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Biological aspects of the two-spotted spider mite on strawberry plants under silicon application

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ABSTRACT

Silicon is an inducer of plant resistance to arthropod pests, being a promising strategy for integrated management. The aim of this study was to evaluate the effect of silicon on biological, reproductive and population aspects of parental and F1 generations of the two-spotted spider mite on strawberry plants. Potassium silicate, nanosilica and water were applied to the plants. Two-spotted spider mite females were confined to strawberry leaf disks for oviposition and, after hatching, larvae were observed until the emergence of adults. Once adults had been obtained, couples were formed in order to evaluate pre-oviposition, oviposition, longevity and fertility, with an estimated net reproduction rate, intrinsic rate of increase, finite rate of increase and the necessary time for doubling the generation. Silicon prolonged the duration of some immature stages of the mites in parental and F1 generations, did not affect the duration of the whole biological cycle, though. The periods of pre-oviposition, oviposition and longevity of the parental generation and the longevity and oviposition of F1 generation of the two-spotted spider mite were negatively affected by potassium silicate and nanosilica. The population parameters of the parental generation of the mites indicated that nanosilica is able to lead to a long-run decrease of this pest population.

Keywords: *Tetranychus urticae*, nanosilica, induced resistance, potassium silicate.

RESUMO

Aspectos biológicos do ácaro-rajado em morangueiro sob aplicação de silício

O silício é indutor de resistência de plantas a artrópodes pragas, constituindo-se em promissora estratégia para o manejo integrado. O trabalho objetivou avaliar o efeito do silício nos aspectos biológicos, reprodutivos e populacionais do ácaro-rajado nas gerações parental e F1 em morangueiro. As plantas receberam aplicações de silicato de potássio, nanossílica e água. Fêmeas do ácaro-rajado foram confinadas sobre os discos foliares de morangueiro para oviposição e, após a eclosão, larvas foram observadas até a emergência dos adultos. Destes, casais foram formados para avaliação da pré-oviposição, oviposição, longevidade e fecundidade, com estimativa da taxa líquida de reprodução, taxa intrínseca de aumento, razão finita de aumento e tempo médio de geração. O silício prolongou a duração de algumas fases imaturas do ácaro-rajado nas gerações parental e F1, mas não afetou a duração total do ciclo de desenvolvimento da praga. Os períodos de pré-oviposição, oviposição e longevidade da geração parental e a longevidade e fecundidade da geração F1 do ácaro-rajado foram afetados negativamente pelo silicato de potássio e nanosilicato. Os parâmetros populacionais da geração parental do ácaro-rajado indicam que a nanossílica pode diminuir a população desta praga a longo prazo.

Palavras-chave: *Tetranychus urticae*, nanossílica, resistência induzida, silicato de potássio.

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Silicon (Si) has been one of the most studied chemical elements for plant disease resistance against arthropods (Reynolds *et al.*, 2016; Catalani *et al.*, 2017). Among the factors which promote resistance induction is an increase in photosynthetic rate and mechanical resistance of cells (Moraes *et al.*, 2005), an increased production of allelochemicals as well as favoring biological control agents (Reynolds *et al.*, 2016). In addition to these mechanisms, there are some hypotheses which relate silicon as a promoter of increased leaf pubescence (Reynolds *et al.*, 2016).

Although plenty of studies, on the effects of silicon on pest biology and on the induction of resistance in plants, can be found in literature, few studies had focused on the effects of Si on mites (Gatarayiha *et al.*, 2010; Sadeghi *et al.*, 2016; Catalani *et al.*, 2017).

Some studies on the effects of silicon on disease management, in nutrition and fruit organoleptic traits of strawberry plants were carried out (Igarashi, 2008; Braga *et al.*, 2009; Carré-Missio *et al.*, 2010); however, few studies are on induction of strawberry plant's resistance to two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae) (Favaro *et al.*, 2019).

Silicon can be used in integrated pest management to control mites, since it does not interfere with the action of other control methods. Gatarayira, *et al.* (2010), in a greenhouse, verified that conidia of fungus *Beauveria bassiana*,

added to potassium silicate, were efficient in the management of the twospotted spider mite on corn. Sadeghi *et al.* (2016) verified that silicon affects negatively population and reproductive parameters of two-spotted spider mites on bean plants.

The high production of *Fragaria X* ananassa strawberries obtained in the Chapada Diamantina region has aroused increasing interest in the crop, increasing growth and expansion potential of this agricultural activity (SEBRAE, 2017). This region is considered one of the main producing regions in the Northeast part of Brazil, harvest reaching 416 thousand tons and productivity of 40 t ha⁻¹ (SEBRAE, 2017), exceeding the national average of 36.1 t ha⁻¹ (Fagherazzi *et al.*, 2017).

However, an arthropod complex which occurs in strawberry cultivation can compromise this productivity. Among them, the two-spotted spider mite stands out as the main pest of strawberry, because it promotes the appearance of chlorosis, loss of vigor, defoliation and wilting of plants, resulting in losses during production (Lourenção *et al.*, 2000; Bortolozzo *et al.*, 2007).

Thus, the aim of this study was to evaluate the effect of silicon on biological, reproductive and population aspects of two-spotted spider mite of parental and F1 generations, using two sources of this element, aiming to improve integrated pest management for strawberry crop.

MATERIAL AND METHODS

The studies were carried out from May to August, 2018, in Laboratório de Entomologia da Universidade Estadual do Sudoeste da Bahia (Entomology Laboratory of the State University of Southwest Bahia), $25^{\circ}C\pm 2$ temperature, relative humidity $70\%\pm10$ and 12-h photophase, using strawberry plants cv. Mojave and two-spotted spider mites *T. urticae*.

The plants were kept in 5-L pots, with substrate composed of soil, sand and goat manure in a 2:1:1 (v/v/v) ratio, under plastic house conditions.

The specimens of *T. urticae* used in the bioassays were obtained from a stock-culture started with individuals collected from commercial strawberry planting in Chapada Diamantina-BA region, being conducted in common bean plants (*Phaseolus vulgaris*), cultivated in 20-L pots in a plastic house. The plants were monitored daily in order to avoid being contaminated by other phytophagous and predatory mites.

We applied three solutions containing 32 mol L⁻¹ of Si in the form of potassium silicate and nanosilica, and deionized water (Si-free control), in 15 pots for each type of solution. The first application was done at the beginning of flowering of the strawberry crop and the other applications were every 10 days, applying 6.9 mL per plant, covering the total area in order to guarantee the product absorption. The sprayed leaves were marked to ensure the use of the leaves which received the three applications. For treatment applications, we used a compression spraver (1.25-L tank capacity), continuous jet, Guaranv®.

Assay using parental generation

An assay was installed in a completely randomized design, with three treatments and 50 replicates, totalizing 150 plots. The treatments consisted of leaves submitted to applications of two Si sources, potassium silicate and nanosilica, and deionized water (Sifree control). Each replicate consisted of leaf disks (2-cm diameter) obtained from 20 marked leaves (submitted to treatments) and collected from the plants 10 days after the last application. The leaves were selected in order to obtain standardization related to physiological age and position on the plant, seeking, thus, homogeneous conditions for mite development.

Two-spotted spider mite adult females from the stock-breeding were confined in Petri dishes 6.0 cm diameter, containing a leaf disk, previously washed and dried, which was pinned into the center of the dish with the aid of hot glue, one female per dish. The authors added water between the dish base and leaf disk to ensure the viability of the plant material and prevent the escape of mites. Water was replenished whenever necessary.

After being confined, the female mites were observed in order to verify the presence of eggs and, once laying was confirmed, two eggs were kept on each leaf and the female was removed to prevent further laying. Those females which had still not laid any egg were observed every four hours until all dishes had one or two eggs. After hatching, only one larva per leaf was maintained, being monitored in all mobile and quiescent stages until adulthood.

Evaluations were done twice a day, in a 12-h interval, until the mites reached adulthood, observing duration and viability of egg, larva, protochrysalis, protonymph, deutochrysalis, deutonymph, telochrysalis and adult phases.

Upon reaching the adult stage, the mites were sexed, transferring each mite to a new experimental unit and forming couples, for which it was necessary to use some males from the stock-breeding of the corresponding treatment. The pre-oviposition, oviposition, fecundity, fertility and longevity periods were determined for each treatment. Data referring to males from stock-breeding and mites that died on cotton (attempted escape) were not used in statistical analysis.

The computer program TWOSEX-MSChart de Chi (2020), available at http://140.120.197.173/ecology/ Download/Twosex-MSChart.rar, was used to analyze the raw data of development and reproduction, as well as for the calculation of population parameters of all individuals, using the "two-sex life table" procedure (Chi & Liu, 1985; Chi, 1988). The population parameters estimated were net reproduction rate (R0), intrinsic rate of increase (r); finite rate of increase (λ) and average generation time (T).

The standard error of data on development, fecundity, reproduction period and data on population parameters was estimated using the bootstrap method, following the procedure proposed by Huang & Chi (2012). During this procedure, the data for each of these biological parameters were re-sampled 100,000 times. Differences between treatments were compared using the paired bootstrap test based on the confidence interval of the differences (Efron & Tibshirani, 1993).

Assay using F1 generation

The experimental design was completely randomized, with three treatments and 50 replicates, totalizing 150 plots. The treatments were identical to the assay with parental generation.

Ten days after the last applications of the products, the leaves of each treatment were collected, standardized in the best possible way, according to physiological age and position on the plant, in order to ensure that the leaves had received the three applications, as well as identical conditions for mite development. Leaf disks identical to the ones used in the previous assay were made, and afterwards the authors deposited one female to each leaf disk.

After oviposition, the female was removed, leaving one or two eggs per dish. After hatching, only one larva was left per dish. The authors continued to observe all phases until adult emergence. After adult emergence, 50 females of each treatment were used (potassium silicate, nanosilica and control) in order to begin the bioassay using the F1 generation, obtained from replicates of the previous bioassay.

The disks were put in Petri dishes, according to the procedure described in the previous item, and each leaf disk had a female mite on it. From then on, the same procedures adopted for parental generation were used for conducting and evaluating the assay and statistical analysis.

RESULTS AND DISCUSSION

Parental generation

No significant differences for eggs, larva, protochrysalis, protonymph, deutochrysalis and total cycle were noticed; considering males and females, no significant difference for mortality rate was also verified. Deutonymph stage was lower in disks which received potassium silicate and teleiochrysalis stage was lower in control and under nano-silica treatment (Table 1). The results found in this study corroborate the values obtained by Sadeghi *et al.* (2016), who noticed that Si altered development time of immature stages of *T. urticae* on bean plants.

Several studies have been showing that silicon affects the initial phases of sucking insects, such as *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae), on bean plants grown in Si-treated soil (Gomes *et al.*, 2008). Dalastra *et al.* (2011) verified that Si reduced the population density of silver thrips nymphs, *Enneothripes flavens* (Thysanoptera: Thripidae), on peanut plants, providing plant protection and increasing crop productivity.

Pre-oviposition, oviposition days and longevity were shorter in potassium silicate treatment compared with the control; oviposition periods and longevity did not differ in relation to the sources of potassium silicate and nanosilica (Table 2). These results are not in accordance with those obtained by Catalani et al. (2017), who observed that silicon did not affect the survival and oviposition of the two-spotted spider mite females in papaya plants. On the other hand, Sadeghi et al. (2016) verified that Si application reduced oviposition period and longevity, both for female and male, on bean plants.

The net reproduction and intrinsic rates and the finite rate of increase were

lower in nanosilica-treated strawberry plants compared with the control, with no significant differences for these variables between silicon sources. Despite that, potassium silicate provided less time to double the mite population (Table 2).

These results, in practical terms, would indicate a favoring for the mite; however, the lower intrinsic rate of increase represented by nanosilica treatment may suggest that plants treated with Si from this source did not demonstrate adequate conditions for the best performance of the mite population (Birch, 1948; Mottaghinia et al., 2011). The finite rate of increase is a parameter that represents a population multiplication factor within a time interval (Moro et al., 2012); therefore, Si-treatments would decrease the reproductive potential of the twospotted spider mite.

The values of the net reproduction rate indicated harmful effects of the nanosilica on the two-spotted spider mite populations. Catalani *et al.* (2017) verified that potassium silicate applications provided a decrease in net reproduction rate of these mites on papaya plants compared with the control.

The average generation time using the nanosilica source, even being equal to the control, is longer than with potassium silicate, which allows

Table 1. Average and standard deviation of immature stage duration (days) of the parental generation of *Tetranychus urticae* on Si-treated strawberry plants. Vitória da Conquista, UESB, 2019.

Treatment	Potassium silicate	Nanosilica	Control
Egg	$5.00 \pm 0.05 \text{ a} (60)$	4.97± 0.03 a (60)	$5.08 \pm 0.04 \ a \ (59)$
Larva	$0.93 \pm 0.05 \; a \; (59)$	$0.93 \pm 0.06 a (60)$	$0.91 \pm 0.03 \ a \ (58)$
Protochrysalis	0.92 ± 0.03 a (59)	$0.96 \pm 0.03 \text{ a} (59)$	$0.95 \pm 0.03 \ a \ (58)$
Protonymph	0.68 ± 0.03 a (59)	0.76 ± 0.03 b (59)	$0.85 \pm 0.04 \ a \ (58)$
Deutochrysalis	$0.79 \pm 0.04 \text{ a} (56)$	$0.86 \pm 0.03 \ a \ (59)$	$0.85 \pm 0.04 \ a \ (58)$
Deutonymph	$0.87 \pm 0.05 \ b \ (56)$	$1.16 \pm 0.04 \text{ a} (58)$	1.08 ±0.06 a (58)
Teleiochrysalis	$1.25 \pm 0.05 \text{ a} (55)$	$0.99 \pm 0.07 \text{ b} (57)$	1.03 ± 0.05 b (57)
Females	10.53± 0.17 a (43)	$10.73 \pm 0.08 \text{ a} (35)$	$10.84 \pm 0.06 \text{ a} (41)$
Males	$10.21 \pm 0.26 a(12)$	$10.34 \pm 0.13 \text{ a}(22)$	$10.53 \pm 0.25 a (16)$
Mortality rate	$0.08\pm0.03\ a$	$0.05\pm0.02\ a$	$0.03 \ \pm 0.02 \ a$

Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 100,000 samples and means were compared by using paired bootstrap test at 5% significance level.

Table 2. Average and standard deviation of reproductive parameters (duration of preoviposition, oviposition, longevity, daily fecundity and fecundity) and population (R_0 = net reproduction rate; r = intrinsic rate of increase; λ = finite rate of increase; T = average generation time of the parental generation of *Tetranychus urticae* on Si-treated strawberry plants. Vitória da Conquista, UESB, 2019.

Treatment	Potassium silicate	Nanosilica	Control
Pre-oviposition (days)	$0.13\pm0.03\ b$	$0.27\pm0.04\;a$	$0.20\pm0.05~a$
Oviposition (days)	$10.66\pm0.16\ b$	$11.00\pm0.10\ ab$	$11.04\pm0.07\;a$
Longevity (days)	$18.36\pm0.51\ b$	$19.54\pm0.52\ ab$	$20.16\pm0.47~a$
Daily fecundity (eggs/day)	$6.81\pm0.43~a$	$7.37\pm0.45\;a$	$7.78\pm0.48\;a$
Eggs/female	$95.26\pm6.80\ a$	$100.71\pm 6.88~a$	$118.59\pm8.41\ a$
R ₀	$68.260\pm7.33~ab$	$58.750 \pm 7.55 \; b$	$82.400 \pm 9.14 \ a$
r (days ⁻¹)	$0.306\pm0.007\ ab$	$0.283\pm0.009\ b$	$0.301 \pm 0.007 \; a$
λ (days ⁻¹)	$1.359\pm0.01\ ab$	$1.327\pm0.01\ b$	$1.352\pm0.009\ a$
T (days ⁻¹)	$13.760\pm0.19\ b$	$14.390\pm0.15\ a$	$14.610\pm0.10\ a$

Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 100,000 samples and means were compared by using paired bootstrap test at 5% significance level.

Table 3. Average and standard deviation of immature stage duration of F1 generation (days) of *Tetranychus urticae* on strawberry plants treated with two Si-sources. Vitória da Conquista, UESB, 2019.

Treatment	Potassium silicate	Nanosilica	Control
Egg	$3.99 \pm 0.04 \ b(47)$	$4.13 \pm 0.04 \ a \ (47)$	$4.06 \pm 0.03 \text{ ab} (49)$
Larva	$1.21 \pm 0.06 \text{ ab} (47)$	$1.36 \pm 0.07 \text{ a} (46)$	$1.06 \pm 0.05 \ b \ (47)$
Protochrysalis	$1.15 \pm 0.06 \text{ a} (46)$	1.04 ± 0.04 a (46)	$1.07 \pm 0.04 \ a \ (44)$
Protonymph	$0.97 \pm 0.05 \ a \ (46)$	0.92 ± 0.04 a (46)	0.92 ± 0.04 a (44)
Deutochrysalis	$1.10 \pm 0.05 \ a \ (45)$	$1.01 \pm 0.05 \text{ a} (46)$	$1.08 \pm 0.04 \ a \ (44)$
Deutonymph	$1.24 \pm 0.05 \ a \ (44)$	$0.96 \pm 0.05 \ b \ (45)$	0.88 ±0.065 b (43)
Teleiochrysalis	$1.09 \pm 0.06 \ b \ (44)$	$1.16 \pm 0.05 \ b \ (45)$	$1.40 \pm 0.06 \ a \ (43)$
Females	$10.88 \pm 0.16 \text{ a} (30)$	10.83 ± 0.11 a (27)	10.60 ± 0.11 a (29)
Males	$10.39 \pm 0.19 \text{ a} (14)$	$10.17 \pm 0.10 \text{ a}(18)$	$10.21 \ \pm 0.19 \ a \ (14)$
Mortality rate	$0.06\pm0.03a$	$0.04\pm0.02a$	$0.14 \ \pm 0.04 \ a$

Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 100,000 samples and means were compared by using paired bootstrap test at 5% significance level.

the authors to state that nanosilica application to strawberry plants has effects on the parental generation of *T. urticae* compared to untreated plants.

F1 generation

The duration of egg phase was longer in nanosilica-treated plants compared with plants treated with potassium silicate and this larva phase duration was also longer in nanosilica-treated plants in relation to the control. No significant differences in the development of immature stages of protochrysalis, protonymph and deutochrysalis were noticed; duration of teleiochrysalis stage was longer in plants which did not receive silicon application. The treatments did not affect the adult emergence period and mortality in immature stages of the two-spotted spider mites (Table 3).

The stiffening of the cell wall caused by silicon may have hampered to feed immature phases of the pest (Moraes *et al.*, 2005) and/or increased leaf pubescence, which, in theory, would decrease the movement of immature

phases on the leaves of plants (Reynolds et al., 2016). A second hypothesis that can be raised is that silicon could have promoted an increase in the production of phenolic compounds in plant defense (Frew et al., 2016; Reynolds et al., 2016). Silicon hampers the arthropod's feeding and decreases palatability and digestibility of plants (Massey & Hartley, 2009; Moraes et al., 2005); so, it could be expected that the life cycle of F1 generation mites could be extended, because the parental females which were on the plants, that received Si-applications, would have inferior nutritional conditions than the control females, showing likely negative impact on their progenies. In addition, the immature phases of the mite would have greater difficulty in breaking the physical barriers created by silicon. The first hypothesis seems to be supported by the longer time of the larva phase in nanosilica treatment, however, without effects on its total development period.

Pre-oviposition and oviposition did not differ among the treatments. Daily fertility and longevity of two-spotted female spider mite were longer for the control and fertility was shorter for plants which received nanosilica applications. All population parameters of the two-spotted spider mites were higher in the control compared to the nanosilica, not differing from the plants which received potassium silicate applications (Table 4).

No study relating effects of silicon on two-spotted spider mites of F1 generation was found. However, considering the parental generation, Sadeghi *et al.* (2016) verified that all silicon concentrations used, from 1.0 to 2.0 ppm, affected negatively all population parameters of two-spotted spider mite on bean plants. Catalani *et al.* (2017) verified that, among the population parameters, net reproduction rate was the most affected, and the lowest values were noticed where silicon was applied.

The small number of studies on effects of silicon on biological aspects of two-spotted spider mites, both on parental and F1 generations, showed that more studies are necessary in order to discuss pertinent issues, such **Table 4.** Average and standard deviation of reproductive parameters (periods of preoviposition, oviposition, longevity, daily fecundity and fertility) and population rate (R_0 = net reproduction rate; r = intrinsic rate of increase; λ = finite rate of increase; T = average generation time) of the F1 generation of *Tetranychus urticae* on strawberry plants treated with two sources of silicon. Vitória da Conquista, UESB, 2019.

Treatment	Potassium silicate	Nanosilica	Control
Pre-oviposition (days)	$0.07\pm0.03~a$	$0.04\pm0.04\;a$	$0.05\pm0.04\;a$
Oviposition days	$10.95\pm0.17\ a$	$10.87\pm0.12~a$	10.66 ± 0.13 a
Longevity (days)	$16.92\pm0.63\ b$	$16.80\pm0.42\ b$	$19.00\pm0.69\ a$
Daily fecundity (eggs/day)	$4.95\pm0.66\ b$	$4.67\pm0.33\ b$	6.86 ± 0.67 a
Fecundity (eggs/female)	60.86 ± 11.11 ab	$42.26\pm3.41\ b$	$88.76 \ \pm 10.43 \ a$
R ₀ (eggs/individual)	$38.290\pm8.12\ ab$	$24.270\pm3.60\ b$	51.480 ± 8.60 a
r (days ⁻¹)	0.260 ± 0.014 ab	$0.242\pm0.011\ b$	$0.279\pm0.011~\text{a}$
λ (days ⁻¹)	$1.297\pm0.018\ ab$	$1.274\pm0.014\ b$	$1.321 \pm 0.015 \; a$
T (days ⁻¹)	$13.980 \pm 0.27 \; a$	$13.130\pm0.14\ b$	$14.120\pm0.21\ a$

Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 100,000 samples and means were compared by using paired bootstrap test at 5% significance level.

as, silicon sources, how to apply this chemical element and how to keep plant resistance.

In general, three silicon applications sprayed on the leaves, using the two sources mentioned in this study, affected negatively some biological and population parameters of the two-spotted spider mites. The authors concluded that an alternate use of these compounds may result in a decrease in subsequent populations of the twospotted spider mites on strawberry plants.

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