

Antixenosis in cotton genotypes (*Gossypium hirsutum* var. *marie galante*) to *Spodoptera frugiperda* (Lepidoptera: Noctuidae) mediated by trichome and gossypol densities

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Abstract

Cotton (*Gossypium hirsutum* L.) is one of the most important crops in Brazil and pest incidence limits its yields. The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), native to tropical and subtropical America, spread and rapidly became an invasive pest worldwide. It is a polyphagous pest that feeds on up to 353 plant species including cotton. The objective of this study was to evaluate the antixenosis to *S. frugiperda* in 27 cotton genotypes, comparing varieties with non-commercial genotypes. The attractiveness was estimated in no-choice and free-choice tests, offering leaf discs of the 27 cotton genotypes to *S. frugiperda*, and counting the number of caterpillars feeding at 3, 5, 10, 15, and 30 minutes and 1, 2, 6, 12, and 24 hours after their release. The numbers of trichomes and gossypol glands per cm² were measured on abaxial and adaxial surface. The free-choice test was performed in a randomized block design with the 27 genotypes (treatments) and 20 arenas (replicates). The no-choice assay was conducted as a completely randomized design with 18 genotypes and 20 replicates. The non-commercial genotypes PI0440, CE0467, PI0416, BA0512 and MA0425 presented antixenosis against *S. frugiperda*. In the free-choice test the cultivar BRS Buriti and the genotype PI0429 were the most attractive to *S. frugiperda*, while in no-choice test, the genotype PI0429 was the most attractive. High trichome density and gossypol glands did not influence the attractiveness of *S. frugiperda* in cotton genotypes, while the absence of trichomes was repellent to the caterpillars. There is polymorphism among cotton genotypes to attractiveness to the pest, and the genotypes with antixenosis can be used in future study in plant breeding program as donor source of resistance to *S. frugiperda*.

Keywords: Attractiveness, Gossypol, Plant Resistance to insect, Trichomes.

Abbreviations: IPM- Integrated Pest Management Programs; UPGMA - Unweighted Pair Group Method with Arithmetic Mean.

Introduction

Gossypium hirsutum var. *marie galante* is an arboretum cotton species with a large center of origin, ranging from Colombian and Venezuelan coasts to the Southeast of El Salvador, the Antilles, and the Semiarid Northeast Brazil (Moreno et al., 2008). In Brazil, *G. hirsutum* r. *marie galante* is known as mocó cotton, bearing large variability, and differing to the Central American (Stephens, 1973). In Semiarid Northeast region of Brazil, mocó cotton coexists with the compatible species *G. barbadense* and *G. mustelinum*, leading to natural crosses and increased variability (Menezes et al., 2017; Menezes, 2014; Menezes, 2010).

Mocó cotton represented the largest area planted with cotton in Brazil in the 1970s, all of it in the Northeast region of Brazil, being grown on approximately 2.5 million hectares, for its high fiber quality and drought resistance (Stephens, 1973; Liu and Wendel, 2002; Menezes et al., 2015). The main producers were found in the states of Paraíba, Rio Grande

do Norte, Ceará, Piauí, Maranhão, and Pernambuco (Freire, 2007). However, in the 1980s, the cultivated area of mocó cotton was reduced due to economic and political changes, favored also by climatic problems, in addition to the dispersion of the boll weevil (*Anthonomus grandis*) (Coleoptera: Curculionidae) (Giband et al., 2010; Menezes et al., 2017). From this decade, cotton lines bred from cotton types domesticated in Mexico and cultivated in North America (*G. hirsutum* var. *latifolium*) started to be cultivated in Brazilian Cerrado (similar to Savannah) in Central Brazil.

Cotton has been one of the most important crops in Brazil, but one of the main factors that limit its sustainability is the incidence of pests (Campos et al., 2012). Among the pests, caterpillars are the second cause of economical loss after boll weevil, even when transgenics are used. *Spodoptera frugiperda* (Lepidoptera: Noctuidae) causes damage in the vegetative and reproductive phase, feeding on leaves,

flower, floral bud, and green cotton boll (Moreno et al., 2008; Jesus et al., 2014).

The use of pest resistant cotton cultivars can be adopted as strategy in integrated pest management program – IPM to increase crop sustainability (Baldin et al., 2019). The mainly morphological characteristics reported in cotton plants that confer resistance are: frego bract, absence of nectaries, glabrous plants, gossypol, tannin, leaf and bract color, and okra leaf (Bastos et al., 2008). Detection of resistance traits within traditional or native Brazilian genotypes may lead to insertion of valuable traits in bred varieties (Menezes et al., 2014).

Plant resistance to insects are commonly referred to in three categories: non-preference (antixenosis), antibiosis and tolerance. These types of resistance can cause changes in insect behavior and/or biology (antixenosis and antibiosis) or even a reaction of the plant due resulting in tolerance (Seifi et al., 2013; Smith, 2005).

The study involving plant resistance to insects in cotton is limited to commercial cultivars in Brazil. Campos et al. (2012) observed lower attractiveness of *S. frugiperda* in the cultivars BRS Acala-90, Fibermax-966 and DeltaPenta, BRS Cedro, BRS Itamarati 90, Coodetec 408 and BRS Aroeira. Jesus et al. (2014) observed antibiosis in the cultivar NuOpal® to *S. frugiperda* and the caterpillars and pupae reared on this cultivar had their biology negatively affected. Given the scarcity of studies evaluating the resistance of local cotton varieties to *S. frugiperda* due the importance of these materials in plant breeding program as donor source of resistance, the objective this study was to evaluate the antixenosis in 27 cotton genotypes to *S. frugiperda*.

Results

Antixenosis

The mocó genotypes collected in farms or backyards (Table 1) PI0440, CE0467, MA0423, RN0512, MA0430, MA0409, BA0502, MA0418, CE0461, MA0438, PI0467, and AC0602 and the cultivars BRS Cedro and IAC 25 were the least attractive to *S. frugiperda* in free-choice test, and the mocó cotton PI0429, the most attractive (Table 2).

In the no-choice test, the collected genotypes PI0467, RN0512, MA0418, MA0425, MA0438, MA0409, BA0477, CE0474, PI0416, CE0467, MA0423, PI0440, BA0502, CE0461, BA0512, BA05101, PI0432, PI0438, AC0602 and the variety BRS Cedro were less attractive to *S. frugiperda* than the varieties BRS Buriti and IAC 25 or the collected genotypes PI0429, MA0407, CE0507, PI0433 and MA0430 (Table 2). According to the attractiveness index in both tests, the genotypes RN0512, MA0418, CE0467 and BA0502 were classified as repellent to *S. frugiperda*.

Morphological characteristics

Trichome densities on the adaxial and abaxial surfaces were greatest in PI0416 genotype (Table 3). The genotypes PI0440, AC0602, RN0512, PI0429, CE0467, PI0432, PI0437, BA05101, IAC25, BA0512, MA0438, MA0418, MA0430, CE0461, MA0407, CE0507, MA0409, CE0474, BA0502, PI0433 and PI0467 and the cultivar BRS Buriti showed the lowest trichome densities on the abaxial surface. The genotypes RN0512, PI0432, PI0429, BA0502, BA0512, CE0467, IAC 25, PI0438, BA05101, MA0438 and the cultivar BRS Buriti, also showed the lowest trichome densities on the adaxial surface. The genotype PI0440 did not present trichomes on any leaf surface.

The genotypes PI0433, CE0507, MA0407, BA0477 and MA0409 and the cultivar BRS Cedro, showed a greatest amount of gossypol glands on the adaxial surface (Table 3). On the abaxial surface, the cultivar BRS Cedro and PI0433, CE0507, BA0477, CE0474, CE0461 and MA0409 genotypes showed highest trichome densities.

The correlation coefficients between trichomes or gossypol glands and attractiveness in free-choice or no-choice tests were not significant, showing that the attractiveness of *S. frugiperda* in cotton genotypes was not influenced by trichome densities or the number of gossypol glands (Table 4).

UPGMA separated the groups by different levels of antixenosis according to attractiveness in free-choice and no-choice tests (Fig. 1; Fig. 2). In free-choice test, the group I was composed by the highly susceptible genotypes: BRS Buriti, PI0429 and PI0433; group II, by the susceptible genotypes: MA0407, CE0507 and MA0430; and group III comprised the moderately resistant genotypes: PI0440, CE0461, MA0423, CE0467, BRS Cedro, RN0512, BA0512, MA0425, CE0474, PI0432, PI0467, BA0477, PI0416, AC0602, PI0437, BA0502, BA05101, IAC 25, MA0418 and MA0409 (Fig. 1).

In non-choice test, the group I, was defined by a single genotype as highly susceptible: PI0429; group III comprised the moderately resistant genotypes: IAC 25, PI0467, MA0418, BRS Cedro, MA0438, PI0437, CE0461, BA0502, MA0409, CE0467, PI0440, MA0430, MA0423 and RN0512; and the intermediary group II was composed by the susceptible genotypes: PI0433, BA05101, AC0602, MA0425, BA0512, MA0407, PI0432, BRS Buriti, CE0474 and PI0416 (Fig. 2).

Discussion

In free-choice and no-choice tests, the cotton genotypes influenced the attractiveness behavior of *S. frugiperda*.

The genotypes PI0440, CE0467, MA0423, RN0512, MA0430, MA0409, BA0502, IAC 25, MA0418, CE0461, MA0438, PI0467, BRS Cedro and AC0602, which were less attractive to *S. frugiperda* in free-choice test may not favor the insect-plant interaction lacking to stimulate the insect to feed (Baldin et al., 2019). Most herbivorous insects are shown to be attracted by visual, olfactory and other stimuli, based on the physic-chemical characteristics of the plant's surface (Yang et al., 1991). Factors such as visual and olfactory stimuli, may have influenced the process of choice of the insect by the host and resulted in the repellency of *S. frugiperda*.

In no-choice test, the genotypes PI0467, RN0512, MA0418, MA0425, MA0438, MA0409, BRS Cedro, BA0477, CE0474, PI0416, CE0467, MA0423, PI0440, BA0502, CE0461, BA0512, BA05101, PI0432, PI0438 and AC0602 were the least attractive to *S. frugiperda*. Positive and negative stimuli from the plant influenced the feeding behavior, which nature may be of chemical (Hoffmann-Campo et al., 2001), physical (Prado et al., 2015) and/or morphological (Silva et al., 2014). Considering the attractiveness index, the genotypes RN0512, MA0418, CE0467 and BA0502, that were the least preferred by *S. frugiperda* both in free-choice and no-choice tests, thus antixenosis is present. Antixenosis is related to changes in the insect's behavior for feeding, oviposition and shelter promoted by the host plant (Baldin et al., 2019). The cause of antixenosis in plants are due to the presence of chemical substances, which influences the insect's behavior by attracting or repelling and/or due to the plant's

Table 1. Relationship and origin of cotton genotypes. State of collection by Embrapa and cotton species (<https://www.embrapa.br/en/alelo>).

Genotype	States of Origin	Species
BRS Buriti ¹	Goiás	<i>G. hirsutum latifolium</i>
BRS Cedro ¹	Piauí	<i>G. hirsutum latifolium</i>
IAC 25 ¹	São Paulo	<i>G. hirsutum latifolium</i>
AC0602 ²	Acre	<i>G. hirsutum r. marie galante</i>
BA0477 ²	Bahia	<i>G. hirsutum r. marie galante</i>
BA0502 ²	Bahia	<i>G. hirsutum r. marie galante</i>
BA05101 ²	Bahia	<i>G. hirsutum r. marie galante</i>
BA0512 ²	Bahia	<i>G. hirsutum r. marie galante</i>
CE0461 ²	Ceará	<i>G. hirsutum r. marie galante</i>
CE0467 ²	Ceará	<i>G. hirsutum r. marie galante</i>
CE0474 ²	Ceará	<i>G. hirsutum r. marie galante</i>
CE0507 ²	Ceará	<i>G. hirsutum r. marie galante</i>
MA0407 ²	Maranhão	<i>G. hirsutum r. marie galante</i>
MA0409 ²	Maranhão	<i>G. hirsutum r. marie galante</i>
MA0418 ²	Maranhão	<i>G. hirsutum r. marie galante</i>
MA0423 ²	Maranhão	<i>G. hirsutum r. marie galante</i>
MA0425 ²	Maranhão	<i>G. hirsutum r. marie galante</i>
MA0430 ²	Maranhão	<i>G. hirsutum r. marie galante</i>
MA0438 ²	Maranhão	<i>G. hirsutum r. marie galante</i>
PI0416 ²	Piauí	<i>G. hirsutum r. marie galante</i>
PI0429 ²	Piauí	<i>G. hirsutum r. marie galante</i>
PI0432 ²	Piauí	<i>G. hirsutum r. marie galante</i>
PI0433 ²	Piauí	<i>G. hirsutum r. marie galante</i>
PI0438 ²	Piauí	<i>G. hirsutum r. marie galante</i>
PI0440 ²	Piauí	<i>G. hirsutum r. marie galante</i>
PI0467 ²	Piauí	<i>G. hirsutum r. marie galante</i>
RN0512 ²	Rio Grande do Norte	<i>G. hirsutum r. marie galante</i>

¹Commercial Cultivars. ² Moco cotton (Landraces).

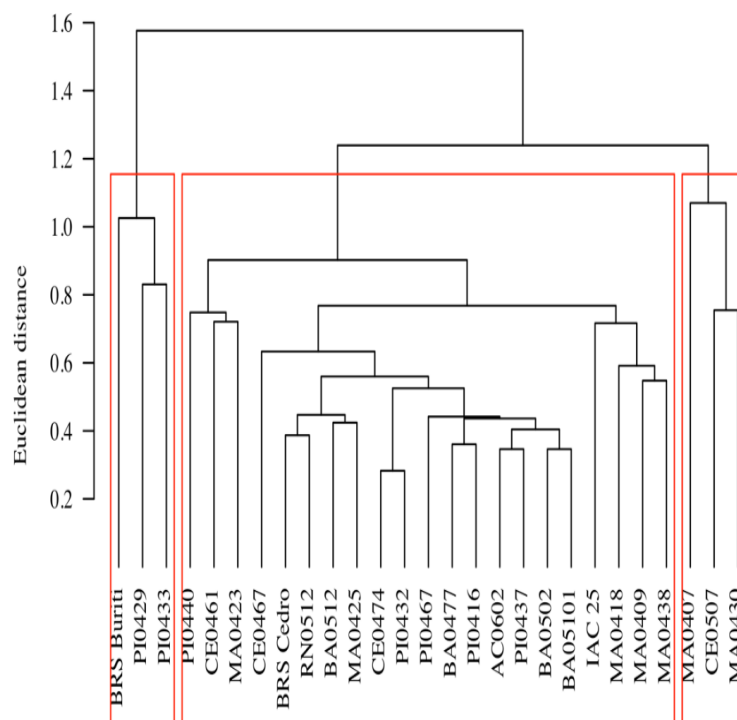


Fig 1. Dendrogram resulting from UPGMA multivariate cluster analysis (Euclidian distance), based on the attractiveness mean of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in free-choice test in 27 cotton genotypes.

Table 2. Mean number of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and attractiveness index in free-choice and no-choice tests in 27 cotton genotypes.

Genotypes	Attractiveness		Attractiveness index	
	Free-choice	No-choice	Free-choice	No-choice
IAC25	0.16±0.03 c	0.46±0.07 a	0.88 D	1.24 S
MA0430	0.11±0.03 c	0.50±0.10 a	0.72 D	1.28 S
PI0467	0.20±0.02 c	0.25±0.06 b	1.00 N	0.94 D
RN0512	0.10±0.04 c	0.26±0.06 b	0.68 D	0.96 D
MA0418	0.17±0.02 c	0.26±0.05 b	0.91 D	0.96 D
MA0425	0.27±0.05 b	0.19±0.05 b	1.14 S	0.81 D
MA0438	0.17±0.02 c	0.32±0.07 b	0.92 D	1.07 S
BRS Buriti	0.27±0.02 b	0.72±0.09 a	1.14 S	1.44 S
MA0409	0.13±0.04 c	0.32±0.07 b	0.78 D	1.14 S
BRS Cedro	0.20±0.02 c	0.28±0.06 b	1.00 SP	1.00 SP
PI0429	0.49±0.05 a	0.70±0.08 a	1.41 S	1.44 S
BA0477	0.30±0.04 b	0.22±0.06 b	1.19 S	0.88 D
CE0474	0.29±0.03 b	0.32±0.06 b	1.17 S	1.07 S
MA0407	0.37±0.06 b	0.62±0.07 a	1.29 S	1.38 S
PI0416	0.26±0.04 b	0.17±0.05 b	1.13 S	0.76 D
CE0467	0.09±0.04 c	0.20±0.05 b	0.63 D	0.83 D
MA0423	0.10±0.04 c	0.36±0.08 b	0.68 D	1.13 S
PI0440	0.09±0.03 c	0.39±0.07 b	0.61 D	1.16 S
BA0502	0.15±0.04 c	0.23±0.06 b	0.85 D	0.90 D
CE0461	0.17±0.03 c	0.34±0.07 b	0.91 D	1.10 S
BA0512	0.34±0.05 b	0.18±0.06 b	1.25 S	0.78 D
BA05101	0.27±0.05 b	0.25±0.50 b	1.14 S	0.94 D
PI0433	0.27±0.05 b	0.54±0.05 a	1.15 S	1.32 S
CE0507	0.34±0.03 b	0.61±0.10 a	1.25 S	1.37 S
PI0432	0.30±0.07 b	0.33±0.06 b	1.19 S	1.08 S
PI0438	0.11±0.03 c	0.31±0.05 b	0.70 D	1.05 S
AC0602	0.22±0.02 c	0.23±0.08 b	1.05 S	0.90 D
F	5.27	2.88	-	-
P Value	<0.0005	<0.0005	-	-

¹Means followed by the same letter in the column do not differ statistically by the Scott-Knott test at 5% probability. SE = standard error. D = Deterrent. N = Neutral. S = Stimulant. SP = Susceptible pattern.

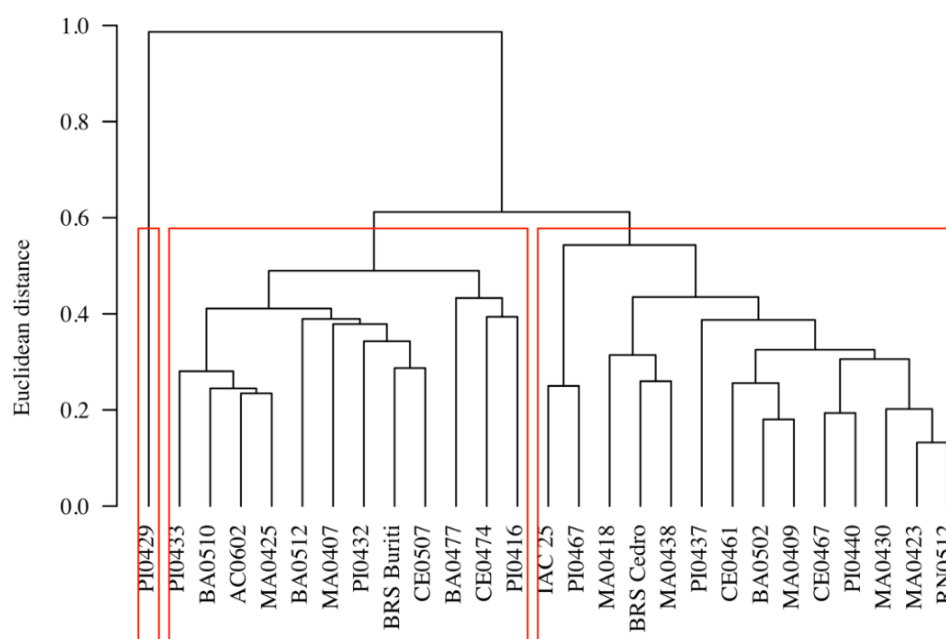


Fig 2. Dendrogram resulting from UPGMA multivariate cluster analysis (Euclidian distance), based on the attractiveness mean of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in no-choice test in 27 cotton genotypes.

Table 3. Number (cm²) of trichomes and gossypol glands in 27 cotton genotypes.

Genotypes	Trichomes		Gossypol Glands	
	Adaxial	Abaxial	Adaxial	Abaxial
IAC25	6.25±6.25 c	16.25±9.56 e	61.00±4.18 b	69.25±6.26 c
MA0430	24.75±1.80 c	67.50±10.52 d	65.25±8.73 b	83.00±6.49 b
PI0467	93.25±30.16 b	139.75±32.47 c	49.25±5.19 c	70.75±6.81 c
RN0512	1.25±1.25 c	1.75±1.75 e	37.25±9.85 c	55.50±6.12 c
MA0418	21.50±7.66 c	60.00±7.31 d	69.00±6.65 b	89.75±9.33 b
MA0425	33.25±4.33 c	62.25±7.36 d	73.25±7.39 b	97.50±5.17 b
MA0438	9.25±4.55 c	28.25±8.13 e	6.00±1.29 d	17.50±2.60 d
BRS Buriti	20.75±13.10 c	30.00±9.91 e	56.50±25.34 b	103.00±13.53 b
MA0409	50.25±13.85 c	124.25±22.86 c	93.00±21.30 a	109.00±9.25 a
BRS Cedro	33.25±8.58 c	35.75±7.69 e	103.00±16.79 a	143.75±13.58 a
PI0429	3.75±2.50 c	7.50±3.12 e	41.00±6.54 c	77.00±8.70 b
BA0477	40.00±16.27 c	176.00±24.77 c	86.00±5.49 a	115.75±11.99 a
CE0474	60.75±6.25 c	94.50±6.14 d	69.00±6.10 b	112.75±8.89 a
MA0407	43.00±9.60 c	70.00±4.55 d	103.50±4.59 a	96.75±4.27 b
PI0416	257.75±61.48 a	293.75±26.74a	46.25±6.79 c	65.00±4.85 c
CE0467	4.00±1.47 c	15.25±6.49 e	7.50±3.88 d	22.00±3.81 d
MA0423	140.00±20.28 b	230.00±15.8 b	70.50±18.52 b	95.00±18.27 b
PI0440	-	-	27.00±12.16 c	53.00±8.45 c
BA0502	63.25±13.35 c	157.75±44.54 c	65.00±6.47 b	96.75±6.26 b
CE0461	26.75±7.77 c	116.50±9.68 c	79.75±17.78 b	111.25±9.45 a
BA0512	7.75±6.49 c	14.50±8.43 e	65.25±9.97 b	82.75±8.44 b
BA05101	5.25±3.35 c	28.25±5.68 e	44.75±8.63 c	45.75±5.51 c
PI0433	116.75±47.47 b	137.00±28.07 c	111.75±16.33 a	135.25±14.63 a
CE0507	45.50±5.25 c	201.50±48.29 b	99.00±21.33 a	124.50±23.26 a
PI0432	4.25±1.65 c	5.00±1.47 e	8.00±6.36 d	14.50±4.41 d
PI0438	4.25±2.10 c	26.00±5.12 e	6.00±2.68 d	14.75±5.92 d
AC0602	0.50±0.29 c	2.75±1.18 e	26.50±7.38 c	59.00±15.76 c
F	98.968	18.664	70.809	12.745
P Value	<0.0005	<0.0005	<0.0005	<0.0005

¹Means followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability. SE = standard error. * Significant at 5%, ** 1% and *** 0.1%.

Table 4. Correlations between the attractiveness of *S. frugiperda*, in free-choice and no-choice tests, and i) the number of trichomes and ii) the number of gossypol glands, obtained on the adaxial and abaxial sides of the leaf in cotton genotypes.

Variables	Attractiveness			
	Free-choice		Nonchoice	
	r ¹	P Value	r ¹	P Value
Attractiveness X T. Adaxial	-0.04 ^{ns}	0.85	0.18 ^{ns}	0.35
Attractiveness X T. Abaxial	-0.11 ^{ns}	0.94	0.10 ^{ns}	0.62
Attractiveness X Gossypol AD.	0.12 ^{ns}	0.54	0.11 ^{ns}	0.59
Attractiveness X Gossypol AB.	0.19 ^{ns}	0.32	0.05 ^{ns}	0.80

¹ Correlation coefficient; ^{ns} not differ statistically by T-test at 5% probability. T. = Trichomes; AD= Adaxial; AB= Abaxial.

morphological characteristics such as color, type of epidermis, presence or absence of trichomes (Seifi et al., 2013; Smith, 2005).

Among the plant morphological characteristics, the presence or absence of trichomes has been associated with antixenosis in several plant varieties (Handley et al., 2005). In cotton, the trichome density has been studied as a source of antixenosis, affecting the insects' feeding and oviposition behavior. Whitefly (*Bemisia tabaci* biotype B Hemiptera: Aleyrodidae) have a preference for ovipositing in cotton plants with a high trichome densities (Prado et al., 2015; Toscano et al., 2003). Cotton with a large amount of trichomes decrease the mobility of 1st instar *Chloridea virescens* (Lepidoptera: Noctuidae) (Ramalho et al., 1984). The PI0416 genotype showed the highest trichome density on both adaxial and abaxial surfaces, while the

attractiveness of *S. frugiperda* to this genotype was intermediary, thus this morphological characteristic seems to have no influence to the pest behavior. On the other hand, the genotype PI0440, which was characterized as glabrous, had the lowest attractiveness to *S. frugiperda*. The genotypes RN0512, MA0418, CE0467 and BA0502, which were repellent to *S. frugiperda*, showed the lowest trichome densities. Thus the absence of trichomes seem to be a source of antixenosis.

Another cause of resistance in cotton is the presence of allelochemicals, mainly the terpenoid gossypol (Campos et al., 2012). In the present study, the genotypes PI0433, CE0507, MA0407, BA0477 and MA0409 and the cultivar BRS Cedro showed a greater amount of gossypol glands in the adaxial surface and BRS Cedro and the genotypes PI0433, CE0507, BA0477, CE0474, CE0461 and MA040 in the abaxial

surface. The terpenoid gossypol do not seem to impede the attractiveness of *S. frugiperda*, since the genotypes PI0433, CE0507 and MA0407, which showed a high number of gossypol glands, were the most attractive to *S. frugiperda*. Thus, the gossypol content do not drive the pest behavior, as was also verified for boll weevil particularly for gossypol as for others terpenoid aldehydes (Magalhães et al., 2020). Other differences among genotypes may explain attractiveness, such as other secondary compounds (nitrogenous, phenolic, terpenoids, organic acids, lipids) and cell wall thickness and hardness (Styrsky et al., 2006). Gossypol glands may not affect the feeding behavior of *S. frugiperda*, because insects such as caterpillars develop morphological and behavioral characteristics to overcome the defense imposed by plant (Young and Moffett, 1979). UPGMA analysis in free-choice and no-choice tests, separated the genotypes in different levels of resistance to *S. frugiperda*, showing to be an important tool to complement the univariate methods in the screening of plants resistant to insects (Pitta et al., 2010). In general, it was observed that the genotypes IAC 25, PI0467, MA0418, MA0438, PI0437, CE0461, BA0502, MA0409, CE0467, PI0440, MA0430 and RN0512 were classified as moderately resistant (antixenosis) to *S. frugiperda*.

Materials and Methods

Colony of *Spodoptera frugiperda*

The study was conducted at the Integrated Pest Management Laboratory of the Goiano Federal Institute (Instituto Federal Goiano), Campus Urutaí, Urutaí, Goiás State, Brazil.

Insects obtained from the Embrapa Arroz e Feijão Entomology Laboratory were used to develop a colony. The pupae were sexed and placed in 20 cm high x 10 cm diameter PVC cages, lined internally with paper (oviposition substrate) for emergence and mating. The adults were fed on 10% honey solution, placed in plastic containers (PET bottle cap), soaked in cotton and changed every two days. The newly hatched caterpillars were placed in 150 mL plastic pots containing an artificial diet (Kasten Junior et al., 1978). After reaching the 3rd instar caterpillars were individualized in plastic containers (50 mL) until the pupal phase. The insects were reared in the laboratory at 25 ± 2 °C, $70 \pm 10\%$ RH, and 12:12 h (L:D).

Plant material

The twenty-seven cotton genotypes of the *Gossypium hirsutum* species, obtained from the Embrapa Cotton Germplasm Bank (Table 1), were planted and conducted grown under field conditions in an experimental area, at the Instituto Federal Goiano, Campus Urutaí (17°29'10"S in latitude and 48°12'38"W in longitude at 823 m altitude).

The seeds were scarified and sown in plastic bags (1 L) with substrate (3: 1: 1 - soil, sand and cattle manure), containing three seeds and thinned out 30 days after planting (DAP). At 45 DAP they were transplanted to the field in an irrigated area. Leaves were obtained from these plants that did not receive insecticide spraying in field.

The attractiveness in free-choice test was performed with 3rd instar caterpillars, offering leaf discs (2.5 diameter) of the cotton genotypes (n = 27) to *S. frugiperda*. The leaves were collected in the apical part of the plant and distributed equidistantly in a circular arena (70 cm in diameter, and 6 cm in height) on moistened filter paper in controlled

temperature of 25 ± 2 °C, photoperiod (12 h) and humidity ($70\% \pm 10$).

The attractiveness in no-choice test was performed offering one leaf disc (2.5 diameter) per arena (Petri dish 9 cm in diameter). In both tests, the attractiveness was assessed by counting the number of caterpillars feeding on each leaf disk at 3, 5, 10, 15, 30 minutes and 1, 2, 6, 12 and 24 hours after release. In the free-choice test the experiment was set in a randomized block design with 27 treatments (genotypes) and 20 replicates (arenas) with two caterpillars per genotype. In non-choice test, a completely randomized design with 18 treatments (genotypes) and 20 replicates was used with one caterpillar per genotype.

Morphological characteristics - trichome density and gossypol glands

Two leaves of each genotype were collected in the median region of the plant 30 days after sowing. The average trichome numbers and gossypol glands per cm² were analyzed in abaxial and adaxial leaf surfaces, both close to the central vein, using a stereoscope microscope (Bel Photonics, Model SZ B LED 89/336, Piracicaba, São Paulo, Brazil).

Statistical analysis

Residual normality and homoscedasticity were evaluated by the Shapiro-Wilk and Bartlett tests. The attractiveness and morphological data were analyzed by Anova and means compared by the Scott Knott test ($P \leq 0.05$). The t-test was used to check the correlation between i) the attractiveness in free-choice test and trichome density; ii) the attractiveness in no choice test and trichome density; iii) the attractiveness in free-choice test and the number of gossypol glands and iv) the attractiveness in the no choice test and the number of gossypol glands. The four comparisons were performed independently for abaxial and adaxial leaf surfaces. A cluster analysis was performed using the Hierarchical Cluster Analysis – UPGMA method based on the Euclidian distance to determine the degree of resistance between the cotton genotypes to *S. frugiperda*. All statistical analyses were performed using R program (R Core Team, 2019). The attractiveness index for *S. frugiperda* was determined using the formula: $IP = 2A/(M + A)$, where: A = Consumption in the genotype and M = susceptibility pattern (BRS Cedro) (Kogan and Goeden, 1970). A genotype was classified as a stimulant if its index was greater than 1, neutral if equal to 1 and deterrent if less than 1.

Conclusions

The genotypes NR0512, MA0418, CE0467 and BA0512 presented characteristics of antixenosis to *S. frugiperda*. The high trichome densities and the number of gossypol glands in cotton plants do not determine the attractiveness of *S. frugiperda*. These cotton genotypes can be used in future study in plant breeding program as donor source of resistance to *S. frugiperda*.

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