CROP PROTECTION

Silicon and Acibenzolar-S-Methyl as Resistance Inducers in Cucumber, against the Whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) Biotype B

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Silício e Acibenzolar-S-Methyl Como Indutores de Resistência em Pepino, à Mosca-Branca *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) Biótipo B

RESUMO - O objetivo deste trabalho foi avaliar o efeito de silicato de cálcio ($CaSiO_3$) e do ativador acibenzolar-S-methyl (BTH) na indução de resistência em pepino à mosca-branca *Bemisia tabaci* (Gennadius) biótipo B. Testes de preferência com e sem chance de escolha e de antibiose foram realizados em casa-de-vegetação e no laboratório do Departamento de Entomologia da Universidade Federal de Lavras/UFLA, MG. O delineamento foi o inteiramente ao acaso com os seguintes tratamentos: 1) testemunha, 2) aplicação de 3 g de silicato de cálcio.kg^{-1} de substrato, 3) duas aplicações de 200 ml de solução a 1% de silicato de cálcio/vaso, 4) duas aplicações de 100 ml de solução a 0,005% de BTH/vaso e 5) aplicação de silicato de cálcio e BTH na mesma dose dos tratamentos 3 e 4. Para eliminar o efeito isolado do cálcio, foi aplicado 1g de carbonato de cálcio.kg^{-1} de substrato no tratamento 1 (testemunha). O silicato de cálcio e BTH causaram efeitos negativos na população da mosca-branca pela redução da oviposição, aumento do ciclo biológico e da mortalidade na fase de ninfa. Apresentaram-se, assim, como produtos alternativos a serem utilizados no manejo integrado de mosca-branca na cultura de pepino.

PALAVRAS-CHAVE: Insecta, silicato de cálcio, BTH, resistência induzida

ABSTRACT - The objective of this work was to evaluate the effect of calcium silicate ($CaSiO_3$) and of the activator acibenzolar-S-methyl (BTH) on resistance induction in cucumber, against the whitefly *Bemisia tabaci* (Gennadius) biotype B. Antibiosis, free-choice and no-choice preference tests were conducted in a greenhouse and at laboratory of the, Departamento de Entomologia of the Universidade Federal de Lavras (UFLA), MG, Brazil. The design was completely randomized, with the following treatments: 1) control; 2) application of 3 g calcium silicate.kg^{-1} of substrate; 3) two 200ml applications of 1% calcium silicate solution per pot; 4) two 100ml applications of 0.005% BTH solution per pot; and 5) application of calcium silicate and BTH at the rates used for treatments 3 and 4. To eliminate the effect of calcium, treatment 1 (control) received 1g of calcium carbonate (control). Calcium silicate and BTH diminished the whitefly population by reducing oviposition and increasing cycle length and mortality at the nymphal stage and can be chemical alternatives for the whitefly integrated management in cucumber.

KEY WORDS: Insecta, calcium silicate, BTH, induced resistance

The whitefly *Bemisia tabaci* (Gennadius) biotype B is an important pest in several crops. Feeding and the excreted honeydew of this insect lead to the formation of sooty mold, which affect cucumber yields in quantitative and qualitative terms (Oliveira *et al.* 2001). One of the most important problems resulting from whitefly attack is the transmission of viral diseases to most of the *Geminivirus* group (Villas Bôas *et al.* 2002).

Infestation by this aleyrodid can significantly inhibit plant development, especially because the toxins resulting from feeding of nymphs may cause plant death (Cohen *et al*. 1992, Lourenção & Nagai 1994). The limitations and high cost of chemical control, in addition to toxicological problems such as insecticide persistence and resistance, justify the adoption of alternative management methods, including plant resistance against the whitefly.

Silicon has not been considered an essential element for plants. However, when it is applied via soil or leaves, it is absorbed by plants as silicic acid and benefits many plant species, especially those in the family Poaceae (grasses) by stimulating growth and yield, providing protection against abiotic stress, and decreasing the incidence of insect pests and diseases. In rice, the application of silicon induced resistance to borer caterpillars of the family Pyralidae (Tayabi & Azizi 1984, Sawant *et al.* 1994), whereas in corn, silicon affected the biological development of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Goussain *et al.* 2002). In sorghum and wheat, silicon negatively affected preference and reduced reproduction rates of the greenbug *Schizaphis graminum* (Rond.) (Carvalho *et al.* 1999, Moraes & Carvalho 2002, Basagli *et al.* 2003). In cucumber, research shows that silicon acts on the plant tissues, resulting in faster activation of defense mechanisms against pathogens (Samuels *et al.* 1991). Induction of this type of defense can also occur when the plant suffers injuries by sucking insects (Dreyer & Campbell 1987).

Synthetic compounds such as salicylic acid and S-methyl benzol[1,2,3]thiadiazole-7-carbothioic [acibenzolar-S-methyl] (BTH) can activate, similarly to biotic inducers, the genes that code for resistance in plants (Kessmann *et al.* 1994). In sorghum, accumulation of phytoalexins in plant tissues occurred as the concentration of BTH increased, indicating plant systemic response to the resistance inducer (Oswald *et al.* 1998). In cotton, the application of BTH induced increases in activity levels of the enzymes chitinase, peroxidase, and 1,3-glucanase in young and old cotton leaves, indicating some local resistance effect (Inbar *et al.* 2001). To date, the interaction among cucumber, whitefly, and silicon-induced resistance has not been investigated.

In view of the potential shown by silicon and the synthetic activator BTH as resistance inducers in plants, and of the actual whitefly population increase in crops of economic importance, we evaluated the effect of calcium silicate ($CaSiO_3$) and BTH on resistance induction to *B. tabaci* biotype B in cucumber plants.

Material and Methods

Stock Rearing of *B. tabaci* Biotype B. Five cucumber seeds, c.v. Caipira Japonês, were sown in a pot containing 2 kg of substrate (dark-red Latosol and rotted cattle manure at the ratio of 3:1) and 6.5 g $.kg^{-1}$ of NPK (4-14-8) substrate, in a greenhouse at the Departamento de Entomologia, Universidade Federal de Lavras (UFLA), MG, Brazil. Thinning was performed after seedling emergence, to ensure a final stand of two plants per pot. A sidedressing fertilization with urea and potassium chloride was applied at dose rates of 0.75 and 0.30 $g.kg^{-1}$ of substrate, respectively, 15 days after seedling emergence. Plants were sprinkler-irrigated daily. Twenty days after seedling emergence, the pots were placed in a cage measuring 5×3×3 m, on a metal bench. *B. tabaci* biotype B whiteflies reared in the laboratory were released into the cage. Inspections of the rearing location were performed periodically, to eliminate plants that were infested with parasitoids or diseased, and to replace pots as plants entered senescence.

General Methodology. The bioassay was conducted in the greenhouse and in the Departamento de Manejo Integrado de Pragas, Universidade Federal de Lavras (UFLA), MG, Brazil, from January 10 to December 20, 2003. The management practices were the same used for the whitefly stock rearing. Assays consisted of the following treatments: 1) control; 2) application of 3 g calcium silicate (62% SiO_2 and 18.5% CaO)$.kg^{-1}$ incorporated to the substrate; 3) two 200 ml applications of 1% calcium silicate solution per pot, sprinkled over the plants five and 15 days after emergence; 4) two 100 ml applications of 0.005% acibenzolar-S-methyl (BTH), Bion 500 WG® solution per pot, sprinkled over the plants five and 15 days after emergence; and 5) application of calcium silicate and BTH, at the same dose rates as used in treatments 3 and 4. To eliminate the effect of calcium, 1g of calcium carbonate was applied in treatment 1 (control).

Free-Choice Preference Test in the Greenhouse. Sixty, 2-liter pots were cultivated with cucumber in a whitefly-free location, with the same corresponding treatments and previously mentioned management practices. Twenty days after seedling emergence, five pots were placed into a 2 x 3 x 2 m cage and ten replicates were randomly arranged on a metal bench. Next, *B. tabaci* biotype B adults were collected, with a manual aspirator, from the stock rearing cucumber plants and placed in the cage for 24h, at the ratio of 100 individuals per plant, in a total of 10,000 individuals. The assay was conducted at the temperature of 25 ± 2°C and 70 ± 10% relative humidity, and recorded with a thermo-hygrograph placed inside the cage. After 24h, the released adults were removed, the eggs counted (five days after release), and 4th-instar nymphs (18 days after release) determined with a 20× magnifying glass, in all leaves of one, randomly chosen plant in each treatment. Survival rates (%) from the egg to the 4th-instar nymphal stage were determined.

No-choice Preference Test in the Greenhouse. The same procedure used in the previous test was adopted, but each of the 50 pots were placed in a separate cage measuring 0.3×0.3×0.5 m, screened with white *voile* fabric, and randomly placed on a metal bench. Next, *B. tabaci* biotype B adults were collected, with a manual aspirator, from the stock rearing cucumber plants and placed in the cage, at the ratio of 100 adults per plant (200 individuals per cage), for 24h. The assay was conducted at a temperature of 25 ± 2°C and 70 ± 10% relative humidity, recorded with a thermo-hygrograph placed inside the cage. The numbers of eggs and 4th-instar nymphs were evaluated, in the same manner as for the free-choice preference test. Survival rates (%) from the egg to the 4th-instar nymphal stages were determined.

Antibiosis. Twenty 2-liter pots were cultivated with cucumber in a whitefly-free location, with the same corresponding treatments and previously mentioned management practices. Twenty days after seedling emergence, five pots, corresponding to the five treatments, were selected according to plant vigor, transferred to the laboratory, and placed in an air-conditioned room at a temperature of 25 ± 2ºC, 70 ± 10% relative humidity, and 12h photophase. Next, ten *B. tabaci* biotype B stock rearing adults were placed in the micro-cages attached to the cucumber leaves, for 24h. The micro-cages were made of clear plastic (2.5 cm in diameter and 2 cm in height), one side being covered with white *voile* fabric, and the rim of the other side covered with foam (5 mm thick), to prevent insects from escaping. The micro-cages were attached to the leaves by means of an aluminum clip, with one of the prongs attached to the fabric and the other to a piece of cardstock with a larger diameter than the cage.

Next, two eggs per micro-cage were isolated, for a total of two to three micro-cages per leaf and 18 eggs per plant, which constituted one plot. Because each pot contained four plants, each treatment was repeated four times. The eggs, and eventually the nymphs, were observed daily and the survival and development time for each stage were recorded.

Statistical Analysis. A completely randomized design was used, except for the free-choice preference test, which was set up as randomized blocks. The data were submitted to analysis of variance using the Sisvar software program, and the means compared by the Scott and Knott test (Scott & Knott 1974). Mortality data for the immature stages of *B. tabaci* biotype B were grouped per plant and transformed into arc sine of the square root of (x / 100).

Results and Discussion

Free-Choice Test. Calcium silicate and BTH affected the oviposition preference of *B. tabaci* biotype B on cucumber leaves. Plants receiving calcium silicate via soil showed a smaller number of whitefly eggs. The most striking effect, nevertheless, was the marked reduction in oviposition by more than three-fold in relation to the control, due to the foliar application of calcium silicate, with or without BTH. The mean number of whitefly nymphs was similar to that for oviposition; survival rate for the egg to fourth-instar nymph period, however, was lower in plants treated with silicon, BTH, or both (Table 1). In the field, the whitefly did not show oviposition non-preference on cotton plants with BTH (Inbar *et al.* 2001), contrary to what was observed in cucumber plants treated with silicon, BTH, or both. When given a choice, the whitefly preferred to oviposit on the most suitable substrates for the development of nymphs (Villas Bôas *et al.* 2002).

No-Choice Test. No significant differences in whitefly oviposition were observed. However, reductions in the survival rate and in approximately half the number of fourth-instar nymphs were observed, in plants receiving foliar applications of calcium silicate, BTH, or both (Table 2).

Silicon application has conferred non-preference resistance to the greenbug *Schizaphis graminum* (Rond.) in

Table 1. Mean number of eggs, 4th-instar nymphs, and survival rate (egg to nymph) (± SEM) of *B. tabaci* biotype B on cucumber plants treated with calcium silicate ($CaSiO_3$) and acibenzolar-S-methyl (BTH) in a free-choice test (at 25 ± 2ºC and 70 ± 10% RH).

Treatment	Egg	Nymph	Survival (%)
Control	487.0 ± 28.11 a	425.6 ± 24.07 a	87.5 ± 1.30 a
Soil $CaSiO_3$	317.2 ± 33.92 b	216.0 ± 17.82 b	70.5 ± 3.48 b
Foliar $CaSiO_3$	223.1 ± 16.47 c	144.5 ± 12.37 c	64.4 ± 1.60 c
Foliar BTH	208.2 ± 17.43 c	147.3 ± 13.80 c	70.2 ± 1.62 b
Foliar $CaSiO_3$ + BTH	220.5 ± 13.61 c	128.2 ± 06.43 c	58.7 ± 1.61 d
CV (%)	23.63	22.42	8.30

Means in a column followed by the same letter are not significantly different by Scott and Knott test (P ≤ 0.05).

Table 2. Mean number of eggs, 4th-instar nymphs, and survival rate (egg to nymph) (± SEM) of *B. tabaci* biotype B on cucumber plants treated with calcium silicate ($CaSiO_3$) and acibenzolar-S-methyl (BTH) in a no-choice test (at 25 ± 2ºC and 70 ± 10% RH).

Treatment	Egg$^{n.s.}$	Nymph	Survival (%)
Control	476.2 ± 32.35	416.0 ± 24.20 a	86.8 ± 1.29 a
Soil $CaSiO_3$	393.0 ± 41.70	310.6 ± 27.00 b	81.6 ± 2.36 a
Foliar $CaSiO_3$	388.7 ± 32.92	233.1 ± 19.37 c	60.0 ± 2.72 b
Foliar BTH	367.0 ± 20.63	207.3 ± 12.30 c	56.7 ± 2.10 b
Foliar $CaSiO_3$ + BTH	359.8 ± 31.01	180.4 ± 14.97 c	51.9 ± 3.81 b
CV (%)	25.20	22.88	10.49

Means in a column followed by the same letter are not significantly different by Scott and Knott test (P ≤ 0.05); $^{n.s.}$ non-significant

Table 3. Mean duration (± SEM) of immature stages and total cycle of *B. tabaci* biotype B on cucumber plants treated with calcium silicate ($CaSiO_3$) and acibenzolar-S-methyl (BTH) (at 25 ± 2°C and 70 ± 10% RH).

Treatment	Duration (days)					
	Egg $^{N.S.}$	Nymph 1	Nymph 2	Nymph 3	Nymph 4	Cycle
Control	6.01 ± 0.09	3.12 ± 0.15 a	2.34 ± 0.10 a	2.50 ± 0.13 a	5.23 ± 0.16 a	19.2 ± 0.31 a
Soil $CaSiO_3$	6.16 ± 0.11	4.62 ± 0.12 b	3.63 ± 0.13 b	3.56 ± 0.14 b	6.35 ± 0.17 b	24.3 ± 0.29 b
Foliar $CaSiO_3$	6.29 ± 0.14	4.55 ± 0.11 b	3.81 ± 0.15 b	3.61 ± 0.12 b	6.58 ± 0.13 b	24.8 ± 0.30 b
Foliar BTH	6.56 ± 0.15	5.24 ± 0.14 c	3.99 ± 0.14 b	3.81 ± 0.12 b	6.80 ± 0.11 b	26.4 ± 0.34 c
Foliar $CaSiO_3$ + BTH	6.36 ± 0.17	5.49 ± 0.14 c	4.06 ± 0.12 b	3.98 ± 0.11 b	6.76 ± 0.11 b	26.8 ± 0.35 c
CV (%)	12.81	17.83	21.45	21.43	13.44	8.10

Means in a column followed by the same letter are not significantly different by Scott and Knott test ($P \leq 0.05$); $^{n.s.}$ non-significant

sorghum and wheat plants (Carvalho *et al.* 1999, Moraes & Carvalho 2002, Basagli *et al.* 2003). Inbar *et al.* (2001) did not observe a reduction in whitefly oviposition in young cotton leaves treated with BTH, similarly to the results obtained in the no-choice test.

Antibiosis. Treatments did not affect the duration of the egg stage of *B. tabaci* biotype B. However, significant differences occurred in the development of first to fourth instar nymphs and in the duration of the total whitefly cycle, when feeding on cucumber plants treated with calcium silicate and BTH, regardless of the application method. All application methods of calcium silicate and BTH caused a significant increase in the duration of the developmental period of second to fourth instar nymphs, as compared to the control. For first instar nymphs, the application of BTH and BTH with calcium silicate delayed molting to the second nymphal instar by more than 70% (Table 3).

The egg-stage survival rate of the whitefly was not affected by any of the tested treatments, ranging from 91.7% in the control to 84.7% in plants treated with calcium silicate and BTH. However, foliar applications of calcium silicate, BTH, or both, caused more than three-fold mortality increase in the nymphal stage and in the total cycle, when compared with the control (Table 4).

The increase in the developmental period of all nymphal stages and in the total cycle of the whitefly can be related to the presence of alelochemicals that caused non-preference for feeding, antibiosis, or both, because of the high mortality. Gatehouse (2002) observed that plant's defense to the attack of herbivore insects can be expressed as a mechanical barrier (lignification or production of resin) or as a biochemical signal, resulting in deterrence of the insect's feeding or oviposition.

The occurrence of mortality in the nymph stage suggests that silicon, BTH, or both, probably acted as elicitors of substances related to the plant's defense, in response to whitefly feeding. Activation of the enzymes chitinase, peroxidase, 1,3-glucanase, polyphenoloxidase and phenylalanine ammonia-lyase proteinase and lipoxygenases, related to the synthesis of secondary compounds, increased with silicon and BTH application in cotton, tomato, and wheat (Stout *et al.*1994, Inbar *et al.* 2001), imparting an antibiosis-type mechanism of resistance to the treated plants.

Our results indicate that silicon and BTH, applied separately or as a mixture on leaves, are absorbed by plants and may induce the synthesis of defense chemicals, reducing

Table 4. Egg viability and mortality (mean ± SEM) of *B. tabaci* biotype B on cucumber plants treated with calcium silicate ($CaSiO_3$) and acibenzolar-S-methyl (BTH) (at 25 ± 2°C and 70 ± 10% RH).

Treatments	Egg $^{N.S.}$ viability (%)	Mortality (%)	
		Nymph	Cycle
Control	91.7 ± 1.60	13.6 ± 1.36 a	20.8 ± 1.39 a
Soil $CaSiO_3$	90.3 ± 1.39	33.8 ± 3.56 b	40.3 ± 2.66 b
Foliar $CaSiO_3$	88.9 ± 2.27	47.9 ± 2.83 c	54.2 ± 1.39 c
Foliar BTH	86.1 ± 3.59	48.5 ± 2.05 c	55.6 ± 3.21 c
Foliar $CaSiO_3$ + BTH	84.7 ± 2.66	53.9 ± 4.55 c	61.2 ± 3.32 c
CV (%)	15.49	8.76	7.17

Means in a column followed by the same letter are not significantly different by Scott and Knott test ($P \leq 0.05$); $^{n.s.}$ non-significant the preference of *B. tabaci* biotype B for oviposition, expanding the insect's developmental period, and increasing nymphal mortality. Therefore, foliar application of silicon, BTH, or both can be efficient for whitefly management in cucumber.

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