

Antixenosis to *Chrysodeixis includens* (Lepidoptera: Noctuidae) among soybean genotypes

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ABSTRACT: The soybean looper *Chrysodeixis includens* is one of the main defoliating pests of soybean cultures in Brazil. This work aimed to evaluate the effect of offering different soybean genotypes to this insect to verify the occurrence of antixenosis. In a greenhouse, assays were carried out to determine the attractiveness of *C. includens* adults given free-choice tests. This experiment used a randomized block design with five replicates. A second experiment assessed oviposition preference using no-choice tests, employing a completely randomized design with five replicates. Seventeen soybean cultivars were used; several of these had previously been reported as carriers of resistance to more than one species of insect. In the laboratory, a morphological analysis was carried out by quantifying the number of trichomes on leaf surfaces and evaluating the color of the leaf

blade of the germplasm. In the attractiveness assay, genotypes ‘IAC 19’, ‘IAC 18’, ‘IAC 23’, L1-1-01, PI 274453, PI 229358, PI 171451, ‘IAC 100’, ‘IAC 24’, ‘IAC 17’ and IAC 74-2832 were classified as repellents. In the oviposition preference experiment, PI 171451, PI 274453, ‘IAC 18’, L1-1-01 and ‘IAC 23’ showed the fewest eggs. The high density of trichomes presented by the genotypes PI 227687 and PI 274453 stand out as a possible *C. includens* resistance factor. The genotype L1-1-01 presented intermediate luminosity (L*) and more intense green (a*) and yellow (b*) colors compared to other genotypes. There was no correlation between oviposition and trichome density or leaf coloration.

Key words: *Glycine max*, soybean looper, host plant resistance to insects, non-preference.

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INTRODUCTION

Brazil is considered to be one of the major soybean producers in the world (Sentelhas et al. 2015). Soybeans are an important economic crop, largely because of derived products such as soybean meal and soybean oil (Thoenes 2015; Singh et al. 2008). Estimated soybean production during 2014-2015 was approximately 96.044.500 tons, an increase of 11.5% over the estimates of previous years, mainly due to an increase in the area of soybean cultivation (Acompanhamento da safra brasileira de grãos 2015).

The soybean looper *C. includens* is one of the main defoliating pests of soybean cultures in Brazil (Bernardi et al. 2012). This insect pest also attacks other hosts, but the adult females apparently prefer to lay eggs on soybean plants (Martin et al. 1976). Lepidopteran females preferentially choose to oviposit in favorable hosts that will support development and larval growth (Cunningham and Zalucki 2014).

In soybean crops, the soybean looper is currently controlled mainly with synthetic insecticides. However, this lepidopterous pest is difficult to control, not only because it is increasingly tolerant to insecticides but also because it is less exposed to spraying due to its habit of remaining sheltered under the plant canopy (Bernardi et al. 2012). Therefore, use of resistant cultivars stands out as a valuable control strategy (Smith and Clement 2012).

Several soybean genotypes have been evaluated for their insect resistance. In the 1970s, three introduced genotypes (PIs), PI 171451, PI 227687 and PI 229358, were identified as being resistant to the Mexican bean beetle, *Epilachna varivestis* Mulsant. (Van Duyn et al. 1971). According to Boethel (1999), these PIs exhibit both antixenosis and antibiosis as modes of resistance to major lepidopterous soybean pests.

Among the causes of antixenosis, morphological factors can influence female behavior, reducing the attraction or oviposition preference of a host plant (Painter 1951; Smith 2005; Cunningham and Zalucki 2014). Different soybean genotypes present several different morphological characteristics that can confer insect resistance. Among these, pubescence, which refers to the density of trichomes, is considered one of the most important (Broersma et al. 1972; Norris and Kogan 1980).

In the search for alternative methods to chemical control for the management of *C. includens* on soybeans, various sources of resistance have been studied to obtain soybean germplasm resistant to *C. includens*. Thus, this work aimed to evaluate different soybean genotypes offered to adults of *C. includens* to verify the occurrence of antixenosis.

MATERIAL AND METHODS

In a greenhouse, attractiveness and oviposition preference assays were conducted with adult moths. In addition, morphological and colorimetric analyses of the soybean leaves were conducted in laboratory conditions (26 ± 2 °C; $65 \pm 10\%$ RH; 14 h light; 10 h dark photophase). Seventeen genotypes were evaluated (Table 1). The plants were supplied by the Instituto Agronômico (IAC, Campinas, SP, Brazil) and are part of the active germplasm bank of the institution. Except for 'Coodetec-208' and 'Conquista', all the other genotypes have already been reported by one or more studies as sustaining little damage from various species of insect pests (Miranda et al. 2003; Valle et al. 2012; Souza et al. 2015).

Table 1. Name and characteristics (genealogy or source) of soybean genotypes.

Name	Characteristics (genealogy or source)
'IAC 17'	D 72-9601-1 × 'IAC 8'
'IAC 18'	D 72-9601 × 'IAC 8'
'IAC 19'	D 72-9601-1 × 'IAC 8'
'IAC 23'	'BR-6' × IAC 83-23
'IAC 24'	IAC 80-1177 × IAC 83-288
'IAC 100'	'IAC 12' × IAC 78-2318
IAC 74-2832	'Hill' × PI 274454
IAC 78-2318	D 72-9601-1 × IAC 73-227
PI 171451	Japão
PI 227687	Okinawa, Japão
PI 229358	Tóquio, Japão
PI 274453	Okinawa, Japão
PI 274454	Okinawa, Japão
D 75-10169	'Govan' × (F4 'Bragg' × PI 229358)
L 1-1-01	'BR-6' × 'IAC 100'
'Coodetec-208'	'OC-4' × Williams 20
'Conquista'	Lo76-4484* × 'Numbaíra'

*Selection of late plant of 'Bragg'.

Stock rearing of *Chrysodeixis includens*

A colony of *C. includens* was initiated from eggs sourced from a colony maintained by the Laboratory of Insect Biology of the Department of Entomology and Zoology, ESALQ-USP (Piracicaba, SP, Brazil) and by DuPont (Paulínia, SP, Brazil). This colony was maintained on an artificial diet following the methodology proposed by Parra et al. (2009), with adaptations.

Plant cultivation

The plants used in the experiments were grown in polyethylene plastic pots (2.5 and 5.0 L) filled with a substrate of a soil mixture (Oxisol), washed coarse sand and organic matter (corral manure) at a ratio of 1:1:1, and maintained in a greenhouse (3 m long × 2 m wide × 2 m high). The plants were fertilized as normally recommended for the culture (Mascarenhas and Tanaka 1997), calculated according to soil analysis. Other necessary cultural practices were also followed (irrigation and thinning).

Attractiveness and oviposition preference

An experiment to evaluate the attractiveness of adults to various soybean genotypes was carried out inside metal cages (2.5 × 3.0 × 2.5 m), covered on top with plastic sheeting and shade cloth (30%) and protected on the sides by white anti-aphid screens. In these cages, pots containing plants of the 17 tested soybean genotypes approximately 55 days after their emergence (V6R2) were arranged in a circle equidistant from the center (Fehr and Caviness 1977). In the center of the cage, two pairs of *C. includens* per genotype were released near the ground (34 pairs per cage).

Attractiveness was evaluated in the late afternoon four days after infestation by counting the number of individuals attracted to the different genotypes. (Campos et al. 2010). Subsequently, the attractiveness index (AI) was determined by the equation $AI = 2T/(T + P)$ (Lin et al. 1990), in which: T is the number of insects attracted to the evaluated genotype and P is the number of insects attracted to the standard susceptible genotype 'Conquista' (a commercial genotype used as a susceptibility standard by Silva et al. 2014). AI values vary between zero and two, where AI = 1 indicates similar attraction between the

evaluated genotype and the susceptible standard; AI < 1 corresponds to lower attraction by the evaluated genotype compared to the susceptible standard; and IA > 1 indicates higher attraction by the evaluated genotype compared to the susceptible standard. The genotypes were classified by comparing the index obtained for the evaluated genotype with the index obtained for the standard; the overall standard error (SE) of the mean for the assay was adopted to discriminate between genotypes (Lin et al. 1990). A randomized complete block design was used involving 17 treatments (genotypes) and five replicates (metallic cages).

Oviposition preferences were evaluated in no-choice tests inside individual cages (30 cm diameter × 70 cm high) covered with organdy fabric in which were placed potted plants of the tested soybean genotypes approximately 45 days after their emergence (V5-R2) (Fehr and Caviness 1977). Inside the cages, two pairs of *C. includens* per genotype were released. Oviposition was evaluated seven days after infestation by visually counting the number of eggs on all plant leaves (Campos et al. 2010). A completely randomized design was performed involving 17 treatments (genotypes) and five replicates (each individualized plant represented one replicate).

Morphological analysis

A morphological analysis was conducted to quantify the number of trichomes per leaf surface. When the plants reached the V5 phenological stage (Fehr and Caviness 1977), leaves were collected from the middle sections of four plants of each genotype. Their quantitative anatomical characteristics were evaluated by making epidermal impressions of the abaxial surface of the leaves using instant glue to create slides, standardized on the middle region of the leaf. Images of the tissues were then made with a projection microscope and digitally recorded using an Olympus camera connected to an Olympus light microscope. The trichomes were counted with the aid of a digitizing tablet and Cell B Olympus software according to the methodology described by Souza et al. (2005). For the analysis of variance, the data obtained were transformed to an area of 16 mm², and the means were compared by Tukey's test ($p < 0.05$) (Valle et al. 2012). This experiment used a completely randomized design with four replicates (each replicate represented an average of 10 valuations).

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Determination of color

To determine the color, we used the colorimetric indices of soybean genotypes. When the plants reached the V5 phenological stage (Fehr and Caviness 1977), we collected leaves from the middle sections of four plants of each genotype.

A color space provides a way to express the color of an object or light source using a standardized type of notation or criterion. The $L^*a^*b^*$ color space (also known as CIE LAB), adopted by the Commission Internationale d'Éclairage (CIE) in 1976, is one of the recognized color spaces. Its parameters are L^* , which represents luminosity, a^* and b^* , which represent chromaticity coordinates. In these coordinates, a^* and b^* indicate the directions of the colors, in which $+a^*$ moves toward the red spectrum, $-a^*$ toward the green spectrum, $+b^*$ toward the yellow spectrum and $-b^*$ toward the blue spectrum. The center is achromatic. As a^* and b^* increase or decrease, the point moves away from the center and the color increases.

Leaf color was determined using a colorimeter (Konica Minolta®, Japan, model Chroma meter CR-400, color space $L^*a^*b^*$, by reflectance). Calibration was conducted with a standard white plate following the manufacturer's instructions. For each leaf, the central portion of both sides of the central vein of the adaxial surface was evaluated. The measured color parameters were: luminosity (L) = (0 = black to 100 = white); a = green (-60) to red ($+60$); b = blue (-60) to yellow ($+60$). A completely randomized study design with four replicates was adopted. Each side of the central vein of each leaf represented a single replicate.

Statistical Analyses

Data obtained from all the assays were submitted to the Kolmogorov-Smirnov test to check for normal distributions. When normal distributions were present, we used the Snedecor test (F); however, when the distributions were not normal, the data were first transformed by $(x + 1.0)^{1/2}$ to normalize them. Pearson's linear correlation coefficients were calculated between trichome density and oviposition and between the colorimetric and oviposition indices from the no-choice test. When a difference occurred between the genotypes, Tukey's tests ($p < 0.05$) were performed to compare the means and the correlations were compared by F-tests ($p < 0.05$), using the SAS Proc Mixed procedure software (SAS Institute Inc., Cary, NC, USA). To analyze the variance in trichome density, the data were first transformed to match an area of 16 mm². These averages were compared by Tukey's tests ($p < 0.05$) and by the SAS Proc Mixed procedure (Valle et al. 2012).

RESULTS AND DISCUSSION

Based on the attractiveness index, the genotypes 'IAC 19', 'IAC 18', 'IAC 23', L1-1-01, PI 274453, PI 229358, PI 171451, 'IAC 100', 'IAC 24', 'IAC 17' and IAC 74-2832 were classified as repellents in relation to the standard susceptible 'Conquista' genotype. The genotypes 'Coodetec 208', D75-10169 and PI 274454 were considered neutral, and IAC 78-2318 and PI 227687 were revealed as attractive (all in comparison to the Conquista standard susceptible) (Figure 1).

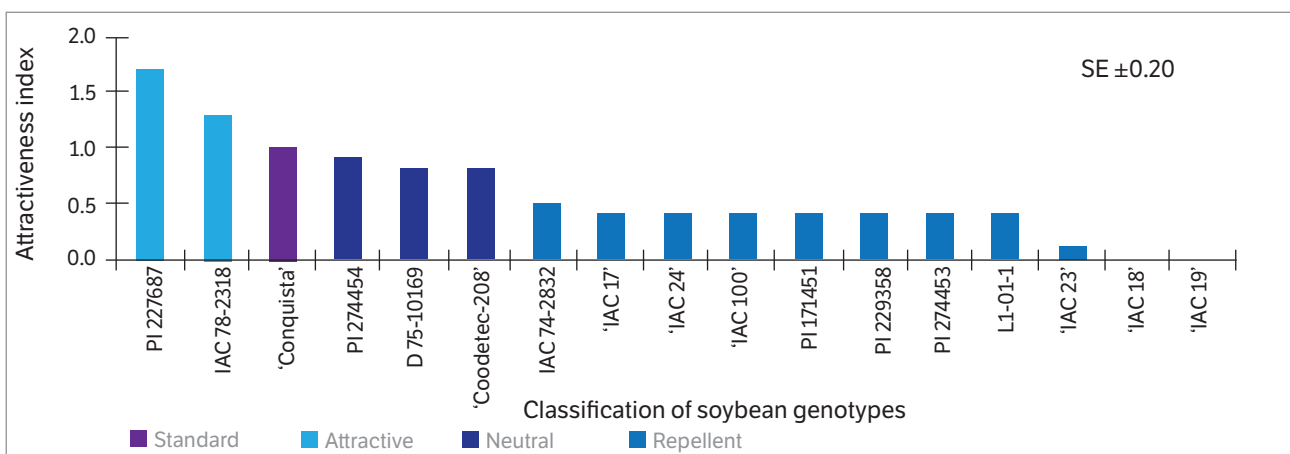


Figure 1. Attractiveness index of adults of *C. includens* and classification of soybean genotypes in free-choice test under greenhouse conditions.

The reduced attraction of some genotypes to adults of *C. includens* is probably related to the production of volatile moth-repellent compounds or inhibitors, and/or with factors related to the color of the substrate, a characteristic that can positively or negatively affect the selection of plant hosts by phytophagous insects (Bruce et al. 2005; Mercader and Scriber 2007). Among the chemical volatiles emitted by plants, kairomones directly influence insects during host plant selection (Bruce et al. 2005). Hartlieb and Rembold (1996) used a steam distillate containing compounds from *Cajanus cajan* L. (guandu) plants as well as a synthetic kairomone composed of a mixture of six pure components (β -caryophyllene, α -humulene, α -guajene, α -muurolene, γ -muurolene and α -bulnesene) to investigate their effects on attractiveness and oviposition of females of the species *Helicoverpa armigera*. The results verified that moths of this species were highly attracted to the steam distillate containing components from *C. cajan*. As for the kairomones, only α -bulnesene was attractive by itself to *H. armigera*, but the six-compound mixture acts as an oviposition stimulant. These results reinforce the importance of olfactory cues for phytophagous insects (Bruce et al. 2005).

Among the genotypes that have repellent effects against moths of *C. includens*, PI 229358 stands out, ratifying the reports that it exhibits resistance to this species (Beach and Todd 1988) as well as to other Lepidoptera (Boethel 1999).

In addition to the PIs, the 'IAC 100' genotype was also classified as less attractive compared to other genotypes. It is important to emphasize that although several studies related to the development and/or preferences of the Noctuidae insect family have been performed for soybeans (Souza et al. 2014; Boiça Júnior et al. 2015), there are no studies specifically concerned with the attractiveness of *C. includens* in relation to the genotype 'IAC 100', which demonstrates the importance of the present research. Recent studies have identified 'IAC-100' as a good progenitor candidate for breeding intended to generate promising lineages that exhibit both insect resistance characteristics and high productivity (Maia et al. 2009).

Regarding the no-choice test (Table 2), lower numbers of eggs were found on the genotypes PI 171451 (22.80), PI 274453 (76.80), 'IAC 18' (87.20), 'IAC 23' (89.80) and L1-1-01 (92.00) compared to the other genotypes, suggesting the occurrence of antixenosis (oviposition) to adults of *C. includens*. When evaluating the oviposition

preferences of *C. includens* for a different host (bean) using no-choice tests, Morando et al. (2015) observed eggs counts ranging from 45.57 – 316.86; this range is higher than the range found in the present study. Kidd and Orr (2001) investigated the oviposition preferences of *C. includens* in soybean and kudzu (*Pueraria montana* Lour.). They observed a greater oviposition preference for soybean leaves using free and no-choice tests (68.5 and 570.9 eggs, respectively) than for kudzu (44.0 and 325.7 eggs, respectively). This study also reported a higher number of eggs in their no-choice tests than were found in the present study, regardless of the host species.

In some cases, the relationship between attractiveness and oviposition can be in conflict, as was observed for the genotypes 'IAC 24' and PI 229358, which were little visited by moths of *C. includens* (Figure 1). Despite these low visitation rates, these two genotypes were among the most oviposited (161.6 and 171.8 eggs, respectively) (Table 2). Other genotypes exhibited both a low attractiveness index (PI 171451, PI 274453, 'IAC 18', 'IAC 23' and L1-1-01) (Figure 1) and low oviposition (22.8, 76.8, 87.2, 89.8 and 92.0 eggs, respectively) (Table 2), suggesting that these genotypes were not preferred by *C. includens* for either shelter or oviposition. This difference in moth behavior may be related to the emission of different odors by host plants, which in addition to interfering in the selection process may also have a positive effect (stimulant) and/or a negative effect (deterrent) on insect oviposition (Cunningham and Zalucki 2014).

Concerning trichome density on the abaxial surfaces of the leaves (Table 2), the genotypes PI 227687 (246.4) and PI 274453 (176.6) had the highest averages, differing from 'IAC 23' (65.5), IAC 74-2832 (68.4), 'Coodetec-208' (69.3), D 75-10169 (70.7), 'Conquista' (75.5), 'IAC 24' (75.5), 'IAC 100' (77.4) and 'IAC 19' (78.3). The stereoscopic microscope images (Figure 2) help to visualize the differences in trichome density between the various genotypes.

The high attractiveness (Figure 1) but only intermediate oviposition of moths of *C. includens* mentioned in the Introduction for PI 227687 for the no-choice test may be associated with the high density of trichomes observed in this genotype (Table 2). Although insects are attracted to this plant species, the plants can induce deterrent effects to insect oviposition due to the presence of chemical

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Table 2. Mean (\pm SE) number of eggs of *C. includens* in soybean genotypes, in no-choice test, in greenhouse and number of trichomes obtained in 16 mm² and colorimetric evaluation of leaves of soybean genotypes.

Genotype	Number of eggs ¹	Number de trichomes ¹	L* ¹	a* ¹	b* ¹
'Conquista'	182.4 \pm 11.80 a	75.5 \pm 14.11 c	41.2 \pm 0.54 ab	-14.2 \pm 0.35 ab	18.7 \pm 0.74 abc
PI 229358	171.8 \pm 13.73 ab	111.6 \pm 13.68 bc	44.0 \pm 2.65 ab	-16.0 \pm 2.79 abc	20.7 \pm 4.56 abc
IAC 78-2318	173.4 \pm 11.28 ab	107.8 \pm 12.39 bc	41.0 \pm 0.62 ab	-14.5 \pm 0.36 abc	18.5 \pm 0.95 bc
'IAC 24'	161.6 \pm 11.61 ab	75.5 \pm 04.86 c	43.0 \pm 0.94 ab	-14.7 \pm 0.71 abc	18.7 \pm 0.95 abc
PI 274454	138.0 \pm 12.12 abc	141.0 \pm 29.46 bc	40.7 \pm 1.08 ab	-16.5 \pm 1.20 bc	19.2 \pm 1.72 abc
'IAC 19'	130.6 \pm 04.29 bcd	78.3 \pm 09.12 c	44.5 \pm 0.91 ab	-16.0 \pm 0.56 abc	23.2 \pm 1.45 ab
PI 227687	112.0 \pm 07.79 cde	246.4 \pm 27.46 a	43.5 \pm 0.61 ab	-17.5 \pm 0.64 bc	21.7 \pm 0.73 abc
'IAC 100'	101.8 \pm 07.57 cde	77.4 \pm 11.92 c	43.7 \pm 0.93 ab	-16.2 \pm 0.94 bc	22.5 \pm 1.26 abc
IAC 74-2832	101.6 \pm 07.06 cde	68.4 \pm 05.53 c	41.2 \pm 1.84 ab	-14.2 \pm 1.55 ab	19.5 \pm 2.67 abc
'IAC 17'	99.8 \pm 05.03 cde	112.1 \pm 22.09 bc	43.2 \pm 1.42 ab	-13.7 \pm 0.96 ab	18.5 \pm 1.63 bc
'Coodetec-208'	98.4 \pm 09.67 cde	69.3 \pm 11.46 c	41.0 \pm 0.87 ab	-14.0 \pm 0.99 ab	18.0 \pm 1.56 bc
D 75-10169	96.8 \pm 03.91 cde	70.7 \pm 09.08 c	40.2 \pm 0.55 ab	-11.7 \pm 0.45 ab	14.5 \pm 1.00 bc
L1-1-01	92.0 \pm 06.16 de	115.4 \pm 14.49 bc	43.7 \pm 1.96 ab	-20.7 \pm 1.62 c	29.0 \pm 2.35 a
'IAC 23'	89.8 \pm 09.53 de	65.5 \pm 07.81 c	44.0 \pm 0.52 ab	-17.0 \pm 0.34 bc	22.5 \pm 0.71 abc
'IAC 18'	87.2 \pm 06.50 e	116.3 \pm 20.82 bc	45.0 \pm 2.33 a	-16.5 \pm 1.68 bc	21.7 \pm 3.12 abc
PI 274453	76.8 \pm 06.98 e	176.6 \pm 18.99 ab	44.0 \pm 1.42 ab	-18.0 \pm 1.88 bc	24.0 \pm 2.91 ab
PI 171451	22.8 \pm 03.44 f	117.3 \pm 11.53 bc	38.0 \pm 0.76 b	-9.7 \pm 0.64 a	12.5 \pm 0.48 c
P	< 0.0001	< 0.0001	0.0325	< 0.0001	0.0003

¹Means followed by the same lower case letter per column do not differ by Tukey's test ($p > 0.05$). L* = luminosity index; a* = index of the variation of the color green to red; b* = index of the variation of the color blue to yellow.

and/or morphological factors (West and Cunningham 2002), as observed in the present research. Luedders and Dickerson (1977) found that PI 227687, among other soybean lines studied, was the most resistant to *Trichoplusia ni* (Lepidoptera: Noctuidae), which is also a defoliating caterpillar of this legume.

The genotype PI 274453 was also classified as repellent to the moths (Figure 1), presenting low oviposition rates and high trichome density (Table 2). Handley et al. (2005) reported that genotypes exhibiting high trichome density tend to be more resistant (low oviposition) compared to less pubescent genotypes. Therefore, in addition to chemical compounds, most plants also produce morphological structures that can function as a source of plant defenses against insect pests (Traw and Dawson 2002). Among these, trichomes are considered the most important structural characteristic according to Sharma et al. (2003) and He et al. (2011). However, in this study, considering 17 soybean genotypes, the correlations between oviposition and trichome density (Table 3) were not statistically significant.

In the colorimetric evaluation, there was a difference at all scales for the different genotypes (Table 2). For

Table 3. Pearson correlation coefficient (r) obtained between oviposition and trichomes number in 16 mm² and colorimetric indexes of 17 soybean genotypes.

Variable	Correlation coefficient ¹
Oviposition \times trichomes	-0.14 ^{ns}
Oviposition \times index L*	0.16 ^{ns}
Oviposition \times index a*	-0.10 ^{ns}
Oviposition \times index b*	0.08 ^{ns}

¹Significant at 5% probability by F test. L* = luminosity index; a* = index of the variation of the color green to red; b* = index of the variation of the color blue to yellow.

luminosity L*, PI 171451 presented itself as less clear (38.00) in relation to 'IAC 18' (45.00). The a* index indicated that the most intense green is present in L1-1-01 (-20.7), quite different from PI 171451 (-9.7). Regarding the b* index, L1-1-01 (29.0) presented the most intense yellow color, again, quite different from PI 171451 (12.5).

Despite all the correlations between the number of eggs and colorimetric indexes, the differences in correlation coefficients are not significant (Table 3). Coloration is considered one of the main factors for host selection from a distance by phytophagous insects (Mercader and Scriber 2007).

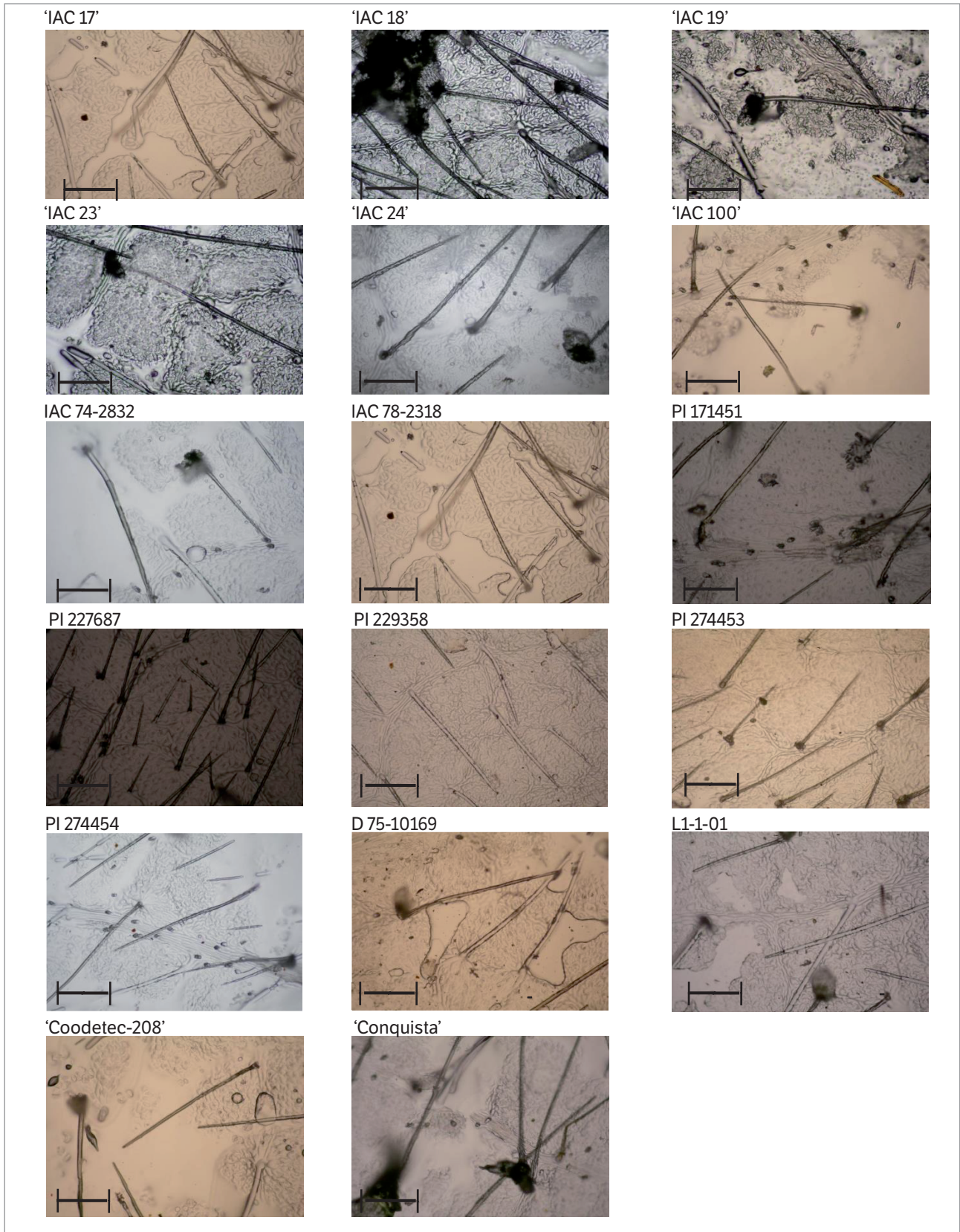


Figure 2. Scanning electron micrographs of trichomes on the abaxial surface of leaflets of 17 soybean genotypes. In the scale every 1 cm equals to 500 μ m.

The results from this work show that L1-1-01 exhibited higher green (– 20.7) and yellow (29.0) index values and was little oviposited (only 92.0 eggs). These results corroborate those found by Morando et al. (2015), who also observed a lower preference for oviposition of *C. includens* on bean genotypes with dark green leaves and a medium preference in relation to bean genotypes with lighter green leaves. In a study of oviposition preferences of other insect species on *Fraxinus americana* L., *Liriodendron tulipifera* L., and *Prunus serotina* Ehrh., which have leaves of different colors, Mercader et al. (2007) observed contrasting results with the present research; *Papilio glaucus* L. (Lepidoptera: Papilionidae) showed an oviposition preference for dark green compared with light green leaves. Although the role of visual cues is an important factor for selection of a host plant, this topic has rarely been studied (Cunningham and Zalucki 2014), highlighting the importance of colorimetric evaluation of leaves in relation to insect oviposition preferences.

Based on all the results obtained (attractiveness and oviposition), it can be inferred that the genotypes PI 171451, PI 274453, 'IAC 18', 'IAC 23' and L1-1-01 express antixenosis as a type of resistance to *C. includens*. These genotypes can be considered as a source of resistance that can be exploited in soybean breeding programs to obtain cultivars resistant to insects, with emphasis on *C. includens*.

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