was counted after 72 h. Then, 10 genotypes were selected (indicative of resistance and susceptibility), which were evaluated for whitefly settling 24, 48, and 72 h after infestation and for oviposition 72 h after infestation. The trichomes of the leaflets were characterized for density, size, and inclination to establish possible correlations with the settling and oviposition in the genotypes. In the first multiple-choice test, involving 15 genotypes, 'IAC-17,' 'IAC-19,' and UX-2569-159 expressed antixenosis against *B. tabaci*. 'Jackson,' 'P98Y11,' and PI-229358 exhibited the same behavior in the no-choice test. In the multiple-choice test, 'Jackson,' 'P98Y11,' and 'TMG1176 RR' were the least attractive and least used for oviposition. The antixenosis shown by 'Jackson,' 'P98Y11,' and PI-229358 may be related to the characteristics of the trichomes (lower density and inclined). Based on the experiments carried out, 'IAC-17,' 'IAC-19,' 'Jackson,' 'P98Y11,' PI-229358, TMG1176 RR, and UX-2569-159 are considered promising for resistance to *B. tabaci* biotype B and may be exploited in soybean breeding programs for resistance to insects.

**Key words:** sweetpotato whitefly, host plant resistance, nonpreference, trichome

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype B is one of the most invasive pests, and destructive to agriculture worldwide, and responsible for losses of billions of dollars annually (Perring et al. 1993, Brown et al. 1995, Oliveira et al. 2013). In Brazil, this insect has limited the productivity of several crops, including soybean [*Glycine max* (L.) Merrill] (Vieira et al. 2011).

*Bemisia tabaci* biotype B is an extremely polyphagous insect, which can directly damage plants when the nymphs and adults feed on the phloem sap and inject toxins, causing changes in the plant's vegetative and reproductive development. The insect can also cause indirect damage due to the excretion of honeydew, which favors the development of fungi (*Capnodium* sp.) that forms blackened colonies on plant tissues (sooty mold) and affects the photosynthetic capacity of plants (Musa and Ren 2005, Naranjo and Legg 2010,

Cameron et al. 2013). Other indirect damage is related to the capacity to transmit several viruses on plants (Navas-Castillo et al. 2011, Polston et al. 2014). In soybean, *B. tabaci* biotype B transmits the stem necrosis virus (*Cowpea mild mottle virus*—CpMMV), belonging to the *Carlavirus* genus (Marubayashi et al. 2010).

The management of this species is mainly done through the application of synthetic insecticides, which causes environmental imbalances, incurs production costs (Toscano et al. 2001, Vieira et al. 2013), and accelerates the insecticide resistance selection (Prabhaker et al. 1998, Sivasupramaniam and Watson 2000). Because of these factors, it is extremely necessary to evaluate more sustainable control alternatives, and also alignment with the integrated pest management (IPM), reducing the abusive use of insecticides. The use of resistant genotypes is a valuable tool

**Table 1.** List of evaluated soybean genotypes, respective genealogies or sources, and justifications for investigation for resistance to *B. tabaci* biotype B

Genotype	Genealogy or Source	History of resistance
'Conquista'	Lo76-4484 x 'Numbaira'	Susceptible commercial (Silva et al. 2012)
'Dowling' (PI 548663)	'Semmes' x PI 200492 (USDA/USA)	Antibiosis to <i>Aphis glycines</i> (Hill et al. 2004)
'IAC-17'	D72-9601-1 x 'IAC-8'	Antixenosis to <i>Bemisia tabaci</i> biotype B (Silva et al. 2012, Valle and Lourenção 2002)
'IAC-19'	D72-9601-1 x 'IAC-8'	Antixenosis to <i>B. tabaci</i> biotype B (Valle and Lourenção 2002)
'IAC-100'	'IAC-12' x 'IAC-78-2318'	Antixenosis to <i>Piezodorus guildinii</i> and <i>Nezara viridula</i> (Hemiptera: Pentatomidae) (Jones and Sullivan 1979, Silva et al. 2014)
'Jackson' (PI 548657)	'Volstate(2)' x 'Palmetto' (USDA/USA)	Antibiosis to <i>A. glycines</i> (Hill et al. 2006)
'KS-4202'	'KS4694' x 'C1842' (USA)	Tolerance to <i>A. glycines</i> (Pierson et al. 2010, Prochaska et al. 2013) and <i>B. tabaci</i> biotype B (Cruz et al. 2016)
PI-229358	Japan	Antixenosis to <i>P. guildinii</i> (Silva et al. 2014), beetles (Van Duyn et al. 1972), and lepidopterans (Hoffmann-Campo et al. 1994)
PI-274454	Japan	Antixenosis to <i>P. guildinii</i> (Silva et al. 2014)
PI-227687	Japan	Antixenosis to <i>P. guildinii</i> (Silva et al. 2014), beetles (Liu and Norris 1989), and lepidopterans (Clark et al. 1972, Reynolds et al. 1984, Valle and Lourenção 2002)
PI-274453	Japan	Antixenosis to <i>P. guildinii</i> (Silva et al. 2014)
'P98Y11'	Pioneer	Commercial material
'TMG132 RR'	Tropical Melhoramento & Genética	Commercial material
'TMG1176 RR'	Tropical Melhoramento & Genética	Commercial material
UX-2569-159	University of Nebraska (USA)	Antibiosis to <i>A. glycines</i> (Baldin et al. 2016) and <i>B. tabaci</i> biotype B (Cruz and Baldin, 2017)

for pest management, making possible the reduction of the insect population below the level of economic injury, without affecting the environment, and thereby allowing the crop to be more profitable for the producer.

A resistant plant is one that, due to the relative sum of its hereditary characteristics, is less damaged than another plant on equal terms. There are three types of resistance: antixenosis (nonpreference), antibiosis, and tolerance. A plant presents resistance as antixenosis when it has chemical, physical, or morphological factors that negatively affect insect behavior during the process of colonization. When the plants have ability to resist or recover from an injury caused by the insect, it is said that the plants have resistance as tolerance. The antibiosis occurs when the plant adversely affects the biology of the insect that uses the plant as host, interfering in its development cycle, reproduction, survival, and other biological parameters (Painter 1951, Smith 2005).

Plant morphology can affect an insect's host-selection process and can contribute to antixenosis. Among the morphological characteristics related to soybean resistance to whitefly, the density and inclination of trichome represent an important feature to be investigated (McAuslane 1996). Soybean genotypes that present more erect or higher densities of trichomes have been highlighted as the most attractive and preferred for oviposition, whereas those with parallel or lightly inclined trichomes relative to the leaf surface have been shown to be more resistant (Lambert et al. 1995, Valle and Lourenção 2002).

Considering the serious damage that *B. tabaci* has caused in soybean, we investigated the possibility of antixenosis in soybean genotypes and determined possible correlations of resistance with trichomes on the abaxial leaf surface. Because the occurrence of multiple resistance (to more than one species of insects) is a highly desirable feature for commercial genotypes, national soybean genotypes promising as the pest resistant (Valle and Lourenção 2002; Silva et al. 2012, 2014) and American soybean genotypes resistant to other sucking insects such as *Aphis glycines* Matsumura (Hemiptera: Aphididae) (Hill et al. 2004, 2006; Pierson et al. 2010; Prochaska et al. 2013) were evaluated.

## Materials and Methods

The tests were performed in a greenhouse in the years 2013 and 2014. The soybean genotypes were grown in pots (3 liter) filled with autoclaved substrate, which was formed with soil (Oxisol), washed coarse sand, and organic material (corral manure) at a ratio 1:1:1. The substrate was fertilized according to that typically recommended for the crop (Mascarenhas and Tanaka 1997). The plants were maintained in a greenhouse, free from insect infestation. The soybean genotypes evaluated in the experiments, their respective genealogies, and justification for choice are described in Table 1.

The initial population of *B. tabaci* biotype B was obtained from the IAC (Agronomic Institute of Campinas) and maintained since 2011 in a screen cage (2.0 by 2.5 by 2 m<sup>3</sup>), covered with plastic sheeting and shade cloth, with the lateral and frontal parts protected with white antiaphid screens. For the maintenance of a colony, collard plants (*Brassica oleracea* var. *acephala* L.) cultivated in plastic pots (2.5 liter) were provided. Frequently, whitefly individuals were collected in field and introduced in colony to maintain the vigor and virulence of the population. The molecular characterization of the insects was made periodically by Dr. Renate Krause-Sakate São Paulo State University, before and during the study, for confirmation of the insect biotype (Walsh et al. 1991, Simon et al. 1994, De Barro et al. 2003).

## Initial Tests

The study was conducted at the São Paulo State University, Botucatu, São Paulo, Brazil. Initially, a multiple-choice study was carried out with 15 soybean genotypes (Table 1) inside screen cages (31.3 °C, 45% relative humidity [RH], and natural light), similar to those described for the colony. Pots containing soybean plants at the V<sub>3</sub>-V<sub>4</sub> phenological stage (Fehr and Caviness 1977) were randomly placed in a circle (spaced at 30 cm) in the screen cages. A randomized block design was adopted, with seven replications. Each cage with 15 pots (one per genotype) was considered a repetition. The cages were infested with 1,500 adults of whitefly (50 couples per

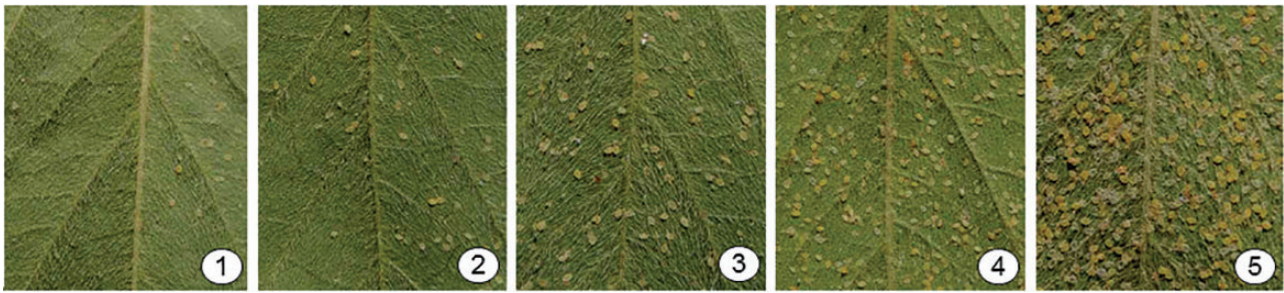


Fig. 1. Growing scale of colonization by nymphs of *B. tabaci* biotype B on soybean leaflet, score of 1 to 5.

genotype), added with the aid of an aspirator (11 by 4 cm), giving preference to whitefly pairs because insect couples usually stay paired (Byrne and Bellows 1991).

Settling, oviposition preference, and colonization experiments followed established methods (Valle and Lourenção 2002, Vieira et al. 2011). At 3 d after infestation (DAI), during 7 to 9 a.m., the number of adults on the abaxial surface of three leaflets per plant (one leaflet in the upper, one in the middle, and one in the lower portion) were counted with the aid of a mirror, to avoid the disturbance to the insects. Furthermore, the leaflets were removed and taken to laboratory, where with the aid of a stereoscopic microscope (magnification 40 $\times$ ), the number of eggs present was evaluated. In addition, the areas of the leaflets were determined by measuring with an LI 3000A (LI-COR Inc., Lincoln, NE) to verify the number of eggs per cm<sup>2</sup>.

At 48 DAI, colonization by *B. tabaci* nymphs was estimated using a visual scale of 1 to 5, where the initial score 1 was a leaf with small nymphs and exuviae, and reached the score 5, when a leaf was totally colonized by nymphs and exuviae. The scores 2 to 4 represented a gradual increase in the colonization by nymphs (Fig. 1). Therefore, nine leaflets were removed from the middle third of each genotype, with a final rate being based on the average of leaflets (Valle and Lourenção 2002, Coelho et al. 2009, Pierson et al. 2010).

In the no-choice test, the same genotypes described earlier were evaluated, except for 'Dowling,' which presented lower germination. Plants (V<sub>3</sub>-V<sub>4</sub>) were placed individually in metal cages (35 cm diameter by 55 cm height), covered with organdy fabric and infested with 50 pairs of *B. tabaci*. After 72 h of infestation, the oviposition was evaluated, as described for the multiple-choice test. Each individual plant represented a repetition in the completely randomized design with eight replications.

### Test With Selected Genotypes

From the results of the initial tests, 10 genotypes were selected for use in a new phase of testing and for the characterization of trichomes. We selected the genotypes 'IAC-17,' 'IAC-19,' 'Jackson,' 'P98Y11,' PI-227687, PI-229358, 'TMG1176 RR,' and UX-2569-159 by presenting indicative of antixenosis, and the genotypes 'Conquista' and 'KS-4202' as susceptible. A multiple-choice test was carried out in a greenhouse (29.5 °C, 49% RH, and natural light) according to the methodology described earlier. However, in this test, we evaluated whitefly settling at three time periods (24, 48, and 72 h after infestation) and evaluated oviposition once at 72 h after infestation.

A correlation was sought for the density and size of the trichomes present in the leaflets of the soybean genotypes with the settling and oviposition preference. For this, plants of selected genotypes were cultivated simultaneously with those used in the

second multiple-choice test and under the same conditions. For the analysis, leaflets from the middle portion of the plants (V<sub>3</sub>-V<sub>4</sub>) were used. The trichome density was quantified by counting the number of structures present in an area of 10 mm<sup>2</sup> on the abaxial surface of the leaflets with a stereoscopic microscope under 32 $\times$  magnification (Valle and Lourenção 2002). The design was completely randomized with 10 treatments (genotypes) and 10 replicates (each leaflet). From each plant was removed just a leaflet which represented one replicate.

For the acquisition of more detailed images of trichomes and measurements of trichome length, samples of the middle region of the leaf blade were prepared (Robards 1978) and analyzed under a scanning electron microscope (SEM) Fei Quanta 200 (FEI, Hillsboro, OR). The lengths of the trichomes were obtained with the aid of Scandium software coupled to the image capture system of the SEM.

### Statistical Analysis

Analysis of variance and F test were performed. The residuals distribution was checked for normality using the Shapiro-Wilk test, and for homogeneity using the Levene test (both considering alpha = 0.05). Tukey's test ( $P < 0.05$ ) was conducted to compare genotype means. Data referring to settling of adults, number of eggs/cm<sup>2</sup>, and number of nymphs/cm<sup>2</sup> were transformed by  $(x + 0.5)^{1/2}$ , and data relating the colonization notes, density and size of trichomes were transformed by  $(x)^{1/2}$ . The Pearson correlation analysis ( $r$ ) was used to assess the settling parameters (mean number of insects present after 24, 48, and 72 h), number of eggs/cm<sup>2</sup>, and density and size of trichomes. Statistical analysis was performed using the PROC GLM procedure in the SAS Software package (SAS Software 2001).

### Results and Discussion

In the first multiple-choice test, 'IAC-17,' 'IAC-19,' and 'TMG1176 RR' were the least infested with *B. tabaci* biotype B at 3 DAI, differing from 'Dowling,' 'KS-4202,' PI-274454, and PI-227687 (Table 2). With regard to oviposition (Table 2), 'IAC-19,' 'TMG1176 RR,' and UX-2569-159 presented the lowest mean of eggs/cm<sup>2</sup> at 3 DAI, differing from 'Dowling,' 'KS-4202,' PI-274454, and PI-227687, which were the most oviposited.

The lower preference of *B. tabaci* biotype B for 'IAC-17' and 'IAC-19' confirms the importance of these genotypes as *B. tabaci* resistance sources (Valle and Lourenção 2002, Vieira et al. 2011, Silva et al. 2012, Valle et al. 2012). In case of PI-227687, the genotype was very infested by *B. tabaci*, similar to observations in previous studies, although it is a genotype with multiple resistance to insects, exhibiting resistance against *Piezodorus guildinii* (Hemiptera:

**Table 2.** Mean ( $\pm$  SE) settled insects and eggs/cm<sup>2</sup> of *B. tabaci* biotype B at 3 DAI and degree of colonization at 48 DAI of 15 soybean genotypes, in a multiple-choice test, in a greenhouse

Genotype	Evaluation		
	3 DAI		48 DAI
	No. of settled insects	No. of eggs/cm <sup>2</sup>	Degree of colonization <sup>a</sup>
'TMG1176 RR'	1.7 $\pm$ 0.38a	0.9 $\pm$ 0.20a	3.3 $\pm$ 0.36b
'IAC-17'	3.0 $\pm$ 0.84a	3.9 $\pm$ 1.47ab	2.4 $\pm$ 0.33ab
'IAC-19'	3.1 $\pm$ 0.75a	1.9 $\pm$ 0.62a	2.1 $\pm$ 0.22ab
UX-2569-159	3.9 $\pm$ 1.23ab	1.8 $\pm$ 0.56a	1.8 $\pm$ 0.23a
'P98Y11'	4.0 $\pm$ 0.62ab	2.9 $\pm$ 0.60ab	2.1 $\pm$ 0.35ab
'IAC-100'	4.2 $\pm$ 1.05ab	3.2 $\pm$ 0.68ab	2.7 $\pm$ 0.26ab
'TMG132 RR'	4.3 $\pm$ 0.69abc	2.4 $\pm$ 0.62ab	3.0 $\pm$ 0.26ab
'Conquista'	4.8 $\pm$ 1.31abc	2.6 $\pm$ 0.59ab	3.0 $\pm$ 0.42ab
'Jackson'	5.8 $\pm$ 0.81abcd	2.5 $\pm$ 0.31ab	2.9 $\pm$ 0.33ab
PI-274453	6.1 $\pm$ 1.42abcd	4.4 $\pm$ 1.01abc	2.7 $\pm$ 0.24ab
PI-229358	6.4 $\pm$ 2.96abcd	3.7 $\pm$ 1.00ab	2.9 $\pm$ 0.44ab
'Dowling'	12.7 $\pm$ 2.81bcd	7.3 $\pm$ 2.07bcd	3.3 $\pm$ 0.34b
PI-274454	13.5 $\pm$ 4.30bcd	11.2 $\pm$ 2.07d	2.7 $\pm$ 0.24ab
'KS-4202'	13.8 $\pm$ 2.93cd	10.9 $\pm$ 2.50cd	3.0 $\pm$ 0.40ab
PI-227687	14.4 $\pm$ 2.25d	12.5 $\pm$ 2.15d	2.7 $\pm$ 0.44ab
F	5.76*	9.90*	2.92*
CV (%)	33.18	29.54	25.11

Means followed by the same letter in the column are not significantly different by Tukey's test ( $P \geq 0.05$ ). For analysis, the original data for the means of No. settled insects and eggs/cm<sup>2</sup> were transformed by  $(x + 0.5)^{1/2}$ , and the means of degree of colonization by  $(x)^{1/2}$ .

<sup>a</sup> Scale of degree of nymphs colonization (1 to 5); \* = significant.

Pentatomidae), *Epicauta vittata* (F.) (Coleoptera: Meloidae), and several lepidopterans (Clark et al. 1972, Rossetto et al. 1977, Reynolds et al. 1984, Valle and Lourenção 2002, Silva et al. 2014).

Regarding the degree of colonization at 48 DAI (Table 2), UX-2569-159 (1.8) was the genotype least favorable to insect colonization, differing from 'Dowling' (3.3) and 'TMG1176 RR' (3.3). The lower colonization in UX-2569-159 may be related to the lower verified oviposition, which indicates the expression of resistance by anti-*xenosis* against *B. tabaci*. Genotypes 'Jackson,' 'P98Y11,' and PI-229358 were intermediate in the oviposition in the multiple-choice test (Table 2), and exhibited the lowest means of eggs/cm<sup>2</sup> in the no-choice test (Table 3), differing from 'KS-4202' and 'TMG1176 RR.'

Considering the initial results, the genotypes selected were as follows: 'Jackson,' 'P98Y11,' and PI-229358 (indicative of anti-*xenosis* in the no-choice test); UX-2569-159 (smaller scale of colonization by nymphs and lower oviposition in the multiple-choice test); 'IAC-17,' 'IAC-19,' and 'TMG1176 RR' (lower infestation); and 'Conquista,' 'KS-4202,' and PI-227687 for the complementary tests and characterization of the trichomes.

For the second multiple-choice test (Table 4), in the two first evaluations (24 and 48 h after infestation), 'TMG1176 RR' was less infested, differing from 'IAC-17,' 'IAC-19,' and PI-227687. At the last evaluation (72 h), 'P98Y11' also stood out as less infested along with 'TMG1176 RR.' Conversely, PI-227687 was the most infested.

Considering the average of the three evaluations (Table 4), 'Jackson,' 'P98Y11,' and 'TMG1176 RR' were the least infested, differing from the other genotypes. With respect to oviposition (Table 4), the least infested genotypes were also the least oviposited. In the same way, PI-227687 was the most infested and more oviposited. During the host-selection process (feeding or oviposition), the insects respond to diverse stimuli from plants, and the absence of such stimuli or the presence of repellents, antifeeding compounds, or feeding deterrents may contribute to different levels of anti-*xenosis* resistance (Panda and Khush 1995). Probably, some of these

**Table 3.** Mean ( $\pm$  SE) eggs/cm<sup>2</sup> of *B. tabaci* biotype B obtained at 3 DAI in leaflets of 14 soybean genotypes, in a no-choice test, in a greenhouse

Genotype	No. of eggs/cm <sup>2</sup>
PI-229358	1.1 $\pm$ 2.61a
'P98Y11'	1.1 $\pm$ 2.75a
'Jackson'	1.2 $\pm$ 2.85a
'IAC-19'	1.6 $\pm$ 3.83ab
'TMG132 RR'	1.7 $\pm$ 4.26ab
'IAC-17'	2.0 $\pm$ 4.79ab
PI-227687	2.7 $\pm$ 6.72ab
UX-2569-159	2.8 $\pm$ 6.86ab
'Conquista'	3.0 $\pm$ 7.38ab
PI-274453	3.5 $\pm$ 8.58ab
'IAC-100'	3.5 $\pm$ 8.55ab
PI-274454	3.8 $\pm$ 9.36ab
'TMG1176 RR'	4.9 $\pm$ 11.94b
'KS-4202'	5.6 $\pm$ 13.72b
F	3.90*
CV (%)	26.55

Means followed by the same letter in the column are not significantly different by Tukey's test ( $P \geq 0.05$ ). For analysis, the original data were transformed by  $(x + 0.5)^{1/2}$ ; \* = significant.

characteristics are associated with the lower infestation and lower oviposition in 'Jackson,' 'P98Y11,' and 'TMG1176 RR.'

The genotype 'TMG1176 RR' exhibited similar expression of resistance in the two multiple-choice tests, showing lower infestation and lower preference for oviposition; 'Jackson' and 'P98Y11' exhibited low levels of oviposition in the no-choice test and showed less infestation and oviposition in the second multiple-choice test, confirming the occurrence of resistance by anti-*xenosis*.

The results obtained with 'Jackson' and 'P98Y11' suggest a greater level of anti-*xenosis* against *B. tabaci*, and presented similar

**Table 4.** Mean ( $\pm$  SE) settled insects and eggs/cm<sup>2</sup> of *B. tabaci* biotype B in 10 soybean genotypes, in a multiple-choice test, in a greenhouse

Genotype	No. of settled insects			Mean <sup>a</sup>	No. of eggs/cm <sup>2</sup>
	24 h	48 h	72 h		
TMG1176 RR	3.5 $\pm$ 0.73a	3.5 $\pm$ 0.98a	3.1 $\pm$ 0.72a	3.4 $\pm$ 0.12a	0.9 $\pm$ 0.05a
'P98Y11'	3.9 $\pm$ 0.64ab	4.5 $\pm$ 0.50ab	3.2 $\pm$ 0.65a	3.9 $\pm$ 0.38a	1.0 $\pm$ 0.28a
'Jackson'	7.1 $\pm$ 0.65abc	7.2 $\pm$ 0.97abc	6.9 $\pm$ 1.19ab	7.1 $\pm$ 0.08b	1.7 $\pm$ 0.29ab
UX-2569-159	11.9 $\pm$ 2.94abcd	9.9 $\pm$ 2.70abc	8.4 $\pm$ 1.92ab	10.1 $\pm$ 1.00c	2.3 $\pm$ 0.51abc
'Conquista'	10.9 $\pm$ 2.48abcd	10.5 $\pm$ 1.82abc	9.3 $\pm$ 1.58ab	10.2 $\pm$ 0.48c	2.9 $\pm$ 0.55abc
'KS-4202'	11.1 $\pm$ 2.28abcd	10.7 $\pm$ 2.31abc	10.7 $\pm$ 2.07ab	10.8 $\pm$ 0.15cd	3.1 $\pm$ 0.66abc
PI-229358	12.5 $\pm$ 1.15abcd	11.8 $\pm$ 1.39abc	9.9 $\pm$ 1.60ab	11.4 $\pm$ 0.78cd	4.4 $\pm$ 0.97bc
'IAC-19'	14.3 $\pm$ 2.58bcd	13.5 $\pm$ 1.95bc	11.5 $\pm$ 1.68ab	13.0 $\pm$ 0.85cd	3.0 $\pm$ 0.67abc
'IAC-17'	17.5 $\pm$ 0.83cd	13.0 $\pm$ 1.96bc	11.6 $\pm$ 1.06b	14.0 $\pm$ 1.77d	4.8 $\pm$ 0.69bc
PI-227687	22.5 $\pm$ 6.47d	18.6 $\pm$ 5.44c	15.1 $\pm$ 4.82b	18.7 $\pm$ 2.13e	6.2 $\pm$ 1.81c
F	5.40*	3.69*	3.73*	69.53*	5.97*
CV (%)	24.47	24.97	24.87	4.84	21.94

Means followed by same letter in the column are not significantly different by Tukey's test ( $P \geq 0.05$ ). For analysis, the original data were transformed by  $(x)^{1/2}$ .

<sup>a</sup> Mean number of settled insects in the three evaluations (24, 48, and 72 h); \* = significant.

**Table 5.** Mean ( $\pm$  SE) density, length, and inclination of the trichomes present on the abaxial surface of leaflets of 10 soybean genotypes

Genotype	No. of trichomes/10 mm <sup>2</sup>	Length of trichomes ( $\mu$ m)	Classification as the inclination
PI-229358	16.4 $\pm$ 1.33a	951.7 $\pm$ 62.56f	Erect
'P98Y11'	19.4 $\pm$ 1.42ab	518.5 $\pm$ 18.75bc	Inclined
'IAC-17'	24.1 $\pm$ 1.96bc	623.5 $\pm$ 28.67bcde	Inclined
UX-2569-159	28.7 $\pm$ 2.20cd	768.8 $\pm$ 43.92ef	Erect
'KS-4202'	30.1 $\pm$ 2.02cd	678.2 $\pm$ 47.76cde	Inclined
TMG1176 RR	30.8 $\pm$ 1.19cd	561.9 $\pm$ 47.99bcd	Erect
'Jackson'	31.5 $\pm$ 1.71cd	492.0 $\pm$ 17.92b	Inclined
'Conquista'	32.4 $\pm$ 2.10d	641.3 $\pm$ 47.59bcde	Inclined
'IAC-19'	35.6 $\pm$ 1.64d	704.9 $\pm$ 48.32de	Erect
PI-227687	73.4 $\pm$ 3.98e	342.1 $\pm$ 26.43a	Inclined
F	52.65*	18.17*	
CV (%)	9.59	11.86	

Means followed by the same letter in the column are not significantly different by Tukey's test ( $P \geq 0.05$ ). For analysis, the original data were transformed by  $(x)^{1/2}$ ; \* = significant.

behavior in the two modalities of test (multiple-choice and no-choice). The lower infestation of a genotype when it is the only option for the insect indicates good application for use in field, where generally, extensive areas are planted with only one genotype (no-choice), and thus, these genotype would be able to reduce the pest population (Lourenço and Yuki 1982).

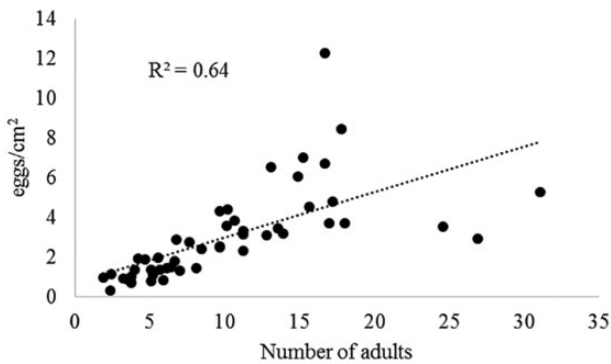
Among the American soybean genotypes, 'Jackson' and UX-2569-159 were the most promising: first due to the low infestation and oviposition, and second, for its lower colonization and oviposition in the multiple-choice test. In the United States, several studies have already reported the occurrence of resistance (antibiosis) of the genotype 'Jackson' against the soybean aphid *A. glycines* (Hill et al. 2004, 2006; Diaz-Montano et al. 2006), results that could stimulate studies of resistance against *B. tabaci* to check the possible occurrence of multiple resistance. Recently, antibiosis of UX-2569-159 was confirmed to *A. glycines* (Baldin et al. 2016) and *B. tabaci* biotype B (Baldin and Cruz, 2017).

Regarding the density of trichomes (Table 5), PI-227687 presented the highest mean of density (73.4/10 mm<sup>2</sup>) but with less length (342.1  $\mu$ m), thereby differing from the others for both parameters. The inverse was observed for PI-229358, which showed the longest trichomes (951.7  $\mu$ m) and the lowest density (16.4/10 mm<sup>2</sup>). Other studies also verified a higher density of trichomes in PI-

227687 and a lower density in PI-229358 (Valle and Lourenço 2002, Lima and Lara 2004).

The correlations between number of eggs/cm<sup>2</sup> and density of trichomes (0.24), eggs and size of trichomes (0.20), number of adults and density of trichomes (-0.02), and number of adults and size of trichomes (0.19) do not indicated significance. However, the correlation between the number of adults and eggs/cm<sup>2</sup> was significant (0.64; Fig. 2), and may be clearly observed in Table 4, where PI-227687, which presented the greatest mean of settling (18.7 adults), was also the most oviposited (6.2 eggs/cm<sup>2</sup>); furthermore, the least infested genotypes were also the least oviposited, for example, 'TMG1176 RR' (3.4 adults and 0.9 eggs/cm<sup>2</sup>). Similar correlations were reported in previous studies (Valle and Lourenço 2002, Valle et al. 2012) and suggests that adults of *B. tabaci* tend simultaneously to select places that are better for feeding and oviposition (Van Lenteren and Noldus 1990).

According to literature, soybean genotypes with a higher density of trichomes are more oviposited by *B. tabaci* (McAuslane 1996). Thus, the high number of eggs in PI-227687 could be associated with the higher number of trichomes present in the leaflets of this genotype (Valle and Lourenço 2002, Lima and Lara 2004, Silva et al. 2012). Despite no significant correlations being found for these parameters in this study, this tendency was observed because in both the multiple-choice tests (Tables 2 and 4), PI-227687 stood out as having



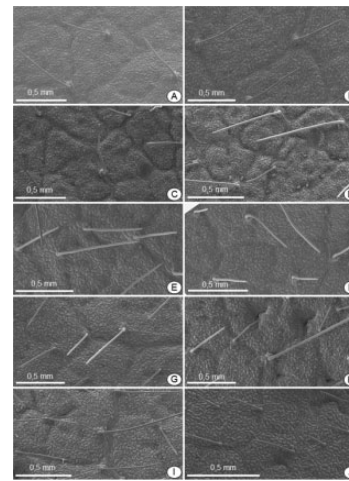
**Fig. 2.** Pearson correlation coefficient ( $r$ ) obtained between the mean settling (mean number of insects present after 24, 48, and 72 h) and number of eggs/cm<sup>2</sup>.

the highest density of trichomes (Table 5) and showed higher oviposition. Similarly, the lower infestation and oviposition preference for ‘P98Y11’ in the multiple-choice test may be associated with the lowest number of trichomes present in the leaflets of this genotype.

PI-229358, which also has a lower number of trichomes, was verified as having intermediate (average) infestation and oviposition in the multiple-choice tests; however, this material was one of the least oviposited in the no-choice test. The intermediate preference observed in PI-229358 in the multiple-choice test may be associated to the larger size of the trichomes and not to the density (Table 5). In addition to the higher density, the greater length of trichomes may also favor permanence and insect oviposition in the plant because these characteristics prevent eggs from being blown away by the wind (Vieira et al. 2011). Furthermore, the trichomes may provide a more suitable microclimate for oviposition (Butter and Vir 1989), besides representing protection against natural enemies, which are more efficient in leaves with a lower density of trichomes or in those that are glabrous (Li et al. 1987).

The Fig. 3 shows the scanning electron micrograph (SEM) of trichomes on the abaxial surface of leaflets of the 10 soybean genotypes evaluated in the second multiple-choice test. Based on the observations under SEM, the genotypes were classified as inclined or erect to indicate the inclination of the trichomes in relation to the leaf surface. In this way, ‘Conquista,’ ‘IAC-17,’ ‘Jackson,’ ‘KS-4202,’ ‘P98Y11,’ and PI-227687 presented inclined trichomes in relation to the leaf surface, whereas ‘IAC-19,’ PI-229358, ‘TMG1176 RR,’ and UX-2569-159 presented more erect trichomes (Table 5). Soybean plants that have parallel or slightly inclined trichomes in relation to the leaf surface, proved to be more resistant to *B. tabaci* than plants with more erect trichomes, suggesting that the inclination of trichomes is also a factor that confers resistance against the insect (Lambert et al. 1995). Other study also observed that some of the genotypes that stood out for lower oviposition presented trichomes that were parallel or slightly inclined, whereas the most oviposited by *B. tabaci* had more erect trichomes (Valle et al. 2012).

In the present study, among the genotypes that indicated resistance by antixenosis, only ‘Jackson’ and ‘P98Y11’ (Fig. 3G and B, respectively) present trichomes more inclined in relation to the leaf surface, corroborating the data of Lambert et al. (1995) and Valle et al. (2012), who also observed such characteristic in materials resistant to *B. tabaci*. On the other hand, PI-227687 (Fig. 3J) shows inclined trichomes as a feature that stands out in the most infested and oviposited genotypes. In this case, the greater settling of this genotype could be associated with the higher number of trichomes



**Fig. 3.** Scanning electron micrograph (SEM) of trichomes on the abaxial surface of leaflets of 10 soybean genotypes: (A) PI-229358; (B) ‘P98Y11’; (C) ‘IAC-17’; (D) UX-2569-159; (E) ‘KS-4202’; (F) ‘TMG1176 RR’; (G) ‘Jackson’ (PI 548657); (H) ‘Conquista’; (I) ‘IAC-19’; (J) PI-227687.

present on the abaxial surface of the leaflets, although other factors might influence on the insect settling.

The results suggest that factors such as the density, size, and inclination of the trichomes have important role in the resistance of some soybean genotypes against *B. tabaci*. Thus, the possible antixenosis demonstrated by ‘Jackson,’ ‘P98Y11,’ and PI-229358 may be related to the features of their trichomes (lower density and inclination), which suggests an interesting source of resistance to *B. tabaci*. However, trichomes should not be solely responsible for the observed differences between the genotypes because biochemical plant factors may have already interfered in the recognition by the insect of a suitable host for feeding, oviposition, mating, or shelter (Smith 2005).

In general, ‘IAC-17,’ ‘IAC-19,’ ‘Jackson,’ ‘P98Y11,’ PI-229358, UX-2569-159, and ‘TMG1176 RR’ express antixenosis as a resistance mechanism to *B. tabaci* biotype B. The configuration of the trichomes of ‘Jackson,’ ‘P98Y11,’ and PI-229358 proves to be an interesting source of resistance in these genotypes, which can be explored in soybean breeding programs. These genotypes represent possible sources of resistance and can be exploited in soybean breeding programs focusing on the management of *B. tabaci* biotype B.

## Acknowledgments

We acknowledge FAPESP, for the financial support (2013/13672-7); CNPq, for the productivity scholarship in research granted to the first author (305649/2013-2); and CAPES, for the doctoral scholarship grants to the second author.

## References Cited

- Baldin, E. L. L., L. Marchi-Werle, L. E. R., Pannuti, A. L., Lourenção, T. M., Heng-Moss, and T. E., Hunt 2016. Evaluating categories of resistance in soybean genotypes from the United States and Brazil to *Aphis glycines* (Hemiptera: Aphididae). *Fla. Entomol.* 99: 487–495.
- Brown, J. K., D. R. Frohlich, and R. C. Rosell. 1995. The sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex? *Annu. Rev. Entomol.* 40: 511–534.
- Butter, N. S., and B. K. Vir. 1989. Morphological basis of resistance in cotton to the whitefly *Bemisia tabaci*. *Phytoparasitica* 17: 251–261.

- Byrne, D. N., and T. S. Bellows Jr. 1991. Whitefly biology. *Annu. Rev. Entomol.* 36: 431–457.
- Cameron, R., E. B. Lang, I. B. Annan, H. E. Portillo, and J. M. Alvarez. 2013. Use of fluorescence, a novel technique to determine reduction in *Bemisia tabaci* (Hemiptera: Aleyrodidae) nymph feeding when exposed to benevia and other insecticides. *J. Econ. Entomol.* 106: 597–603.
- Clark, W. J., F. A. Harris, F. G. Maxwell, E. E. Hartwig. 1972. Resistance of certain soybean cultivars to bean leaf beetle, Striped blister beetle, and bollworm. *J. Econ. Entomol.* 65: 1669–1672.
- Coelho, S.A.M.P., A. L. Lourenção, A.M.T. Melo, and E. A. Schammas. 2009. Resistência de meloeiro a *Bemisia tabaci* biótipo B. *Bragantia* 68, 1025–1035.
- Cruz, P. L., E. L. L., Baldin, L. R. P., Guimaraes, L. E. R., Pannuti, G. P. P., Lima, T. M., Heng-Moss, and T. E., Hunt 2016. Tolerance of KS-4202 soybean to the attack of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae). *Fla. Entomol.* 99: 600–607.
- Cruz, P. L., and E. L. L., Baldin 2017. Performance of *Bemisia tabaci* Biotype B on soybean genotypes. *Neotrop. Entomol.* 46: 210–215.
- De Barro, P. J., K. D. Scott, G. C. Graham, C. L. Lange, and M. K. Schutze. 2003. Isolation and characterization of microsatellite loci in *Bemisia tabaci*. *Mol. Ecol. Notes* 3: 40–43.
- Diaz-Montano, J., J. C. Reese, W. T. Schapaugh, and L. R. Campbell. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. *J. Econ. Entomol.* 99: 1884–1889.
- Fehr, W. R., and C. E. Caviness. 1977. Stages of soybean development. Iowa State University Cooperative Extension Service Special Rep. 80. Iowa State University, Ames, IA.
- Hill, C. B., Y. Li, and G. L. Hartman. 2004. Resistance to the soybean aphid in soybean germplasm. *Crop Sci.* 44: 98–106.
- Hill, C. B., Y. Li, and G. L. Hartman. 2006. Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. *Crop Sci.* 46: 1606–1608.
- Hoffmann-Campo, C. B., R. M. Mazzarin, P. R. Lustosa. 1994. Mecanismos de resistência de genótipos de soja: Teste de não-preferência para *Anticarsia gemmatalis* Hubner, 1818. (Lep.: Noctuidae). *Pesqui Agropecu Brasileira* 29:513–519.
- Jones, W. A., and J. M. Sullivan. 1979. Soybean resistance to the southern green stink bug, *Nezara viridula*. *J. Econ. Entomol.* 72: 628–632.
- Lambert, A. L., R. M. McPherson, and K. E. Espelie. 1995. Soybean host plant resistance mechanisms that alter abundance of whiteflies (Homoptera: Aleyrodidae). *Environ. Entomol.* 24: 1381–1386.
- Li, Z. H., F. Lammes, J. C. Van Lenteren, P. W. T. Huisman, A. Van Vianen, and O.M.B. De Ponti. 1987. The parasite-host relationship between *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). XXV. Influence of leaf structure on the searching activity of *Encarsia formosa*. *J. Appl. Entomol.* 104: 297–304.
- Lima, A.C.S., and F. M. Lara. 2004. Resistência de genótipos de soja à mosca branca *Bemisia tabaci* (Genn.) biótipo B (Hemiptera: Aleyrodidae). *Neotrop. Entomol.* 33: 71–75.
- Liu, S. H., D. M. Norris. 1989. Volatiles from the foliage of soybean, *Glycine max*, and lima bean, *Phaseolus lunatus*: their behavioral effects on the insects *Trichoplusia ni* and *Epilachna varivestis*. *J. Agric. Food Chem.* 37:496–501
- Lourenção, A. L., and V. A. Yuki. 1982. Oviposição de *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) em três variedades de soja sem chance de escolha. *Bragantia* 41: 199–202.
- Marubayashi, J.M., V. A. Yuki, and E. B. Wutke. 2010. Transmissão do *Cowpea mild mottle virus* pela mosca branca *Bemisia tabaci* biótipo B para plantas de feijão e soja. *Summa Phytopathol.* 36: 158–160.
- Mascarenhas, H.A.A., and R. T. Tanaka. 1997. Soja, pp. 202–203. In: B. Raij, V. H. Cantarella, J. A. Quaggio, and A.M.C. Furlani (eds.), *Recomendações de adubação e calagem para o Estado de São Paulo*. IAC, Campinas.
- McAuslane, H. J. 1996. Influence of leaf pubescence on ovipositional preference of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on soybean. *Environ. Entomol.* 25: 834–841.
- Musa, P. D., and S. Ren. 2005. Development and reproduction of *Bemisia tabaci* (Homoptera: Aleyrodidae) on three bean species. *Insect Sci.* 12: 25–30.
- Naranjo, S. E., and J. P. Legg. 2010. Biology and ecology of *Bemisia tabaci*, pp. 105–107. In: P. A. Stansly and S. E. Naranjo (Eds.), *Bemisia: bionomics and management of a global pest*. Springer, Dordrecht.
- Navas-Castillo, J., E. F. Olivé, and S. C. Campos. 2011. Emerging virus diseases transmitted by whiteflies. *Annu. Rev. Phytopathol.* 49: 219–248.
- Oliveira, C. M., A. M. Auad, S. M. Mendes, and M. R. Frizzas. 2013. Economic impact of exotic insect pests in Brazilian agriculture. *J. Appl. Entomol.* 137: 1–15.
- Painter, R. H. 1951. *Insect resistance in crop plants*. McMillan, New York.
- Panda, N., and G. S. Khush. 1995. *Host plant resistance to insects*. CAB International, Wallingford, United Kingdom.
- Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar, T. S. Bellows Jr. 1993. Identification of a whitefly species by genomic and behavioural studies. *Science* 259: 74–77.
- Pierson, L. M., T. M. Heng-Moss, T. E. Hunt, and J. Reese. 2010. Categorizing the resistance of soybean genotypes to the soybean aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 103: 1405–1411.
- Polston, J. E., P. De Barro, and L. M. Boykin. 2014. Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Manag. Sci.* 70: 1547–1552.
- Prabhaker, N., N. C. Toscano, and T. J. Henneberry. 1998. Evaluation of Insecticide Rotations and Mixtures as Resistance management strategies for *Bemisia argentifolii* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 91: 820–826.
- Prochaska, T. J., L. M. Pierson, E.L.L. Baldin, T. E. Hunt, T. M Heng-Moss, and J. Reese. 2013. Evaluation of reproductive stage soybeans for resistance to soybean aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 106: 1036–1044.
- Reynolds, G. W., C. M. Smith, and K. M. Kester. 1984. Reductions in consumption utilization, and growth rate of soybean looper (Lepidoptera: Noctuidae) larvae fed foliage of soybean genotype PI 227687. *J. Econ. Entomol.* 77: 1371–1375.
- Rossetto, D., A. S. Costa, M.A.C. Miranda, V. Nagai, and E. Abramides. 1977. Diferenças na oviposição de *Bemisia tabaci* em variedades de soja. *Ann. Soc. Entomol. Bras.* 6: 256–263.
- Robards, A. W. 1978. An introduction to techniques for scanning electron microscopy of plant cells. pp. 343–403. In: J. L. Hall (eds.), *Electron microscopy and cytochemistry of plant cells*. Elsevier, New York.
- SAS Software 2001. *SAS/STAT: User's guide, version 8.1*. SAS Institute, Cary, NC.
- Silva, J.P.G.F., E.L.L. Baldin, E. S. Souza, and A. L. Lourenção. 2012. Assessing *Bemisia tabaci* (Gann.) biotype B resistance in soybean genotypes: Antixenosis and antibiosis. *Chil. J. Agric. Res.* 72: 516–522.
- Silva, J.P.G.F., E.L.L. Baldin, V. F. Canassa, E. S. Souza, and A. L. Lourenção. 2014. Assessing antixenosis of soybean entries against *Piezodorus guildinii* (Hemiptera: Pentatomidae). *Arthropod Plant Interact.* 8: 349–359.
- Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Ann. Entomol. Soc. Am.* 87: 651–701.
- Sivasupramaniam, S., and T. F. Watson. 2000. Selection for fenpropathrin and fenpropathrin+acefate resistance in the silverleaf whitefly (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 93: 949–954.
- Smith, C. M. 2005. *Plant resistance to arthropods*. Springer Science & Business Dordrecht, The Netherlands.
- Toscano, N. C., N. Prabhaker, S. J. Castle, and T. J. Henneberry. 2001. Interregional differences in baseline toxicity of *Bemisia argentifolii* (Homoptera: Aleyrodidae) to the two insect growth regulators, buprofezin and pyriproxyfen. *J. Econ. Entomol.* 94: 1538–1546.



- Valle G. E., and A. L. Lourenção. 2002. Resistência de genótipos de soja a *Bemisia tabaci* biótipo B (Hemiptera: Aleyrodidae). *Neotrop. Entomol.* 31: 285–295.
- Valle, G. E., A. L. Lourenção, and J. B. Pinheiro. 2012. Adult attractiveness and oviposition preference of *Bemisia tabaci* biotype B in soybean genotypes with different trichome density. *J. Pest Sci.* 85: 431–442.
- Van Duyn, J. W., S. G. Turnipseed, J. D. Maxwell. 1972. Resistance in soybeans to the Mexican bean beetle: II. Reactions of the beetle to resistant plants. *Crop Sci.* 12: 561.
- Van Lenteren, J. C., and P. J. Noldus. 1990. Whitefly-plant relationships: behavioural and ecological aspects. pp. 47–89. In: D. Gerling (ed.), *Whiteflies: their bionomics, pest status and management*. Intercept, Andover.
- Vieira, S. S., A. F. Bueno, R.C.O.F. Bueno, and C. B. Hoffman-Campo. 2011. Resistance of soybean genotypes to *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae). *Neotrop. Entomol.* 40: 117–122.
- Vieira, S. S., A. F. Bueno, R.C.O.F. Bueno, M.I.C. Boff, and A. L. Gobbi. 2013. Different timing of whitefly control and soybean yield. *Ciênc Rural* 43: 247–253.
- Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 4: 506–513.