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Resistance induction in chrysanthemum due to silicon application in the management of whitefly *Bemisia tabaci* Biotype B (Hemiptera: Aleyrodidae)

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ABSTRACT
Whitefly *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) is a key pest in many cultures worldwide, among them chrysanthemum. The aim of this study was to evaluate whether silicon application in chrysanthemum plants can induce resistance to whitefly. The experiments were conducted in chrysanthemum cv. Rage in a greenhouse at EPAMIG in São João Del Rei, MG. The treatments were: 1) silicon applied as soil drench (dosage of 2t SiO_2/ha) on the 4th day after planting, 2) two silicon applied as foliar spray on the 4th and 12th days after planting, 3) control, with 10 replications each. The plants were infested with 100 whitefly adults/pot, released 16 days after planting. The number and viability of whitefly eggs and number of nymphs and adults were evaluated. The production of fresh and dry matter of shoots and the silicon content in plants were also evaluated. Silicon did not affect the whitefly oviposition preference; however, it affected the development of nymphs in free-choice test. Foliar silicon applications reduced the viability of whitefly eggs. There was no difference of fresh and dry weight and silicon content in plants with the application of silicon. Therefore, foliar silicon application on chrysanthemum can reduce the viability of eggs and delay the development of nymphs and thus contribute to the integrated whitefly management in commercial crops.

Palavras-chave:
MIP
controle alternativo
inseto-praga
resistência induzida

Indução de resistência em plantas de crisântemo pela aplicação de silício no manejo de mosca-branca *Bemisia tabaci* Biótipo B (Hemiptera: Aleyrodidae)

RESUMO
A mosca-branca *Bemisia tabaci* biótipo B (Hemiptera: Aleyrodidae) é praga chave em diversas culturas no mundo, dentre elas o crisântemo. Assim, o objetivo neste trabalho foi avaliar se a aplicação de silício em plantas de crisântemo pode induzir resistência à mosca-branca. Os experimentos foram realizados com crisântemo cv. Rage, em casa de vegetação, na EPAMIG em São João Del Rei-MG. Os tratamentos foram: 1) aplicação de silício no solo (drench) (dosagem de 2t de SiO2/ha) no 4º dia após o plantio; 2) duas aplicações foliares de silício realizadas no 4º e 12º dias após o plantio; 3) testemunha, com 10 repetições cada. As plantas foram infestadas com 100 adultos da mosca-branca/vaso, liberados 16 dias após o plantio. Foram avaliados o número e a viabilidade dos ovos e o número de ninfas e adultos de mosca-branca. Também foi avaliada a produção de matéria verde e seca da parte aérea e o teor de silício nas plantas. O silício não interferiu na preferência para oviposição da mosca-branca, todavia afetou o desenvolvimento das ninfas no teste com chance de escolha. A aplicação foliar de silício reduziu a viabilidade dos ovos da mosca-branca. Não foi verificada diferença no peso verde e seco e no teor de silício das plantas com a aplicação de silício. Portanto, o silício quando aplicado via foliar em crisântemo pode reduzir a viabilidade dos ovos e retardar o desenvolvimento de ninfas e, assim, contribuir para o manejo integrado de mosca-branca em cultivos comerciais.

Introduction

Chrysanthemum, *Dendranthema grandiflorum* (Asteraceae) is one of the most important ornamental flowers grown and marketed worldwide. In Brazil, this species is highly appreciated due to the beauty and durability, besides this represents a high volume of sales, both as pot or cut flowers (BARBOSA et al., 2012).

Whitefly *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) is a polyphagous insect which has caused problems for chrysanthemum growers in Brazil since 1991 (LOURENÇÃO & NAGAI, 1994). Its presence is associated with direct economic losses and especially to the possibility of transmission of viral diseases (ROCHA et al., 2013). There are several reports associated with reduced efficiency of insecticides in the whitefly management, and whitefly populations resistant to various chemical groups including pyrethroids, organophosphate, carbamate, neonicotinoid and growth regulators such as buprofezin and pyriproxyfen have already been observed (SILVA et al., 2009; BASIT et al., 2011).

By the other hand, the difficulty in manage whitefly by chemical control has stimulated the search for alternative methods that contribute to the integrated management of this pest. Studies have shown the potential of resistance induction by silicon application as a method for controlling whitefly on cucumber (CORREA et al., 2005), bean (PEIXOTO et al., 2011) and soybean crops (MORAES et al., 2009). In rosebush, Locarno et al. (2011) found relationship between silicon fertilization and chlorophyll content in leaves, which possibly can enhance rates of photosynthesis. In chrysanthemum, silicon application contributed in reducing leafminer and aphid populations (PARRELLA et al., 2007; JEONGL et al., 2012).

In general, silicon can enhance plant resistance against insect by two main mechanisms. Physical resistance (constitutive) associated in silica deposition on leaves which results in digestibility reduction and/or increase hardness and abrasiveness of plant tissues. On the other side, soluble silicon can induced chemical defenses to insect herbivory by increasing production of defensive enzymes (REYNOLD et al., 2009). Therefore, in view of the need to identify viable alternatives for pest management in chrysanthemum and knowing the potential of silicon in the control of pest insects through the induction of resistance in plants, the aim of this study was to evaluate whether silicon application in chrysanthemum can induce resistance to whitefly.

Material and Methods

The experiments were conducted in a greenhouse at the Agricultural Research Enterprise of Minas Gerais (EPAMIG), São João Del Rei, MG, from March to June 2012. A thermo-hygrometer was installed inside the greenhouse to daily evaluate temperature and relative air humidity. Potted chrysanthemum cuttings (cv. Rage) were planted in pots (pot 11, with capacity of 1liter volume) containing substrate (Plantimax®). The cultivation procedures followed the practices used by chrysanthemum growers, except for pest control. Drip irrigation system and fertirrigation were applied to supply plants necessities (BARBOSA et al., 2012).

A stock rearing of *B. tabaci* biotype B started with populations collected in the Department of Entomology, Federal University of Lavras. Whiteflies were kept in greenhouse on potted cabbage plants (*Brassica oleracea* var. *acephala*) and inspections of the rearing occurred weekly to replace plants which entered senescence.

Three treatments with 10 replications each were assessed in a completely randomized block design, and experimental unit was a pot, 1liter containing two plants per pot. Source of Si were 1% silicic acid solution ($SiO_2.XH_2O$) (Vetec Fine Chemicals [Vetec Química Fina], Duque de Caxias, Brazil). The treatments were: 1) Silicon applied as soil drench by using one application of 100 ml of 1% silicic acid solution (dosage equivalent to 2t of SiO_2/ha-1), around the stems of plants 4 days after the planting of seedlings, 2) Silicon applied as foliar spray, two times of 1% silicic acid up to spray runoff, applied in the 4th and 12th day after the planting of seedlings, 3) control.

Free-choice test for oviposition preference and nymphal development

On the 16th day after planting, pruning and thinning of plants were performed, keeping two plants per pot (plants A and B), after this process thirty pots were selected and randomly distributed in 10 blocks (each block contained one pot from each treatment). Each block was individualized in PVC cages (50 x 50 x 70 cm) coated with organza fabric to prevent the escape of insects. At the same day, each cage was infested with 300 unsexed whitefly adults from the stock rearing, at the ratio of 100 insects per pot.

About 48 hours after infestation, the adults were removed from plants for egg number assessment. One plant (plant A) was cut from each pot, identified and brought to the laboratory for counting the number of eggs present on the abaxial side in five leaves of each plant, counted from the top of the plant. This work was carried out with the aid of a stereoscopic microscope, with magnification of 40X. The other chrysanthemum plant (Plant B) remained in the greenhouse for the assessment of the effect of silicon on nymphal stage development. Sixteen days after the infestation, 3rd and/or 4th nymphal stages were accounted on each plant following the same procedure used to assess the number of eggs.

No-choice test for oviposition preference and nymph development

The same procedures used in the previous test were used for this assay, except that only one pot was placed in each PVC cage. For infestation, 100 unsexed whitefly adults were released in each cage, at the ratio of 100 insects per pot. The number of eggs and 3rd and/or 4th nymphal stages were evaluated as the same way used for free-choice test.

Effects of silicon on the biological parameters of *B. tabaci* biotype B

Chrysanthemum plants used in the test were grown following the same procedure of previous tests. After 16 days of planting, six pots of each treatment were selected, which corresponded to 12 plants per treatment. Each pot was individualized in PVC cages and unsexed whitefly adults were released inside the cage at a ratio of 100 insects per pot.

About 24 hours after infestation, adults were removed and whitefly eggs were selected and isolated in micro-cages that were attached to the leaves, each plant contained two micro-cages with 10 eggs each. The micro-cages were made of transparent plastic discs (2.5 cm diameter x 2 cm), having one side covered with organza fabric and the edge of the other side covered with foam (3 mm thick) to prevent the escape of insects. The micro-cages were attached on plant leaves by means of an aluminum holder which had one of the handles attached to the plastic disc and the other to a plastic ring with diameter equal to that of the micro-cage (CORREIA et al., 2005).

Egg viability was assessed seven days after infestation by counting the number of eggs hatched on this day. Subsequently, only two nymphs were kept in each micro-cage and 20 days after counting the number of hatched eggs, the presence of nymphs and adults was measured. The nymphs were divided into two groups, nymphs of 1st and 2nd instar and nymphs of 3rd and 4th instar, characterized by the absence or presence of "red eyes", respectively, according to SALAS & MENDOZA (1995).

Effect of silicon on the vegetative development of chrysanthemum plants

At the end of the free-choice test, chrysanthemum plants were identified, weighed on a precision scales, placed individually in paper bags and transferred to an incubator at 60°C to constant weight. Then, the dry weight of each plant was determined and, after crushed in a Willy TE-648 grinder, 50-mesh sieve, samples were sent to the Laboratory of Fertilizers, Federal University of Uberlândia to determine the silicon percentage.

Statistical Analysis

Data were submitted to analysis of variance, and count data were transformed into $\sqrt{X+0,5}$ prior to analysis. Means were compared by Tukey test ($p \leq 0.05$).

Results and Discussion

In the free-choice test, no significant differences ($p > 0.05$) in the mean number of whitefly eggs between treatments were observed. However, silicon applications affect the development of whitefly nymphs of 3rd and/or 4th instar present in chrysanthemum plants. Plants that received silicon had fewer nymphs, compared with control (Table 1).

Some changes in the insect's development could be associated with modifications in the characteristics of plants caused by the use of silicon, resulting in effects on the feeding behavior and/or biology of insects. Studies have shown that resistance induction by silicon application can cause chemical changes in plants such as increased levels of tannins and lignin, which can alter the biology of insects (GOUSSAIN et al., 2005; GOMES et al., 2008; MORAES et al., 2009). Gatehouse (2002) reported that the defense of plants against herbivore insects can be expressed by a mechanical barrier (lignification or resin production) or through biochemical pathway, which may cause a deterrent effect on the feeding behavior of insects. In addition, the presence of silicon in plants can also change nutrient absorption by insects and this fact can affect their biology (REYNOLDS et al., 2009).

In Assis et al. (2013) silicon applied to sunflower changed the palatability of leaves, reducing the injury percentage and the leaf area consumed by the caterpillar *Chlosyne saundersii lacinia* (Lepidoptera: Nymphalidae). Jeongl et al. (2012) reported that colonies of aphids (*Macrosiphoniellas anborni*) were also reduced in silicon-treated chrysanthemum, showing 40-57% lower than those of control plants. In the no-choice test, no significant differences (p> 0.05) on oviposition and development of whitefly were observed (Table 2).

Table 1. Mean number of eggs and nymphs of 3rd and/or 4th instar per chrysanthemum leaf (± SE) of *Bemisia tabaci* biotype B in the free-choice test in chrysanthemum plants (24 ± 5ºC and 65 ± 10% RH).

Treatment	Number of eggs ns	Number of nymphs*
Si as soil drench	11.8 ± 5.00	2.9 ± 1.37 b
Si as foliar spray	15.5 ± 9.48	2.7 ± 2.13 b
Control	18.8 ± 10.80	4.8 ± 1.96 a

ns Means with no significant differences by the F test (p> 0.05). * Means followed by the same letter in column do not statistically differ by Tukey test (p ≤ 0.05).

In this test, foliar or soil silicon applications were probably insufficient to promote resistance in plants. In no-choice test, similar to this study, no significant differences in the number of eggs and nymphs of *B. tabaci* on soybean (MORAES et al., 2009), beans (PEIXOTO et al., 2011) and cucumber (CORREA et al., 2005) were observed in plants treated with silicon.

With other insects, similar results were obtained by Dogramaci et al. (2013) which reported that pepper plants treated with potassium silicate had not enough silicon on leaves to protect them against thrips *Scirtothrips dorsalis* (Thysanoptera: Thripidae). Also Hogendorp et al. (2010) observed that applications of up to 800 ppm of potassium silicate on poinsettia *Euphorbia pulcherrima* (Euphorbiaceae) did not affect whitefly development on this plant.

Among the biological parameters of whiteflies assessed only egg viability showed significant difference (Table 3). It was observed that the foliar silicon application reduced the viability of whitefly eggs around 20%, compared to soil application and control. According to BUCKNER et al. (2002), whitefly eggs receive water or water vapor from the plant via their pedicel which are inserted into leaves. Here we suggest that the female whiteflies faced some difficulties to insert the egg pedicel, probably due to the presence of silicon crystals on leaves. Consequently, eggs suffered dehydration and this fact may have caused the reduction of egg viability observed.

Table 2. Mean number of eggs and nymphs of 3rd and/or 4th instar per leaf (± SE) of *Bemisia tabaci* biotype B in the no-choice test in chrysanthemum plants (24 ± 5ºC and 65 ± 10% RH).

Treatment	Number of eggs ns	Number of nymphs ns
Si as soil drench	14.8 ± 8.30	6.2 ± 2.50
Si as foliar spray	17.8 ± 11.40	5.4 ± 2.76
Control	28.0 ± 15.41	4.0 ± 2.78

ns Means with no significant differences by the F test (p> 0.05).

Table 3. Viability (%) of eggs, nymphs and adults (± SE) in chrysanthemum plants treated with silicon (24 ± 5ºC and 65 ± 10% RH).

Treatment	Eggs	Nymphs of 1st and 2nd instar ns	Nymphs of 3rd and 4th instar ns	Adults ns
Si as soil drench	91.6±7.06 a	22.9±22.93	43.7± 3.38	33.3±27.0
Si as foliar spray	71.2±16.96 b	29.2±21.88	39.6±24.25	31.2±32.35
Control	93.0±5.06 a	14.6±9.40	37.5±19.36	47.9±21.53

ns Means with no significant differences by the F test (p> 0.05). * Means followed by the same letter in column do not statistically differ by Tukey test (p ≤ 0.05).

In this study, silicon applied as foliar spray was more effective in reducing the viability of whitefly eggs compared to silicon applied as soil drench, corroborating with Moraes et al. (2005), who found reductions of aphids *Rhopalosiphum maidis* (Hemiptera: Aphididae) in corn plants treated with two foliar silicon applications or one soil silicon application and an additional foliar silicon application.

There were no significant differences (p> 0.05) in the application of silicon in the soil or foliar silicon on whitefly nymphs and adults (Table 3). Similar results for nymphs and adults were observed by Alcantra (2010) when trials were conducted to evaluate the behavior of *Aphis gossypii* in cotton plants treated with silicon.

There were no significant differences (p> 0.05) in the production of fresh and dry matter and in the silicon content in chrysanthemum plants (Table 4). Some studies also have shown no effect of silicon application on the agricultural parameters of various plants such as fresh weight and dry weight of the shoots of plants, height, stem diameter, chlorophyll content and silicon content, for example, in wheat (COSTA et al., 2007), soybean (MORAES et al., 2009; FERREIRA et al., 2011) and sunflower (ASSIS et al., 2013).

It was observed that the silicon content present in chrysanthemum plants was low, between 0.20 to 0.22%, i.e., only a small amount of silicon (<0.5% v/v) accumulated on the plant tissue, both by foliar and soil application. Dogramaci et al. (2013) reported that the tissues of pepper plants that received soil silicon were able to absorb an amount of silicon by roots close to 2.5% (v/v); however, silicon was not translocated to leaf and stem tissues in equivalent standard.

Regarding the silicon content, the results of this study differed from studies carried out by Carvalho-Zanão et al. (2012), who studied chrysanthemum plants cultivars Coral Charm, White Reagan and Indianapolis, and observed a greater amount of silicon (from 2.5 to 2.8%) in plants treated with silicon. However, the absence of significant differences in this study with cv. Rage do not mean that chrysanthemum plants can not respond to silicon fertilization. Silicon absorption by plants may vary depending on the genotype and even in relation to the amount, way of application and period between silicon application and evaluation (MA et al., 2007; PARRELLA et al., 2007).

Qualitative characteristics, number and diameter of buds, stem length, among other parameters, were not evaluated. However, in study carried out by Carvalho-Zanão et al. (2012), silicon applications did not change these characteristics. Ribeiro et al. (2006) also found no effect of silicon application on growth and production of chrysanthemum cv. Rage.

Table 4. Fresh and dry weight of the shoots (± SE) and silicon content in chrysanthemum plants.

Treatment	Green weight (g)^{ns}	Dry weight (g)^{ns}	Silicon (%)^{ns}
Si as soil drench	7.9±2.54	1.6±0.41	0.20
Si as foliar spray	9.2±2.86	1.9±0.50	0.22
Control	7.8±2.63	1.6±0.47	0.24

^{ns} Means with no significant differences by the F test (p> 0.05).

In some cases, silicon applied to ornamental plants can contribute to quality aspects (PARRELLA et al., 2007; HOGENDORP et al., 2010; LOCARNO et al., 2011); nevertheless, studies should be carried out to clarify the development of different species in relation to the amount and source of silicon used as fertilizer. According to Reynolds et al. (2009), the presence of silicon provides resistance to plants, which may be related to the formation of a mechanical barrier that interferes with the performance of insects that feed on these plants. However, according to the authors, there are still difficulties in measuring these changes, and therefore, further studies are needed to elucidate the potential use of silicon against insects in the induction resistance context.

In general, the results obtained here for the free-choice test showed that silicon applied to chrysanthemum can affect the development of nymphs of *B. tabaci* and, besides this, foliar can negatively affect the viability of the whitefly eggs. From this point, silicon has the potential to reduce the presence of pests in chrysanthemum plants. However, more research is necessary to understand the feasibility of this tool as a complement in integrated management to *B. tabaci* when this pest is associated with chrysanthemum culture.

Conclusions

Soil or foliar silicon applications in chrysanthemum plants can affects the development of nymphs of *B. tabaci* biotype B in free-choice test.

In no-choice test, silicon does not affect oviposition and development of nymphs of *B. tabaci* biotype B in chrysanthemum plants.

Silicon applied as foliar spray can reduce the viability of whitefly eggs in chrysanthemum plants.

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