Tolerance of KS-4202 Soybean to the Attack of Bemisia tabaci Biotype B (Hemiptera: Aleyrodidae)

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Tolerance of KS-4202 soybean to the attack of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae)

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Abstract

Bemisia tabaci (Gennadius) biotype B (Hemiptera: Aleyrodidae) is considered one of the most important pests of soybean, *Glycine max* L. (Merrill) (Fabaceae), in Brazil and worldwide. Although chemical control still represents the principal strategy used to control this insect, less aggressive strategies such as the use of resistant genotypes stand out as potentially efficient alternatives for integrated pest management programs. This study aimed to evaluate the possible occurrence of tolerance to *B. tabaci* biotype B in the 'KS-4202' soybean genotype, which is already recognized as tolerant to *Aphis glycines* Matsumura (Hemiptera: Aphididae) in the United States. The 'Conquista' Brazilian cultivar was used as a susceptible control. In a greenhouse, plants (stages V3–V4) of both genotypes were individualized and subjected to 6 patterns of infestation: 1) uninfested and without chemical control; 2) infested and without chemical control; 3) infested and sprayed at 15 d after infestation (DAI); 4) infested and sprayed at 30 DAI; 5) infested and sprayed at 45 DAI; and 6) infested and sprayed at 60 DAI. The study was performed in a completely randomized design with 6 replications for each pattern of infestation. We evaluated the following parameters of productivity: number of pods per plant, dry weight of pods per plant, number of seeds per plant, dry weight of seeds per plant, and dry weight of biomass per plant. A 2-by-2 factorial bioassay was carried out to evaluate the plant responses to whitefly feeding, with 5 replications for each combination. The factors were 2 soybean genotypes ('KS-4202' and 'Conquista') and 2 levels of infestation (0 and 25 pairs), with 4 collection dates of leaflets (7, 14, 21, and 28 DAI). The protein contents and enzyme activities (dismutase superoxide, peroxidase, and polyphenoloxidase) were also determined for each collection date. Whitefly infestation had a negative effect on the weight of seeds and dry weight of biomass of 'Conquista' plants for even the shortest period of infestation (15 d). In contrast, for 'KS-4202', there was no difference in the number of pods per plant, number of seeds per plant, or dry weight of biomass between infested (15, 30, 45, and 60 d) and uninfested plants. Our results demonstrated that the 'KS-4202' genotype is tolerant to *B. tabaci* biotype B feeding. However, studies are still necessary to better understand the causes of this tolerance because the main factors of tolerance found in this genotype are not the oxidative enzymes studied here.

Key Words: *Glycine max*; whitefly; host plant resistance to insects; oxidative enzymes

Resumo

Bemisia tabaci (Gennadius) biótipo B (Hemiptera: Aleyrodidae) é considerada uma das mais importantes pragas para a cultura da soja, *Glycine max* L. (Merrill) (Fabaceae), no Brasil e no mundo. Embora o controle químico ainda represente a principal estratégia utilizada para o manejo desse inseto, medidas menos agressivas como o uso de genótipos resistentes destacam-se como alternativas potencialmente eficientes para os programas de Manejo Integrado de Pragas. Este estudo teve como objetivo avaliar a possível expressão de tolerância da soja 'KS-4202'a *B. tabaci* biótipo B, genótipo reconhecidamente tolerante a *Aphis glycines* Matsumura nos Estados Unidos da América. A cultivar brasileira 'Conquista' foi utilizada como padrão suscetível. Em casa de vegetação, plantas dos dois genótipos (V3–V4) foram individualizadas e submetidas a seis diferentes padrões de infestação: 1) sem infestação e sem controle químico; 2) com infestação e sem controle químico; 3) infestado e com pulverização aos 15 dias após a infestação (DAI); 4) infestado e com pulverização aos 30 DAI; 5) infestado e com pulverização aos 45 DAI e 6) infestado e com pulverização aos 60 DAI. O estudo foi realizado em delineamento inteiramente casualizado, com seis repetições para cada padrão de infestação. Foram avaliados os seguintes parâmetros de produtividade: número de vagens por planta, peso seco de vagens por planta, número de sementes por planta, peso seco de sementes por planta e peso seco de biomassa por planta. Visando avaliar a resposta das plantas à alimentação da mosca-branca foi realizado um ensaio em esquema fatorial 2 × 2, com cinco repetições para cada combinação. Os fatores foram dois genótipos de soja ('KS-4202' e 'Conquista') e dois níveis de infestação (0 e 25 casais), com quatro datas de coleta de folíolos (7, 14, 21 e 28 DAI). Para cada data de coleta determinou-se o conteúdo de proteínas e a atividade de enzimas (superóxido dismutase, peroxidase e polifenoloxidase). A infestação com a mosca-branca afetou negativamente o peso de sementes e a biomassa total das plantas de 'Conquista', mesmo sob o mais curto período de infestação (15 dias). De maneira oposta, para 'KS-4202' não foram verificadas diferenças quanto ao número de vagens por planta, número de sementes por planta ou peso seco de biomassa entre plantas infestadas (15, 30, 45 e 60 dias) e não infestadas. Nossos resultados demonstraram que o genótipo 'KS-4202' é tolerante à alimentação de *B. tabaci* biótipo B. No entanto, estudos complementares são ainda necessários, a fim de melhor esclarecer as causas dessa tolerância, uma vez que as enzimas oxidativas estudadas não são as principais causas da resistência encontrada nesse genótipo.

Palavras Chave: *Glycine max*; mosca-branca; resistência de plantas a insetos; enzimas oxidativas

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Bemisia tabaci (Gennadius) biotype B (Hemiptera: Aleyrodidae) is one of the most harmful crop pests worldwide (Brown et al. 1995; De Barro 2011; Oliveira et al. 2013). Since its detection in Brazil in 1991 (Lourenção & Nagai 1994), this insect has caused a great deal of damage to various plant species (Torres et al. 2007; Baldin & Beneduzzi 2010; Baldin et al. 2013; Cruz et al. 2014), including soybean crops [*Glycine max* L. (Merrill); Fabaceae] (Tamai et al. 2006; Vieira et al. 2011; Silva et al. 2012; Valle et al. 2012), causing economic losses estimated at 714 million dollars annually (Oliveira et al. 2013).

This insect causes both direct and indirect damage to plants. Direct damage occurs due to the nymphs and adults feeding on the phloem sap, which compromises the plant's vegetative and reproductive development. Indirect damage is due to the insects' excretion of honeydew during the feeding process, which serves as a substrate for the growth of sooty mold (*Capnodium* sp.; Cnapodiaceae). Sooty mold darkens foliage, affecting the plants' ability to photosynthesize (Perring 2001; Naranjo & Legg 2010). Furthermore, whiteflies are also considered one of the most important virus vectors for several economically important crops (Jones 2003). In soybeans, there are reports of *B. tabaci* biotype B acting as a vector for the stem necrosis virus (*Cowpea mild mottle virus*, CpMMV) (Almeida et al. 2005; Marubayashi et al. 2010).

Considering the damage caused by whiteflies and the fact that controlling them mainly involves massive spraying of synthetic insecticides, it is important to search for new tools that can be used to manage this pest. In this sense, the adoption of resistant genotypes may represent an important avenue of investigation to determine its efficiency and suitability to act in concert with other control strategies employed in integrated pest management (Painter 1951; Smith 2005).

Tolerance is classified as a type of horizontal resistance conferred by several genes and generally more stable and durable than vertical or monogenetic resistance, which is commonplace in antibiosis (Smith 2005). In addition, because tolerance is a plant response and not an insect response, tolerant plants do not impose the same levels of selection pressure imposed by carriers of antibiosis and antixenosis resistance mechanisms, where high selection pressure may result in the appearance of new insect biotypes (Stinchcombe 2002; Smith 2005). Tolerance may be conferred by various plant compensatory mechanisms such as a high relative growth rate, an increase in the photosynthetic rate after insect damage, and an increase in the production of hormones, allelochemical compounds, and oxidative enzymes (Strauss & Agrawal 1999; Heng-Moss et al. 2004; Franzen et al. 2007).

In general, most of the studies undertaken in Brazil to select soybean genotypes resistant to whiteflies have focused primarily on characterizing the occurrence of antixenosis and antibiosis (Lima et al. 2002; Valle & Lourenção 2002; Lima & Lara 2004; Vieira et al. 2011; Silva et al. 2012; Valle et al. 2012). We know of no studies that identify promising candidates for expression of tolerance.

In the United States, some authors have reported that the KS-4202 genotype shows tolerance to the soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae), which is considered one of the most harmful soybean pests in North America (Pierson et al. 2010; Prochaska et al. 2013; Marchi-Werle et al. 2014). The tolerance of KS-4202 against soybean aphids may be directly related to higher peroxidase activities in infested plants (Pierson et al. 2011; Marchi-Werle et al. 2014). Peroxidases and other oxidative enzymes are involved in several plant physiological response mechanisms; they are responsible for degrading toxic compounds and are synthesized by the plant in response to any stress (Apel & Hirt 2004).

Considering that *B. tabaci* biotype B and *A. glycines* belong to the same order (Hemiptera), exhibit similar feeding behaviors (sap-feeding), and often infest the same soybean guilds, the focus of this study was to evaluate whether the KS-4202 genotype also presents tolerance to attacks from *B. tabaci* biotype B, as well as to investigate plant response when subjected to insect infestation by using biochemical analysis (total soluble protein content and the activity of dismutase superoxide, peroxidase, and polyphenoloxidase). Finding multiple avenues of resistance in this genotype may be useful for soybean breeding programs focused on insect resistance.

Materials and Methods

The study was carried out in greenhouse conditions without ambient control (mean temperature = 30.5 °C, with a maximum of 38.4 °C and a minimum of 16.4 °C; mean relative humidity = 58%, with a maximum of 97% and a minimum of 35%; and natural lighting). Two soybean, *G. max*, genotypes were used: KS-4202 [KS4694 × C1842 (EUA)], which is tolerant to *A. glycines* (Pierson et al. 2010; Prochaska et al. 2013; Marchi-Werle et al. 2014) and, as a control, Conquista (Lo76-4484 × Numbaíra), a commercial Brazilian strain that is susceptible to *B. tabaci* biotype B (Silva et al. 2012).

REARING OF *B. TABACI* BIOTYPE B

The initial population of *B. tabaci* biotype B was collected from the Agronomic Institute of Campinas, Brazil, and maintained in a screened cage (2.0 × 2.5 × 2.0 m) covered with plastic sheeting and shade cloth (30%). The front and sides of the cage were protected with white anti-aphid screens (200 mesh). Pots (2.5 L) containing cabbage plants (*Brassica oleracea* var. *acephala* L.; Brassicaceae) were placed in the cage for colony maintenance. The plants were monitored on a weekly basis. A molecular characterization of the insects was performed according to Walsh et al. (1991), Simon et al. (1994), and De Barro et al. (2003) to confirm the biotype B strain used in the current study. Subsequently, this identification was performed periodically through the cultivation of squash plants within the greenhouse as these insects induce the plants to express leaf silvering, a typical physiological disorder caused by the feeding of immature biotype B insects on this crop (Yokomi et al. 1990).

BIOASSAYS

The KS-4202 and Conquista soybean genotypes were cultivated in 5 L pots with autoclaved substrate. The substrate was composed of soil (dark red latosol), washed coarse sand, and organic matter (corral manure) in a 1:1:1 ratio. The substrate was fertilized according to the crop recommendations (Mascarenhas & Tanaka, 1997). When the plants reached the V3–V4 vegetative stages (Fehr & Caviness 1977), they were placed individually into metallic cages (35 cm in diameter × 55 cm high) covered with voile fabric for the beginning of the assays.

The 2 soybean genotypes were subjected to 6 patterns of infestation: 1) uninfested and without chemical control; 2) infested and without chemical control; 3) infested and sprayed at 15 d after infestation (DAI); 4) infested and sprayed at 30 DAI; 5) infested and sprayed at 45 DAI; and 6) infested and sprayed at 60 DAI. The infestation was performed by releasing 25 whitefly pairs per plant. The insects were collected from rearing by using an aspirator (11 cm high × 4 cm in diameter). During insect collection, preference was given to whitefly pairs because, according to Byrne & Bellows Junior (1991), insect couples are usually paired. The assay was carried out using a completely randomized design, with 6 replications for each infestation pattern. Each replication consisted of 1 plant.

The following insecticides were sprayed to control the whiteflies: lambda-cyhalothrin + thiamethoxam (Engeo Pleno®) at 250 mL per ha and pyriproxyfen (Tiger®) at 300 mL per ha in combination, with a spray volume of 200 L per ha. Insecticides were sprayed using an FT-

16 backpack spraying equipment (Yamaho Inc., Campinas, SP, Brazil), which had a capacity of 16 L and an adjustable cone nozzle. At the time of spraying, plants were temporarily removed from the cages until they were totally dry. Spraying was performed once per treatment.

Prior to each spraying (15, 30, 45, and 60 DAI) and 15 d after the last spraying (75 DAI), 3 leaflets from each treatment were removed and taken to the laboratory. In the laboratory, the number of live nymphs on the abaxial surface of the leaflets was counted using a stereomicroscope (40× magnification) to monitor the pattern of infestation across the treatments. After counting, the leaf area was measured with an LI 3000A area meter (LI-COR Inc., Lincoln, Nebraska) to determine the number of live nymphs per cm².

The plants were allowed to grow until the end of their cycle. After maturation, the pods were collected and placed into paper bags for drying (until 13% humidity) using air circulation at 40 °C. The parameters of productivity for each treatment were calculated by evaluating the following: number of pods per plant, dry weight of pods per plant, number of seeds per plant, dry weight of seeds per plant, and dry weight of biomass per plant (weight of stem + pods + seeds). The dry weights were obtained using an AY 220 analytical scale (Marte Inc., São Paulo, SP, Brazil). Comparisons between the percentages of productivity were calculated by comparing the averages of the dry weight of seed per plant obtained in the treatments with insect infestation (with or without spraying) with those obtained from plants without infestation for each genotype.

Another assay was conducted to investigate the plants' physiological responses to the insect infestation. For that, the contents of the total soluble protein and the activities of dismutase superoxide, peroxidase, and polyphenoloxidase enzymes were determined. This assay was conducted in a completely randomized design (2 by 2 factorial), with 5 replications for each combination. The factors were as follows: 2 soybean genotypes (KS-4202 and Conquista) and 2 levels of infestation (0 and 25 couples of whitefly), with 4 sampling dates (7, 14, 21, and 28 DAI). For the enzyme analysis, each replication was analyzed in triplicate. The same infestation procedure described in the previous assay was adopted here. The youngest fully expanded trifoliate leaf was collected on each sampling date. After collection, the leaf was first frozen in liquid nitrogen and then stored at −20 °C for subsequent processing (Marchi-Werle et al. 2014).

The determination of the total soluble protein content (µg protein per g fresh weight) was performed according to the method proposed by Bradford (1976). The results of the protein content analysis were used to calculate the enzyme activity. The method described by Beauchamp & Fridovich (1973) was used to determine the activity of dismutase superoxide (units per g fresh weight). The peroxidase activity (µmol of decomposed H_2O_2 per min per g fresh weight) was determined using the method described by Lima et al. (1999). Polyphenoloxidase activity (µmol oxidized catechol per min per g fresh weight) was determined according to the method described by Kar & Mishra (1976) and modified by Lima et al. (1999).

STATISTICAL ANALYSES

Data were subjected to analysis of variance using the F-test. Normality was verified using the Shapiro–Wilk test, and homogeneity was analyzed using Levene's test. When the F-test result was significant, the means were compared using Tukey's test ($P < 0.05$) using SAS 9.2 software (SAS Institute 2001). Data related to the percentages of productivity reduction were transformed to arc sine $(x + 0.5)^{1/2}$.

Results

During this study, higher infestations were observed in the KS-4202 plants (infested and not sprayed) as compared with the Conquista

plants. However, application of insecticides halted insect population increases when applied during the respective treatments (Fig. 1). No differences in the numbers of pods per plant for the KS-4202 genotype were observed in any of the compared patterns of infestation ($F = 0.51$; df = 5; $P = 0.7631$). The Conquista plants (infested and not sprayed) presented a reduced number of pods per plant, which differed from the other treatments ($F = 5.19$; df = 5; $P = 0.0078$) (Table 1). For the dry weight of pods, the KS-4202 plants (infested and not sprayed) showed lower mean weights, which differed from the other treatments except for those infested plants sprayed at 60 DAI ($F = 3.98$; df = 5; $P = 0.0169$). For the Conquista plants, pod weight differed only in uninfested plants and the plants infested and sprayed at 15 DAI compared with plants infested and not sprayed ($F = 5.66$; df = 5; $P = 0.0055$) (Table 1). The KS-4202 plants showed no differences between the patterns of infestation with regard to the number of seeds per plant ($F = 0.81$; df = 5; $P = 0.5600$). The Conquista plants (infested and not sprayed) presented a reduced number of seeds per plant, which differed from the other treatments ($F = 7.20$; df = 5; $P = 0.0020$) (Table 1).

The mean weight of the KS-4202 seeds that were infested and not sprayed was less compared with the uninfested plants, and with those infested and sprayed at 15 DAI ($F = 3.64$; df = 5; $P = 0.0234$). For the Conquista genotype, all the infested plants (whether sprayed or not) showed a lower mean seed weight, differing from those without infestation ($F = 11.77$; df = 5; $P = 0.0002$) (Table 2). The dry weight of biomass parameter was not affected for the KS-4202 plants for any compared infestation pattern ($F = 2.83$; df = 5; $P = 0.0539$); however, for
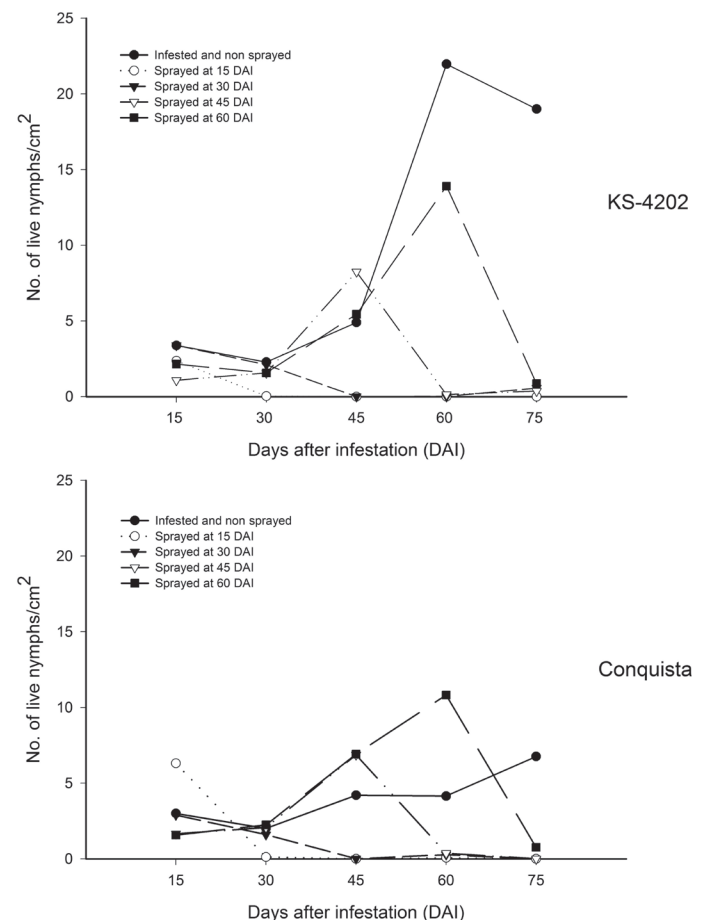

Fig. 1. Mean number of live *Bemisia tabaci* biotype B nymphs per cm² for the KS-4202 and Conquista genotypes for each pattern of infestation at 5 periods of evaluation.

Table 1. Means (± SE) of pods, dry pod weight, and seeds produced by KS-4202 and Conquista plants subjected to 6 patterns of *Bemisia tabaci* biotype B infestation.

Pattern of infestation	No. of pods per plant[a]		Dry weight of pods (g) per plant[a]		No. of seeds per plant[a]	
	KS-4202	Conquista	KS-4202	Conquista	KS-4202	Conquista
Uninfested and without control	21.3 ± 0.63 a	46.8 ± 5.28 a	0.23 ± 0.01 a	0.19 ± 0.02 a	47.3 ± 2.78 a	91.3 ± 8.69 a
Infested and sprayed at 15 DAI	24.0 ± 2.71 a	42.3 ± 4.42 a	0.23 ± 0.01 a	0.16 ± 0.01 ab	54.8 ± 4.80 a	81.0 ± 5.96 a
Infested and sprayed at 30 DAI	21.5 ± 1.26 a	51.5 ± 2.22 a	0.23 ± 0.01 a	0.15 ± 0.01 abc	51.3 ± 3.09 a	95.8 ± 2.50 a
Infested and sprayed at 45 DAI	24.3 ± 1.93 a	47.5 ± 4.99 a	0.24 ± 0.02 a	0.15 ± 0.01 abc	56.5 ± 4.05 a	93.0 ± 10.12 a
Infested and sprayed at 60 DAI	23.3 ± 1.11 a	41.0 ± 2.12 a	0.21 ± 0.01 ab	0.12 ± 0.01 bc	48.5 ± 2.40 a	80.5 ± 4.92 a
Infested and without control	23.3 ± 1.75 a	24.0 ± 2.71 b	0.18 ± 0.00 b	0.10 ± 0.02 c	53.5 ± 5.69 a	37.0 ± 10.61 b
P	0.7631	0.0078	0.0169	0.0055	0.5600	0.0020

[a]Means in a column followed by different lowercase letters are significantly different ($P \leq 0.05$; ANOVA and Tukey's test).

Conquista, all infested treatments (whether sprayed or not) presented lower dry biomass weight than uninfested plants. Those not sprayed had the lowest value and stood out significantly ($F = 14.05$; df = 5; $P = 0.0001$) (Table 2).

Comparing the percentages of productivity reduction between KS-4202 and Conquista plants under different patterns of infestation (Fig. 2), no differences were observed between the treatments of infested and not sprayed ($F = 0.45$; df = 3; $P = 0.5507$), infested and sprayed at 30 DAI ($F = 4.99$; df = 3; $P = 0.1116$), and infested and sprayed at 60 DAI ($F = 4.53$; df = 3; $P = 0.1231$). However, the KS-4202 plants infested and sprayed at 15 DAI ($F = 17.65$; df = 3; $P = 0.0246$) and 45 DAI ($F = 20.21$; df = 3; $P = 0.0205$) showed lower productivity reductions when compared with the Conquista plants.

Regarding the protein content (Table 3), the interaction was significant at 14 DAI ($F = 10.40$; df = 3; $P = 0.0053$), with KS-4202 plants achieving higher protein content than Conquista plants for the control treatment and a reduction in infested plants when compared with the healthy ones. The KS-4202 plants (whether infested or not) showed a higher protein content when compared with Conquista plants at 7 DAI ($F = 15.11$; df = 3; $P = 0.0013$), at 21 DAI ($F = 8.13$; df = 3; $P = 0.0115$), and at the last evaluation (28 DAI) ($F = 20.56$; df = 3; $P = 0.0004$). At 28 DAI, a higher protein content was also verified in the plants without infestation ($F = 8.23$; df = 3; $P = 0.0111$).

The activity of dismutase peroxidase (Table 3) showed a difference at only 7 DAI between all the treatments ($F = 5.82$; df = 3; $P = 0.0282$), with Conquista presenting a higher enzyme activity than KS-4202. This interaction was also significant at 14 DAI ($F = 5.98$; df = 3; $P = 0.0264$), where the non-infested Conquista plants presented higher enzyme activity than the KS-4202 plants. The KS-4202 plants showed an increase in this enzyme activity in infested plants compared with those without infestation. At 21 DAI, there was a difference only between the 2 genotypes ($F = 10.40$; df = 3; $P = 0.0053$), with superior values for Conquista. The interaction was significant at 28 DAI ($F = 5.58$; df = 3; $P = 0.0311$), where the infested Conquista plants showed higher enzyme activity

both in relation to uninfested Conquista plants and in relation to KS-4202 infested plants.

In the analysis of peroxidase activity (Table 4), all the interactions were significant. At 14 DAI, regardless of genotype, the infested plants showed lower peroxidase activity than the control plants ($F = 6.40$; df = 3; $P = 0.0223$). A higher level of peroxidase activity was found in Conquista plants at 28 DAI ($F = 11.68$; df = 3; $P = 0.0035$). Infested plants of both genotypes showed a significant increase in peroxidase activity ($F = 7.01$; df = 3; $P = 0.0175$) in comparison with those without infestation.

For the polyphenoloxidase enzyme (Table 4), the soybean genotypes differed only at 7 DAI ($F = 21.66$; df = 3; $P = 0.0003$), where the Conquista strain obtained the higher average. At 14 DAI, the interaction was significant ($F = 10.28$; df = 3; $P = 0.0055$), showing a reduction in this enzyme activity in the Conquista infested plants. At 21 DAI, the Conquista plants had higher polyphenoloxidase activity than the KS-4202 plants ($F = 9.17$; df = 3; $P = 0.0080$). For the evaluation at 28 DAI, the interaction was significant, with the Conquista infested plants showing the highest enzymatic activity ($F = 5.67$; df = 3; $P = 0.0301$).

Discussion

Whitefly colonization developed in both genotypes over time and continued to increase until the insecticides were sprayed. In all post-spraying evaluations, the number of live nymphs per cm^2 decreased in the treatments where the insects were chemically controlled. However, at the last count (75 DAI), the insect population had risen to 3 times greater in the KS-4202 unsprayed plants, demonstrating their suitability for insect colonization, which is expected in plants that exhibit tolerance (Smith & Clement 2012). This result is in agreement with Prochaska et al. (2013), who verified that KS-4202 plants were more infested by *A. glycines* in the field compared with other commercial soybean genotypes.

Based on the productivity parameters obtained in this study, the KS-4202 genotype was demonstrated to be more tolerant to the dam-

Table 2. Means (± SE) of dry seed weight and dry weight of biomass from KS-4202 and Conquista plants subjected to 6 patterns of *Bemisia tabaci* biotype B infestation.

Pattern of infestation	Weight of seeds (g)[a]		Total biomass (g)[a]	
	KS-4202	Conquista	KS-4202	Conquista
Uninfested and without control	0.21 ± 0.01 a	0.23 ± 0.01 a	19.5 ± 1.24 a	43.1 ± 2.39 a
Infested and sprayed at 15 DAI	0.20 ± 0.00 a	0.17 ± 0.01 b	20.5 ± 1.45 a	32.5 ± 1.82 b
Infested and sprayed at 30 DAI	0.18 ± 0.01 ab	0.16 ± 0.01 b	18.0 ± 1.37 a	34.2 ± 2.15 b
Infested and sprayed at 45 DAI	0.19 ± 0.00 ab	0.18 ± 0.01 b	21.0 ± 1.25 a	34.8 ± 2.66 b
Infested and sprayed at 60 DAI	0.18 ± 0.02 ab	0.15 ± 0.01 b	16.7 ± 1.32 a	27.3 ± 1.41 b
Infested and without control	0.15 ± 0.00 b	0.15 ± 0.00 b	15.0 ± 1.22 a	16.9 ± 3.00 c
P	0.0234	0.0002	0.0539	0.0001

[a]Means in a column followed by different lowercase letters are significantly different ($P \leq 0.05$; ANOVA and Tukey's test).
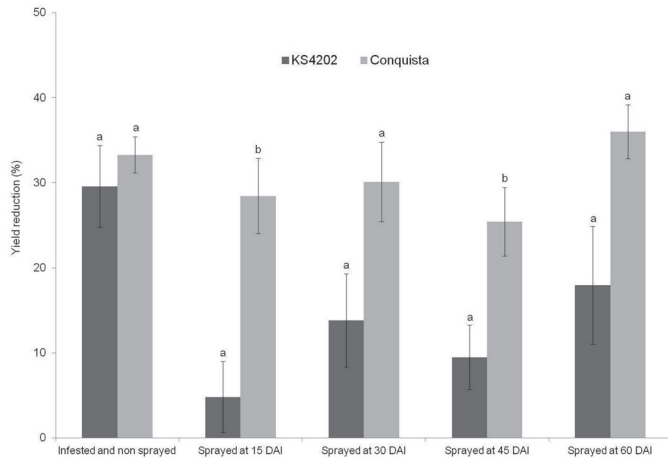
Fig. 2. Comparison of the percentages of reduction in productivity between KS-4202 and Conquista for each pattern of *Bemisia tabaci* biotype B infestation. The means of the columns labeled with the same letter for each pattern of infestation do not differ according to Tukey's test ($P > 0.05$); ns = not significant. From the left, the columns represent the treatments as follows: infested with no chemical control ($F = 0.45$; df = 3; $P = 0.5507$), infested and sprayed at 15 DAI ($F = 17.65$; df = 3; $P = 0.0246$), infested and sprayed at 30 DAI ($F = 4.99$; df = 3; $P = 0.1116$), infested and sprayed at 45 DAI ($F = 20.21$; df = 3; $P = 0.0205$), and infested and sprayed at 60 DAI ($F = 4.53$; df = 3; $P= 0.1231$). DAI = days after infestation.

age caused by whitefly feeding compared with the Conquista genotype. Infested KS-4202 plants (with or without chemical control) showed a number of pods similar to uninfested plants. In contrast, Conquista plants with no chemical control produced approximately 48% fewer pods than uninfested Conquista plants.

Whitefly infestation affected the mean weight of the pods produced by both genotypes. The KS-4202 plants infested but without chemical control (not sprayed) showed an average reduction in pod weight of approximately 22% compared with those without infestation. However, there was no difference between the plants without insect infestation and those infested and sprayed (15, 30, 45, and 60 DAI), suggesting that insecticide (control) in KS-4202 may be applied until 60 d after the beginning of a *B. tabaci* biotype B infestation without risking a significant reduction in pod weight. For the Conquista genotype, plants infested with whiteflies that were not sprayed suffered a reduction of approximately 47% in pod weight compared with the uninfested plants.

When analyzing tolerance as a mechanism of *Thrips palmi* Karny (Thysanoptera: Thripidae) resistance in common beans (*Phaseolus vulgaris* L.; Fabaceae), Frei et al. (2004) verified that the EMP 486 genotype, under field conditions, did not show significant losses in productivity even when highly infested by the insect; the infested plants showed a reduction of 7.3% in the number of empty pods and 5.0% in the pod weight compared with uninfested plants. In contrast, in the susceptible genotypes, these reductions were 50.0 and 23.8%, respectively. These results demonstrated that tolerant plants possess the ability to withstand insect attacks without significantly impacting their productivity.

The number of seeds per plant was similar among the infested and uninfested KS-4202 plants (whether sprayed or not), which demonstrates the ability of this genotype to tolerate damage caused by *B. tabaci* biotype B. The amount of seeds produced was similar regardless of attacks by this insect. A different result was observed for Conquista, whose infested and unsprayed plants produced approximately 60% fewer seeds than those without insect infestation, demonstrating the sensitivity of this genotype to whitefly feeding.

Although KS-4202 did not show a reduction in the number of seeds per plant when infested by the insect, plants infested and not sprayed had a 29% reduction in seed weight compared with the uninfested plants and a reduction of 25% compared with those where the insects were controlled at 15 DAI. This result suggests that only one spraying of insecticide at the beginning of whitefly infestation is required for the plants to produce seed weights similar to uninfested plants. However, for the Conquista plants, even early insect control provided little protection against the infestation's damage to seed weight because all the infested plants (controlled or not) showed reduced averages compared with uninfested plants.

The biomass of the KS-4202 plants was not affected by the whitefly infestation; however, for the Conquista plants, this parameter was affected by even the shortest period of infestation (15 d) compared with the uninfested plants. In addition, the Conquista plants that were infested and not sprayed (uncontrolled) presented a total biomass approximately 60% lower than plants without infestation and 40% lower than those that had the insects controlled at 60 DAI.

In comparing the productivity percentage of reduction, both genotypes in both the infested and uninfested treatments showed differences at 15 and 45 DAI, with a greater reduction for the Conquista genotype. Nevertheless, the reduction was always higher for Conquista in absolute values, which reinforces the higher capacity of KS-4202 to withstand damage caused by *B. tabaci* biotype B without an impact on its productivity. Prochaska et al. (2013) evaluated the resistance of soybean genotypes in field conditions, demonstrating that KS-4202 plants suffered minimal loss of productivity while tolerating soybean aphid populations that would cause extensive losses for most genotypes. Considering the results for the parameters observed in this study, we can infer that KS-4202 exhibits tolerance to *B. tabaci* biotype B feeding because insect infestation affected its vegetative and reproductive development in a less pronounced way compared with the more susceptible Conquista plants.

Tolerance is a very interesting feature for soybean genotypes because, in addition to exerting lower selection pressure on the insects (Stinchcombe 2002; Smith 2005), tolerant genotypes present a higher economic threshold and economic injury level compared with susceptible genotypes; therefore, they require a reduced number of insecticide sprayings. The results found here support the strategy of using tolerant genotypes as being compatible with biological control by favoring the maintenance and action of natural enemies in crop systems (Panda & Khush 1995).

The biochemical analysis performed in this study showed that KS-4202 plants infested with whiteflies presented a significant increase in superoxide dismutase activity at 14 DAI compared with the uninfested plants of that genotype. For the Conquista cultivar, the difference in enzyme activity between infested and uninfested plants was observed only for the last evaluation at 28 DAI, with higher activity in infested plants. Superoxide dismutase activity was always higher in Conquista when compared with KS-4202, which may be due to genetic differences in the metabolic pathways used to eliminate possible free radicals formed during infestation (Taggar et al. 2012). Our results showed that this enzyme was activated faster in KS-4202 plants (14 DAI) than in Conquista plants, likely due to the formation of free radicals in response to whitefly feeding. Generally, the increase in superoxide dismutase activity associated with *B. tabaci* infestation may be a defensive response, reflected in a reduced production of superoxide radicals or in an elevated ability to eliminate O_2^- (Zhang et al. 2014). The faster superoxide dismutase response of KS-4202 may be indicative of its tolerance in relation to Conquista, thus corroborating earlier results.

After 21 DAI, there was an increasing trend in peroxidase activity in infested plants of both the KS-4202 and Conquista genotypes.

Table 3. Total soluble protein content for the dismutase enzyme in the soybean genotypes at 7, 14, 21, and 28 d after *Bemisia tabaci* biotype B infestation.

	Total soluble protein (µg protein per g fresh weight)			Superoxide dismutase (units per g fresh weight)		
	7 DAI					
	Treatment[a]			Treatment[a]		
Genotype	Control	Infested	Mean	Control	Infested	Mean
Conquista	10.56	14.04	12.30 b	0.290	0.182	0.236 a
KS-4202	17.03	18.10	17.56 a	0.150	0.139	0.144 b
Mean	13.80 A	16.07 A		0.220 A	0.160 A	
P (Genotype)	0.0013			0.0282		
P (Treatment)	0.1122			0.1328		
P (Interaction)	0.3840			0.2213		
	14 DAI					
	Control	Infested	Mean	Control	Infested	Mean
Conquista	8.11 bA	7.85 aA	7.98	0.329 aA	0.321 aA	0.325
KS-4202	13.63 aA	8.19 aB	10.91	0.184 bB	0.325 aA	0.254
Mean	10.87	8.02		0.256	0.323	
P (Genotype)	0.0022			0.0338		
P (Treatment)	0.0027			0.0452		
P (Interaction)	0.0053			0.0264		
	21 DAI					
	Control	Infested	Mean	Control	Infested	Mean
Conquista	5.17	4.96	5.06 b	0.529	0.597	0.563 a
KS-4202	9.84	8.77	9.30 a	0.263	0.374	0.318 b
Mean	6.86 A	7.50 A		0.396 A	0.485 A	
P (Genotype)	0.0115			0.0053		
P (Treatment)	0.6744			0.2564		
P (Interaction)	0.7779			0.7725		
	28 DAI					
	Control	Infested	Mean	Control	Infested	Mean
Conquista	3.13	1.17	2.15 b	0.883 aB	2.280 aA	1.581
KS-4202	8.10	4.40	6.25 a	0.313 aA	0.762 bA	0.537
Mean	5.61 A	2.78 B		0.598	1.521	
P (Genotype)	0.0004			0.0001		
P (Treatment)	0.0111			0.0003		
P (Interaction)	0.3602			0.0311		

[a]Means in a column followed by different lowercase letters or means in a row followed by different uppercase letters are significantly different ($P \geq 0.05$; ANOVA and Tukey's test).

The increases in peroxidase activity may be related to a possible increase in H_2O_2 in the cells as a result of superoxide dismutase's action (Kawano 2003; Apel & Hirt 2004). The action of peroxidase on phenolic compounds may induce the formation of phenols and other oxidative radicals that may hamper herbivorous insect feeding and/or produce toxins that reduce leaf digestibility (Felton et al. 1989).

The tolerance of KS-4202 to soybean aphids has been related to a higher level of peroxidase activity in plants subjected to feeding by aphids because plants of susceptible genotypes (infested or not) showed similar levels of enzyme activity (Pierson et al. 2011; Marchi-Werle et al. 2014). The increase in peroxidase activity has also been associated with resistance to pests in *Buchloe dactyloides* (Nuttall) (Poaceae) (Heng-Moss et al. 2004), wheat (Franzen et al. 2007), cabbage (Khattab 2007), and barley (Gutsche et al. 2009).

A higher level of polyphenoloxidase activity was observed in Conquista plants subjected to whitefly infestation when compared with uninfested plants at 28 DAI. This increase probably occurred in response to the stress caused by the longer period during which the plants were exposed to whitefly feeding. Wang et al. (2014) observed an increase in polyphenoloxidase activity after just 1 d of *A. glycines* feeding in soybean plants, with a progressive increase during the infestation period.

Our results corroborate the results of previous studies that showed that oxidative enzymes play important roles in plants' responses to stresses caused by insects (Khattab 2007; Gutsche et al. 2009; Wang et al. 2014). In general, alterations in levels of oxidative enzymes occurred in response to whitefly feeding for both genotypes. However, for Conquista, the actions of these enzymes were insufficient to compensate for the productivity damage caused by the insects' feeding.

The North American genotype KS-4202 exhibited tolerance to *B. tabaci* biotype B feeding, representing an important source of resistance to be taken into account in soybean breeding programs aiming at insect resistance. However, further studies are still necessary to better understand the causes of this tolerance because the oxidative enzymes studied are apparently not the major causes of tolerance in this genotype; however, these enzymes may represent important tools that can aid in the study of host plant resistance to insects.

Table 4. Activities of the peroxidase and polyphenoloxidase enzymes in soybean genotypes at 7, 14, 21, and 28 d after *Bemisia tabaci* biotype B infestation.

	Peroxidase (μmol of decomposed H_2O_2 per min per g fresh weight)			Polyphenoloxidase (μmol oxidized catechol per min per g fresh weight)		
		7 DAI				
	Treatment[a]			Treatment[a]		
Genotype	Control	Infested	Mean	Control	Infested	Mean
Conquista	5.98×10^{-3}	4.56×10^{-3}	5.27×10^{-3} a	2.42×10^{-3}	2.16×10^{-3}	2.29×10^{-3} a
KS-4202	3.64×10^{-3}	3.34×10^{-3}	3.49×10^{-3} a	1.40×10^{-3}	1.20×10^{-3}	1.30×10^{-3} b
Mean	4.81×10^{-3} A	3.95×10^{-3} A		1.91×10^{-3} A	1.68×10^{-3} A	
P (Genotype)	0.0645			0.0003		
P (Treatment)	0.3517			0.2956		
P (Interaction)	0.5410			0.8896		
		14 DAI				
	Control	Infested	Mean	Control	Infested	Mean
Conquista	7.08×10^{-3}	4.86×10^{-3}	5.97×10^{-3} a	3.48×10^{-3} aA	2.24×10^{-3} bB	2.86×10^{-3}
KS-4202	6.28×10^{-3}	4.72×10^{-3}	5.50×10^{-3} a	3.04×10^{-3} aA	3.56×10^{-3} aA	3.30×10^{-3}
Mean	6.68×10^{-3} A	4.79×10^{-3} B		3.26×10^{-3}	2.90×10^{-3}	
P (Genotype)	0.5381			0.1284		
P (Treatment)	0.0223			0.2081		
P (Interaction)	0.6646			0.0055		
		21 DAI				
	Control	Infested	Mean	Control	Infested	Mean
Conquista	17.56×10^{-3}	19.38×10^{-3}	18.47×10^{-3} a	6.82×10^{-3}	9.26×10^{-3}	8.04×10^{-3} a
KS-4202	12.02×10^{-3}	21.30×10^{-3}	16.66×10^{-3} a	3.84×10^{-3}	4.90×10^{-3}	4.37×10^{-3} b
Mean	14.79×10^{-3} A	20.34×10^{-3} A		5.33×10^{-3} A	7.08×10^{-3} A	
P (Genotype)	0.6284			0.0080		
P (Treatment)	0.1498			0.1680		
P (Interaction)	0.3243			0.5770		
		28 DAI				
	Control	Infested	Mean	Control	Infested	Mean
Conquista	25.90×10^{-3}	57.48×10^{-3}	41.69×10^{-3} a	8.20×10^{-3} aB	20.72×10^{-3} aA	14.46×10^{-3}
KS-4202	15.84×10^{-3}	20.62×10^{-3}	18.23×10^{-3} b	3.40×10^{-3} aA	6.30×10^{-3} bA	4.85×10^{-3}
Mean	20.87×10^{-3} A	39.05×10^{-3} B		5.80×10^{-3}	13.51×10^{-3}	
P (Genotype)	0.0035			0.0002		
P (Treatment)	0.0175			0.0015		
P (Interaction)	0.0686			0.0301		

[a]Means in a column followed by different lowercase letters or means in a row followed by different uppercase letters are significantly different ($P \geq 0.05$; ANOVA and Tukey's test).

Acknowledgments

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