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PEST MANAGEMENT

Resistance of Soybean Genotypes to *Bemisia tabaci* (Genn.) Biotype B (Hemiptera: Aleyrodidae)

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Abstract

The silverleaf whitefly *Bemisia tabaci* (Genn.) biotype B has become a serious problem for soybean cultivation because it can significantly reduce soybean productivity. The use of soybean cultivars resistant to whitefly attack is an important strategy in an integrated pest management (IPM) program. This study evaluated the preference for oviposition and colonization by *B. tabaci* biotype B on different soybean genotypes. In the free-choice test, the genotypes studied were 'IAC 17' and 'IAC 19' as the standards for resistance and 'IAC Holambra Stwart' as the standard for susceptibility, as well as BABR01-0492, BABR01-0173, BABR01-1259, BABR01-1576, BABR99-4021HC, BABR99-4021HP, 'Barreiras', 'Conquista', 'Corisco', 'BRS Gralha', PI274454, PI227687, and PI171451. In the no-choice test, the four best genotypes selected in the free-choice test, in addition to the susceptible and resistant standards were evaluated. Our data indicated 'Barreiras' as the most resistant genotype against *B. tabaci* biotype B. 'BRS Gralha', which was the least attractive to whitefly adults in the free-choice test, did not show resistance to insect attack when they were confined in cages in the no-choice test. Despite the high number of eggs observed, BABR01-1576 and BABR99-4021HC showed a reduced number of nymphs, indicating antibiosis. The genotypes with a high level of resistance can be used as a tool against *B. tabaci* in IPM or as a source of resistance in plant-breeding programs.

Introduction

The soybean, *Glycine max*, is one of the most important crops in Brazil, with around 23 million hectares cultivated in the 2009/2010 season (Conab 2010), but its productivity can be reduced by several insect pests. The silverleaf whitefly, *Bemisia tabaci* biotype B, has become a serious problem (Lourenção *et al* 1999, Tamai 2006). The honeydew excrete by whiteflies favors the development of a fungus of the genus *Capnodium*, which forms a dark layer of mycelia on the leaf surface, reducing the photosynthetic capacity and other plant physiological functions (Ferreira & Avidos 1998).

In soybeans, whiteflies are almost exclusively managed by insecticide sprays, which frequently do not provide efficient control. The use of cultivars resistant to the attack of this insect is an important strategy because it is completely compatible with other control techniques. A resistant soybean cultivar might impact the occurrence of a whitefly outbreak in different ways, including the presence of traits (e.g., trichome type, size, density) that affect the oviposition behavior of *B. tabaci* (Dahlin *et al* 1992, Valle & Lourenção 2002). Therefore, this study assessed the preferences of *B. tabaci* biotype B for oviposition and colonization on different soybean genotypes, and evaluated the effect of size and density of the foliar trichomes of these genotypes on insect preference.

Material and Methods

The experiments were carried out during 2007 and 2008 at the Embrapa Arroz e Feijão experimental farm in Santo Antônio de Goiás, state of Goiás, Brazil, in greenhouse conditions (25 ± 5°C and 50 ± 15% UR), using a randomized block design with ten replicates per treatment.

Insect colony

The colony of silverleaf whitefly was maintained on soybean plants in a greenhouse at Embrapa Arroz e Feijão in Santo Antônio de Goiás, GO, Brazil. The insects used to initiate the colony were identified by PCR-RAPD of genomic DNA and confirmed as *B. tabaci* biotype B.

Oviposition and colonization preference: free-choice test

The oviposition preference of *B. tabaci* biotype B was studied according to Valle & Lourenção (2002) on 16 soybean genotypes chosen from several cultivars adapted for the Central Brazil region and some genotypes (lines and plant introductions) known to be resistant to whiteflies and used in breeding programs. The genotypes used were: 'IAC 17' and 'IAC 19' as standards for resistance, 'IAC Holambra Stwart' as standard for susceptibility (Valle & Lourenção 2002), and BABR01-0492, BABR01-0173, BABR01-1259, BABR01-1576, BABR99-4021HC, BABR99-4021HP, 'Barreiras', 'Conquista', 'Corisco', 'BRS Gralha', PI274454, PI227687, and PI171451.

The soybean plants were grown under greenhouse conditions (25 ± 5°C and 50 ± 15% UR), in 16-L plastic pots filled with clayey latosol Typic Haplustox soil. A plastic dish was placed underneath each pot, and was periodically filled with water to provide plants with adequate moisture during the experimental period. Pots with previously infested soybean plants (from a laboratory colony maintained on soybean plants for several generations) were used for the artificial infestation procedure. The infestation was carried out when plants were at the V_4 vegetative stage (Fehr & Caviness 1977). The replicates of each genotype were arranged equidistantly in a circle around a soybean plant previously infested with *B. tabaci* from the insect colony.

The infestations were evaluated weekly for six weeks until the beginning of leaf senescence by selecting two leaflets per plant from the third completely developed apical leaf, totaling four leaflets per replicate. A visual count of adult whiteflies on the underside of selected leaflets was made at the greenhouse. The number of nymphs (all nymphal instars) and eggs on 4 cm^2 of the central region of each leaflet were counted in the laboratory with the help of a stereomicroscope.

Oviposition and colonization preference: no-choice test

The preference of *B. tabaci* biotype B for oviposition was evaluated on the six genotypes with the lowest mean number of eggs/cm^2 and the most susceptible genotype selected in the previous experiment (free-choice test). The genotypes selected were 'IAC 17' and 'IAC 19' as standards for resistance, 'IAC Holambra Stwart' as the standard for susceptibility, and 'Barreiras', 'Corisco', 'BRS Gralha', and BABR01-1576.

In this experiment, each genotype was individually caged in an iron-framed cage covered with fine-mesh fabric (50 cm diameter x 120 cm tall). Individual plants were infested at the V_3 or V_4 vegetative stage by introducing approximately 200 *B. tabaci* adults (without age control) per cage, as described by Valle & Lourenção (2002). The whiteflies remained in the cages for four days. After this period, all whitefly adults were removed from the cages, and plants were kept in the greenhouse (25 ± 5°C and 50 ± 15% UR). The oviposition preference was evaluated by the same procedures described for the free-choice test. Colonization was also evaluated weekly by counting the number of nymphs on the abaxial leaf surface of four leaflets per replicate.

Morphological characteristics of leaf trichomes (size and density)

The density and length of the soybean genotypes' leaf trichomes were evaluated by means of a permanent slide technique, using an optical microscope with an ocular measuring grid. Leaves were collected when plants were at the R_1 stage (Fehr & Caviness 1977). In the laboratory, a drop of glue (Super Bonder™, Loctite) was placed in the center of a microscope slide, the abaxial surface of each leaf was pressed against the slide for about two seconds and then carefully removed. This produced a permanent print of at least 2 cm^2 of the abaxial surface of the leaf, where the number and size of the trichomes could be evaluated. Two leaflets per replicate were evaluated.

Data analysis

The data obtained from all experiments were analyzed for normality (Shapiro & Wilk 1965) and homocedasticity (Burr & Foster 1972). Data were transformed prior to the analysis of variance (ANOVA) whenever necessary. When the F statistic showed significant values, means were compared by the Tukey test ($P \leq 0.05$) using the SAS statistics package (SAS Institute 2001).

Results and Discussion

The genotypes 'BRS Gralha', 'Barreiras', 'Corisco', BABR01-1259, and BABR99-4021 HP, as well as the resistant cultivars IAC 19 and IAC 17, were the least-preferred by whiteflies for oviposition in the free-choice test (Table 1). Similar antixenosis plant resistance response was previously reported by Lambert *et al* (1997) for the 'Perrin', 'Cook', and 'N88-91' soybean genotypes when compared to the susceptible varieties 'Braxton' and 'Cobb' in field conditions. Lambert *et al* (1995a) also observed significant differences in soybean varietal response to whitefly population densities and demonstrated that the greenhouse can be used effectively to screen soybean for resistance to whiteflies.

The highest number of eggs was observed on genotype PI171451, with 11.2 eggs/4 cm^2, followed by BABR01-1576, PI274454, and BABR01-0492 and the known-susceptibility standard 'IAC Holambra Stwart', with 10.3, 10.1, 7.0, and 6.5 eggs/4 cm^2, respectively. The remaining genotypes showed intermediate levels of preference for oviposition (Table 1). Previous studies on insect-resistant soybean genotypes have shown that PI171451, as well as PI227687, possessed multiple resistance (Clark *et al* 1972, Hatchett *et al* 1976, Turnipseed & Kogan 1976, Lourenção & Miranda 1987). However, when these genotypes were evaluated for resistance to *B. tabaci*, they were found to be susceptible (Valle & Lourenção 2002). Moreover, line PI274454 is resistant to stinkbugs and to caterpillars, such as *Hedylepta indicata* (= *Omiodes indicata*) (Fabricius) (Pyralidae) (Lourenção *et al* 1985), but susceptible to *B. tabaci* biotype B, with high attractiveness to adults and higher preference for oviposition than the other genotypes. Other investigators (Lourenção & Miranda 1987, Lima *et al* 2002, Valle & Lourenção 2002) have found similar results.

The lowest numbers of nymphs (insect colonization) were also observed on 'BRS Gralha' and 'Barreiras'. Despite the high number of eggs observed, the breeding line BABR01-1576 showed few nymphs (10.3 eggs produced only 3.0 nymphs). The genotype BABR99-4021HC, which showed intermediate attractiveness and oviposition rates, displayed a low colonization rate, with a mean of 4.2 nymphs (Table 1). Therefore, despite being attractive to adults, these genotypes are unsuitable to sustain the whitefly immature development probably because they induce a high mortality rate for young nymphs, mainly on BABR01-1576, indicating an antibiosis type of resistance.

Table 1 Number (mean ± SE) of different developmental stages of *Bemisia tabaci* biotype B on different soybean genotypes in the free-choice test.

Genotype	Eggs/4 cm^2	Nymphs/4 cm^2	Adults/leaflet
'IAC Holambra Stwart'	6.5 ± 0.87 abcd	17.6 ± 3.01 a	4.7 ± 1.72 abcd
'IAC 17'	3.4 ± 0.95 cdef	5.6 ± 1.19 bcde	2.5 ± 0.36 bcd
'IAC 19'	2.7 ± 0.78 def	4.2 ± 0.52 cde	2.3 ± 0.6 bcd
BABR 01-0173	5.4 ± 0.71 abcde	5.5 ± 1.71 cde	5.9 ± 1.43 abc
BABR 01-0492	7.0 ± 0.92 abc	9.1 ± 1.77 abcd	5.7 ± 0.95 abc
BABR 01-1259	3.9 ± 0.93 cdef	7.7 ± 2.03 abcde	3.9 ± 0.89 bcd
BABR 01-1576	10.3 ± 1.18 ab	3.1 ± 0.63 de	4.6 ± 0.91 Abcd
BABR 99-4021HP	4.5 ± 0.95 bcdef	4.5 ± 0.78 cde	1.9 ± 0.43 cd
BABR 99-4021HC	3.6 ± 0.95 cdef	4.2 ± 1.06 de	5.5 ± 1.25 abc
'Barreiras'	1.6 ± 0.32 f	3.8 ± 0.52 cde	2.9 ± 0.97 bcd
'BRS Gralha'	1.5 ± 0.33 f	3.1 ± 0.86 e	1.2 ± 0.18 d
'Conquista'	6.9 ± 1.44 abcd	11.2 ± 1.72 abc	6.1 ± 1.26 abc
'Corisco'	2.3 ± 0.47 ef	6.6 ± 1.37 abcde	3.9 ± 0.92 bcd
PI 171451	11.2 ± 2.27 a	14.0 ± 2.6 ab	10.3 ± 2.21 a
PI 227687	8.5 ± 1.84 abc	11.9 ± 2.61 abc	3.4 ± 0.7 bcd
PI 274454	10.1 ± 1.53 ab	9.6 ± 2.80 abcde	6.5 ± 1.01 ab
CV (%)	29.09	30.66	37.88

Means followed by the same letter within columns are not statistically different by the Tukey test ($P > 0.05$).

Cultivars IAC 17 and IAC 19 were less preferred, for both attractiveness of adults and oviposition and colonization, clearly distinguishing them from the other genotypes studied. These cultivars were developed by the Instituto Agronômico de Campinas – IAC in trials aiming at insect resistance, and originated from crossings involving line D 72-9601, which possesses resistance to soybean caterpillars and is derived from the introduction of genotype PI229358 (Valle & Lourenção 2002).

The no-choice test for oviposition and colonization preference was carried out with the genotypes selected based on the free-choice test conducted. The results confirmed the susceptibility of 'IAC Holambra Stwart' to silverleaf whitefly, since it showed more nymphs than the other genotypes throughout the experimental period (Table 2). 'Barreiras' was the least infested, with significantly fewer nymphs than the known resistant standard genotypes, 'IAC 17' and 'IAC 19', at 21 and 28 days after infestation (DAI), respectively (Table 2). These results confirm the resistance to the silverleaf whitefly observed in the free-choice test. Cultivar BRS Gralha, which was the least attractive to adults, and consequently had the lowest infestation of eggs and nymphs in the free-choice test (Table 1), did not show resistance to insect attack in the no-choice test (Table 2). In this situation, the infestation was always high and similar to the susceptible standard 'IAC Holambra Stwart' (Table 2). In contrast, the number of nymphs observed on BABR01-1576 decreased 54% and 32% in the evaluations performed at 21 DAI and 28 DAI, respectively, as compared to 14 DAI; whereas for all the other genotypes tested this number increased or remained at the same level. In the last evaluation, the number of nymphs observed on BABR01-1576 was lower than that observed for the selected resistant standards ('IAC 17' and 'IAC 19').

The density and length of trichomes are generally considered one of the mechanisms involved in the susceptibility or resistance of soybean genotypes to silverleaf whitefly attack. In this present study, the soybean genotypes were evaluated for the length and density of trichomes on the leaf blade and veins (Table 3). Cultivar IAC Holambra Stwart had the longest and densest trichomes, both on the veins (40.4 trichomes/16 mm^2) and on the leaf blade (69.6 trichomes/16 mm^2). The large number of trichomes on the leaf, in general, and the longer trichomes on leaves of 'IAC Holambra Stwart', may be important traits for insect colonization, helping adults to remain on the leaf surface and avoid being blown off by the wind. McAuslane (1996) observed that soybean genotypes with a high density of trichomes were preferred for oviposition when compared to glabrous ones.

A high density of trichomes is positively correlated with oviposition by *B. tabaci* biotype B on several different host crops, such as cotton (Mound 1965, Flint & Parks 1990), soybean (McAuslane *et al* 1995, Lima & Lara 2004), and tomato (Heinz & Zalon 1995). Butter & Vir (1989) suggested that densely haired genotypes may offer a more appropriate microhabitat for oviposition to *B. tabaci* females, which prefer to lay their eggs at the base of the trichome insertion (Omram & El-Khidir 1978, Berlinger 1986). This behavior may be an evolutionary response to selection pressure exerted by predators and parasitoids (Butter & Vir l989), as natural enemies are more efficient on glabrous or on less-hairy leaves than on densely haired ones (Li *et al* 1987).

Our data allowed us to conclude that the most resistant genotypes to *B. tabaci* biotype B were 'Barreiras', 'IAC 17', and 'IAC 19'. 'BRS Gralha' was the least attractive to whitefly adults in the free-choice test, but did not display

Table 2 Number (mean ± SE) of nymphs of *Bemisia tabaci* biotype B on different soybean genotypes, on different days after infestation (DAI) in the no-choice test.

Genotype	Nymphs/leaflet[1]		
	14 DAI	21 DAI	28 DAI
'IAC Holambra Stwart'	67.3 ± 14.29 a	84.4 ± 5.60 a	76.2 ± 7.39 a
'BRS Gralha'	30.0 ± 6.47 ab	61.6 ± 3.49 ab	50.0 ± 3.08 ab
'IAC 19'	40.4 ± 9.64 ab	41.0 ± 3.42 bc	39.6 ± 5.22 bc
'IAC 17'	33.4 ± 5.48 ab	43.2 ± 6.90 bc	35.2 ± 4.63 bc
'Corisco'	35.4 ± 9.92 ab	37.3 ± 4.56 cd	33.8 ± 4.74 bcd
'Barreiras'	13.6 ± 5.81 b	24.2 ± 5.90 de	24.2 ± 4.56 cd
BABR 01-1576	35.8 ± 10.77 ab	16.6 ± 2.25 e	20.6 ± 4.61 d
CV (%)	48.68	19.82	19.98

Means followed by the same letter within columns are not statistically different by the Tukey test (P > 0.05). [1]Original data followed by statistics (including CV) done on data transformed by $\sqrt{X}+1$ as required for ANOVA according to Burr & Foster (1972).

Table 3 Density and size (mean ± SE) of leaf trichomes of different soybean genotypes.

Genotype	Length of trichomes (mm)		Density of trichomes (on 16 mm^2)	
	Leaf blade[1]	Vein	Leaf blade[2]	Vein
'IAC Holambra Stwart'	1.8 ± 0.03 e	3.1 ± 0.08 cd	69.6 ± 3.37 a	40.4 ± 2.38 a
'IAC 19'	1.8 ± 0.04 de	3.1 ± 0.09 bcd	34.7 ± 1.85 b	25.1 ± 1.17 b
BABR99-4021HP	1.9 ± 0.06 de	2.9 ± 0.07 cd	27.3 ± 1.41 bcd	22.6 ± 1.79 b
BABR99-4021HC	1.9 ± 0.10 cde	2.9 ± 0.08 cd	25.6 ± 1.21 bcd	22.3 ± 1.22 b
PI227687	2.0 ± 0.08 cde	2.7 ± 0.07 d	30.8 ± 2.82 bc	26.1 ± 2.18 b
'BRS Gralha'	2.0 ± 0.13 cde	3.0 ± 0.08 cd	33.4 ± 2.27 B	21.9 ± 2.61 b
BABR01-0492	2.0 ± 0.08 cde	3.6 ± 0.13 ab	30.4 ± 1.88 BC	25.9 ± 1.42 b
'IAC 17'	2.1 ± 0.06 bcde	3.8 ± 0.08 a	33.6 ± 2.49 b	25.7 ± 1.60 b
'Barreiras'	2.1 ± 0.10 bcde	3.1 ± 0.09 bcd	22.2 ± 1.21 cd	19.4 ± 1.74 b
BABR01-0173	2.1 ± 0.07 abcde	3.4 ± 0.16 abc	29.8 ± 1.56 bc	22.2 ± 1.80 b
BABR01-1259	2.1 ± 0.05 abcde	3.0 ± 0.14 cd	22.4 ± 1.18 cd	21.3 ± 1,26 b
'Corisco'	2.2 ± 0.08 abcd	3.3 ± 0.11 abc	34.8 ± 3.95 b	24.6 ± 2.47 b
PI171451	2.3 ± 0.06 abc	3.1 ± 0.09 cd	34.8 ± 2.50 b	27.4 ± 2.02 b
'Conquista'	2.3 ± 0.21 abc	3.7 ± 0.17 a	20.5 ± 1.34 d	20.3 ± 1.23 b
PI274454	2.5 ± 0.17 abc	3.3 ± 0.13 abc	29.5 ± 1.63 bc	22.8 ± 1.99 b
BABR01-1576	2.5 ± 0.07 a	3.6 ± 0.05 ab	32.7 ± 2.04 b	26.6 ± 0.93 b
CV (%)	16.35	10.68	10.02	20.70

Means followed by the same letter within columns are not statistically different by the Tukey test (P > 0.05); [1]Original data followed by statistics (including CV) done on data transformed to log (x) as required for ANOVA according to Burr & Foster (1972); [2]Original data followed by statistics (including CV) done on data transformed to $\sqrt{X}$ as required for ANOVA according to Burr & Foster (1972).

any resistance to insect attack in the no-choice test. Despite the high number of eggs observed, BABR01-1576 and BABR99-4021HC showed relatively few nymphs, indicating resistance of the antibiosis type. However, the size and number of trichomes were not the only factors responsible for the differences in resistance observed among the genotypes evaluated, because only small differences were observed, as already reported by others (Lambert *et al* 1995b). Therefore, chemical features of the plants might play an important role as well, and plants with upeol usually have higher whitefly populations (Lambert at al 1995b). The chemical characteristics of the resistant cultivars 'Barreiras' and standards used in this study ('IAC 17' and 'IAC 19') may also be chemically evaluated in future trials to better establish the resistance mechanism involved. It is important to point out that the soybean genotypes with high resistance levels can be used as a tool against *B. tabaci* in IPM, or as a source of resistance in plant breeding programs.

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