Neotropical Entomology

PEST MANAGEMENT

Silicon Influence on Resistance Induction against *Bemisia tabaci* Biotype B (Genn.) (Hemiptera: Aleyrodidae) and on Vegetative Development in Two Soybean Cultivars

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Abstract

The potential of populations of *Bemisia tabaci* (Genn.) to become resistant to insecticides has stimulated research into alternative tactics of integrated pest management such as the induction of host-plant resistance. Recent data have shown that silicon can increase the degree of resistance of host plants to insect pests. Therefore the aim of our work was to study the effects of silicon application on the vegetative development of soybean plants and on the induction of resistance to the silverleaf whitefly, *B. tabaci* biotype B. We performed choice and no-choice tests of oviposition preference on two soybean cultivars, IAC-19 (moderately resistant to *B. tabaci* biotype B) and MONSOY-8001 (susceptible), with and without application of silicon. Silicon did not affect silverleaf whitefly oviposition preferences, but caused significant mortality in nymphs. Thus, silicon increased the degree of resistance to silverleaf whitefly. Silicon decreased the production of phenolic compounds, but did not affect lignin production. However, when applied to cultivar IAC-19, it increased the production of non-protein organic nitrogen. Silicon had no effect on the vegetative development of soybean plants, but it increased the degree of resistance to the silverleaf whitefly. We conclude that silicon applications combined with cultivar IAC-19 can significantly decrease silverleaf whitefly populations, having a positive impact both on the soybean plant and on the environment.

Introduction

Soybean holds a prominent place in national and world landscapes. Indeed, Brazil is the second largest soybean producer and exporter (after the United States) in the world. The total area used for soybean production in Brazil equals 20.7 million hectares and is rapidly expanding due to biodiesel production from soybean oil (Dall'Agnol *et al* 2010). However, several factors can interfere with soybean production. Besides climate conditions, another important cause of yield losses is the attack by insect pests. The whitefly *Bemisia tabaci* (Genn.) has for a long time been considered only an occasional pest of soybean in Brazil. However, the biotype B of *B. tabaci,* which was introduced in the early 90s, is becoming more and more problematic year after year (Lima & Lara 2004).

The biotype B of *B. tabaci* is considered the most aggressive and virulent biotype, as it becomes easily adapted to new host plants and to diverse climate conditions (Villas Boas *et al* 1997). It also has higher oviposition and feeding rates, and produces larger quantities of honeydew, in addition to inducing physiological disorders in infested plants (Perring 2001, Lima & Campos 2008).

Several studies report the importance of this pest species in other crops, such as beans, courgettes, okras, sweet peppers, tomatoes and watermelons (Lima & Campos 2008, Oriani *et al* 2008, Baldin *et al* 2009, Coelho *et al* 2009, Silva *et al* 2009). For soybean, the main damage stems from virus transmission, which is characterised by severe nanism, leaf curling, intense chlorosis and reduced seed production (Valle & Lourenção 2002).

Bemisia tabaci control is mainly based on insecticide application, but some physiological and behavioral characteristics of the insect favour the development of resistance to insecticides from different chemical groups (Ahmad *et al* 2002). Therefore, the potential of whitefly populations to become resistant as a consequence of intensive chemical use has stimulated studies on alternative tactics of integrated pest management (IPM) (Baldin *et al* 2005, Bleicher *et al* 2007).

In this context, induced host plant resistance represents a crucial strategy, as it offers a practical, cheap and long lasting solution to maintain low levels of whitefly populations, and ultimately reduces yield losses (Bellotti & Arias 2001).

Silicon foliar application has been reported to reduce oviposition preference and increase both the development time and nymph mortality in *B. tabaci* in cucumber (Correa *et al* 2005), and may be a potential tool for whitefly management. It has also been shown that silicon can indirectly stimulate the vegetal development and plant yield due to the protection against abiotic, such as the hydric stress (Korndörfer *et al* 2004), and biotic ones, such as insect pests (Gomes *et al* 2008, Costa *et al* 2009, Moraes *et al* 2009) and diseases (Moraes *et al* 2006). Some of these responses might be due to the fact that silicon application can probably trigger natural plant defensive mechanisms, such as the production of phenolic compounds, chitinases, peroxidases and lignin (Chérif *et al* 1994, Epstein 1999, Gomes *et al* 2008), which can all interfere with the physiology and development of insect pests.

In this paper we evaluated whether silicon application affects the vegetative development and induces resistance in soybean plants to the whitefly *B. tabaci* biotype B.

Material and Methods

The experiments were conducted in a greenhouse exposed to outside natural conditions of temperature and luminosity and in a climatic chamber (30 ± 2°C, RH 70 ± 10% and photophase of 12h). The whiteflies used in the experiments were collected from the rearing stock, which was kept at 30 ± 2°C, 70 ± 10% RH and a 12h photophase, and reared on tomato (*Solanum lycopersicum*), cabbage (*Brassica oleracea*), cucumber (*Cucumis sativus*), cotton (*Gossypium hirsutum*) and poinsettia (*Euphorbia pulcherrima*).

Soybean seeds from cultivars IAC-19 (moderately resistant to *B. tabaci* biotype B) (Valle & Lourenção 2002) and MONSOY-8001 (susceptible) were treated immediately before planting with the fungicide Captan, according to the recommendations on the product label. Seeds were planted on pots with a 3:1 mix of soil and organic matter. For each soybean cultivar, 20 pots were planted with six seeds per pot, and only four plants were kept after thinning. Soybean plants were irrigated daily in order to maintain soil moisture.

Ten days after seedling emergence, 250 ml of a 1% silicic acid solution (equivalent to one tonne of silicon per hectare) was applied to the soil around the seedlings.

Silicon-based resistance induction of soybeans to B. tabaci *biotype B*

Free-choice tests for oviposition preference and nymphal development

Twenty days after seedling emergence, five pots per treatment, (IAC-19 with silicon, IAC-19 without silicon, MONSOY-8001 with silicon and MONSOY-8001 without silicon), totalling 20 pots, were placed randomly in the climatic chamber. A new thinning was done leaving two plants per pot prior to infestation with approximately 2000 unsexed adult whiteflies. Adults were released in the climatic chamber with free access to all plants. After 48h of infestation, whiteflies were removed from the plants and the number of eggs laid on each plant was counted. We selected the third fully developed apical leaf of both plants in each pot (adapted from Valle & Lourenção 2002), totalling two leaves per pot, and all eggs on the abaxial side of each leaf were counted under a stereoscopic microscope, leaving the leaves intact on the plants. Each leaf was then marked for further evaluation on the nymphs.

Fifteen days after the removal of the whiteflies, the number of 3rd and/or 4th instars on the marked leaves was assessed, totalling two leaves per pot. Leaves were removed from the plants, placed in paper bags and then examined under a stereoscopic microscope to calculate the survival rate (%) from egg to the 3rd-4th instars.

No-choice tests for oviposition preference and nymph development

Seeds were potted and maintained as described earlier, but after the second thinning, two plants were randomly selected and dried at 60°C in an oven for later analysis of secondary compounds. Moreover, in the no-choice tests, each pot was placed in nylon-covered individual cages (0.3 x 0.3 x 0.8 m), which were randomly arranged in the climatic chamber. Nearly 100 unsexed adult whiteflies from the rearing stock were collected and released

into each cage where they remained for 60 h. Data was collected as described for the free choice tests.

Nymphal survival

As nymphal survival is independent of whether or not the nymphs developed from eggs in which adult whiteflies had a free-choice or a no-choice to lay their eggs, we combined the nymphal survival data from the free-choice and no-choice experiments prior to statistical analyses.

Production of secondary defence compounds

Dried leaves obtained as described were crushed in a Willy grinder until they formed a fine powder and subjected to chemical analysis at the "Laboratório de Produtos Vegetais, Departamento de Ciências dos Alimentos, Universidade Federal de Lavras", MG. Samples were used for the analysis of phenolic compounds, lignin and non-protein organic nitrogen following Gomes *et al* (2008).

Effects of silicon on the vegetative development of soybean

After the number of nymphs was determined, two plants from each pot were cut just above soil level and the roots were washed under running water. Plants were placed in paper bags and dried to constant weight at 60°C, and the dry weight of aerial parts and roots was determined.

Statistics

The experimental design to test the number of eggs and nymphs, the percentage of phenolic compounds, lignin and non-protein organic nitrogen was completely randomized with five replicates in a 2x2 factorial design, with two levels of silicon (with and without silicon) and two soybean cultivars (IAC-19 and MONSOY-8001), in which each replicate corresponded to the mean of the two leaves or plants per pot, totalling 20 pots (five pots per treatment). The experimental design to test the nymphal survival rate was the same as above, but with ten replicates for each treatment (totalling 40 pots), as the data from the free-choice and the no-choice tests were pooled (see Nymphal survival).

Count data was $\sqrt{X}$ **-** transformed before analysis of variance in order to meet the assumptions of the ANOVA. As this is a 2x2 factorial design, comparison of means were not necessary.

Results and Discussion

Silicon-based resistance induction of soybeans to B. tabaci *biotype B*

Free-choice tests for oviposition preference and nymphal development

No interaction was observed between cultivar and silicon application for any of the variables assessed (Table 1). However, a non-preference for oviposition in the IAC-19 cultivar as compared to MONSOY-8001 cultivar was observed (Table 1), as already reported by Lourenção & Valle (2002). IAC-19 also showed a lower number of nymphs of *B. tabaci* biotype B than MONSOY-8001. Furthermore, silicon application did not affect oviposition or the mean number of *B. tabaci* nymphs on soybean plants (Table 1).

No-choice tests for oviposition preference and nymphal development

The cultivars tested and the application of silicon independently affected the biological parameters evaluated (Table 1). All biological parameters observed for whiteflies in no-choice tests on IAC-19 and MONSOY-8001 cultivars were similar to those observed in the free-choice tests (Table 1). However, an effect of silicon could be observed on the number of nymphs; treated plants had fewer nymphs than the untreated ones (Table 1). This result differs from that reported by Moraes *et al* (2009), wherein no significant differences were observed in the number of eggs and nymphs of *B. tabaci* in treated soybean IAC-19 and MONSOY-8001 cultivars.

Nymphal survival

There was a significant interaction between soybean cultivars and the application of silicon on nymphal survival of *B. tabaci* biotype B (P = 0.0128) (Table 2). Nymphal survival, at least through the 3rd and 4th instars, was not significantly different between the two untreated

Table 1 Mean number of eggs and nymphs of *Bemisia tabaci* biotype B per leaf (± SE) on two soybean cultivars (A) and on control (no silicon) and silicon-treated plants (B). FCT: free-choice test and NCT: no-choice test.

Test		Treatment	Eggs	Nymphs
FCT	(A)	IAC-19	83.7 ± 21.19 b	49.8 ± 13.91 b
		MONSOY-8001	184.6 ± 34.89 a	125.3 ± 20.87 a
	(B)	Control	142.6 ± 38.23 a	101.0 ± 24.27 a
		Silicon	125.6 ± 27.46 a	74.2 ± 17.80 a
		CV(%)	37.1	35.7
NCT	(A)	IAC-19	51.4 ± 6.85 b	34.0 ± 6.86 b
		MONSOY-8001	79.2 ± 10.96 a	48.0 ± 5,74 a
	(B)	Control	75.3 ± 10.72 a	49.6 ± 6.49 a
		Silicon	55.3 ± 8.54 a	32.4 ± 5.68 b
		CV(%)	21.2	21.6

Means followed by the same lower case letter in the column in each group (A or B) do not differ significantly (F test; P > 0.05).

Table 2 Mean nymphal survival (%) of *Bemisia tabaci* biotype B until the third or fourth instars (± SE) on two soybean cultivars and on control and silicon-treated plants. Data from the free-choice and no-choice tests are pooled.

Soybean	Nymphal survival (%)	
	Control	Silicon
IAC-19	74.2 ± 4.69 aA	47.5 ± 5.09 bB
MONSOY-8001	68.0 ± 3.45 aA	67.1 ± 6.26 aA
CV(%)	22.05	

Means followed by the same lower case letter in the columns, and upper case letter in the rows, do not differ significantly (F test; P > 0.05).

cultivars, which indicates that the genotypic resistance of IAC-19, while negatively affecting the oviposition of *B. tabaci*, did not influence nymphal development of this insect.

However, nymphal survival on silicon-treated IAC-19 plants was significantly lower than that of untreated IAC-19 plants and from silicon-treated MONSOY-8001 (Table 2). These results are similar to those of Correa *et al* (2005) who observed a higher nymphal mortality of *B. tabaci* biotype B after foliar application of silicon on cucumber plants, probably due to an increase in the plants defensive compounds induced by a higher activity of oxidative enzymes (Gomes *et al* 2008).

Production of secondary defensive compounds

No significant interaction between cultivars and application of silicon could be observed on the concentration of phenolic compounds or lignin (Table 3). In addition, no differences were observed for the phenolic compounds between the two tested soybean cultivars; however, MONSOY-8001 had a higher percentage of lignin than IAC-19 (Table 3). Although this difference may be due to the genetic properties of the cultivars themselves, no such difference between these cultivars was reported

Table 3 Mean percentages (dry weight) of phenolic compounds and lignin (± SE) in two soybean cultivars (A) and in control and silicon-treated plants (B).

	Treatment	Phenolic compounds (%)	Lignin (%)
(A)	IAC-19	2.3 ± 0.17 a	4.5 ± 0.10 b
	MONSOY-8001	2.2 ± 0.10 a	6.5 ± 0.14 a
(B)	Control	2.4 ± 0.12 a	5.5 ± 0.52 a
	Silicon	2.0 ± 0.12 b	5.5 ± 0.48 a
	CV(%)	12.4	5.2

Means followed by the same letter in the column in each group (A or B) do not differ significantly (F test; P > 0.05).

on an earlier study (Moraes *et al* 2009).

A lower percentage of phenolic compounds was observed for soybean plants treated with silicon as compared to untreated plants. However, silicon did not seem to affect lignin production (Table 3). Conversely, Moraes *et al* (2009) observed a significant interaction between cultivar and inducer, with the highest production of this compound in silicon-treated IAC-19 soybean plants. The lack of change in lignin production as a response to silicon observed here could be due to the fact that the plant defensive responses depend on several factors, like the induction of gene expression, the type and concentration of the elicitor, the plant used and the duration of the protective effect (Roncatto & Pascholati 1998).

Furthermore, there was a significant interaction between cultivars and application of silicon for non-protein organic nitrogen (P = 0.0244) (Table 4). There was a significant increase for non-protein organic nitrogen in IAC-19 in response to silicon application, but not for MONSOY 8001 (Table 4). The increase in non-protein organic nitrogen in IAC-19 may have contributed to nymph mortality in this cultivar. Nitrogen-based organic compounds increase in concentration when nitrogen availability also increases in plants. Moreover, some of the non-protein organic nitrogen present in its free form acts as a protective substance, exerting toxicity in several ways (as non-protein amino acids by blocking the synthesis or absorption of protein amino acids, or by being erroneously incorporated into proteins, leading to the production non-functional enzymes), and can possibly alter the insect metabolism and impair its development (Taiz & Zeiger 2004).

Untreated MONSOY-8001 cultivar showed a higher mean percentage of non-protein organic nitrogen than untreated IAC-19 (Table 4). However, it is unlikely that a differential production of these defence compounds alone is sufficient to affect development of *B. tabaci* biotype B on MONSOY-8001, since this cultivar does not present any resistance to the insect.

Given that the protection of soybean plants that have a certain degree of insect resistance can still be increased when combined with other control tactics (Valle &

Table 4 Mean percentage (dry weight) of non-protein organic nitrogen (± SE) in silicon treated soybean plants.

Soybean	Non-protein organic nitrogen (%)	
	Control	Silicon
IAC-19	4.5 ± 0.12 bB	4.9 ± 0.14 aA
MONSOY-8001	5.3 ± 0.10 aA	5.1 ± 0.10 aA
CV(%)	5.2	

Means followed by the same lower case letter in the columns, and upper case letter in the rows, do not differ significantly (F test; P > 0.05).

Table 5 Mean dry weights of roots (g), aerial parts (g) and mean root: aerial part ratio (± SE) of two soybean cultivars (A) and of control and silicon-treated plants (B).

	Treatment	Roots (R)	Aerial parts (AP)	Ratio R: AP
(A)	IAC-19	0.3 ± 0.02 a	1.7 ± 0.10 a	0.20 ± 0.01 b
	MONSOY-8001	0.4 ± 0.03 a	1.5 ± 0.09 a	0.24 ± 0.02 a
(B)	Control	0.4 ± 0.03 a	1.6 ± 0.12 a	0.22 ± 0.02 a
	Silicon	0.4 ± 0.03 a	1.6 ± 0.07 a	0.21 ± 0.02 a
	CV(%)	7.7	6.7	8.1

Means followed by the same lower case letter in the columns in each group (A or B) do not differ significantly (F test; P > 0.05).

Lourenção 2002), the application of silicon is a viable alternative to the management of whitefly in soybean cultivars like IAC-19.

Effect of silicon on the vegetative development of soybean

No significant interaction between cultivars and application of silicon could be observed on the vegetative development of soybean plants (Table 5). IAC-19 and MONSOY-8001 showed no significant differences in the mean dry weight of roots and aerial parts (Table 5). However, we observed a higher ratio root:aerial part in MONSOY-8001 than in IAC-19, which may be due to their intrinsic agronomical traits. In addition, no effect of silicon on the vegetative development of soybeans was observed; the mean weights found for root and aerial parts, and the root:aerial parts ratio were the same for silicon-treated or untreated (Table 5).

Although silicon has been demonstrated not to affect the dry weight of the aerial parts of several plants (Costa *et al* 2007, Tokura *et al* 2007, Gomes *et al* 2008, Smith *et al* 2008, Moraes *et al* 2009), changes in the root:aerial parts ratio observed in our laboratory tests must be investigated under field conditions.

In conclusion, although silicon application did not affect oviposition preference of the silverleaf whitefly, it did impair nymphal development by reducing nymphal survival. Therefore, the positive results on plant resistance obtained here suggest that silicon, combined with resistant cultivars such as the IAC-19, can be considered as a good alternative for the management of silverleaf whitefly in soybean.

Acknowledgments

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