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ORIGINAL ARTICLE

Life table and fertility rate of *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae) in tomato with silicon

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Abstract

The miner fly *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae) is an insect of economic importance for tomato culture. The conventional control with insecticides is complex due to the mining eating habit that provides protection to the larvae inside the leaves. Therefore, farmers can opt for biological control agents, or substances that provide protection to the plant. Thus, the objective of our research was to evaluate the use of silicon to induce resistance in tomato plants against *L. sativae*. The results showed that in tomato plants treated with SiO_2/F and K_2SiO_3/F there was a reduction in the net reproduction rate (*Ro*), in the intrinsic rate of increase in number (r_m), in the finite rate of increase (λ), in the average interval between generations (*IMG*), in the doubling time (*TD*), in the number of eggs/female/day and the accumulated egg laying of F1 females of *L. sativae*. The products SiO_2/F and K_2SiO_3/F gave the tomato a protective effect against injuries caused by *L. sativae*.

Keywords: biology study, leafminer, pest management, silicon, tomato

Introduction

The miner fly *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae) causes serious damage to agricultural ecosystems. When the larvae of *L. sativae* develop and open galleries in the leaf mesophile, they cause a reduction in the photosynthetic area, and allow the entry of primarily pathogenic microorganisms into the tomato culture (Musundire *et al.* 2012; Araujo *et al.* 2013).

The injuries caused by *Liriomyza* spp. in tomatoes can compromise up to 65% of the leaves (Pratissoli *et al.* 2015). To reduce the attack of *Liriomyza*, in addition to synthetic insecticides, farmers can use biological control agents such as predators (Pohl *et al.* 2012), parasitoids (Foba *et al.* 2015), and entomopathogenic microorganisms (Migiro *et al.* 2011), as well as substances that induce plant resistance (Vieira *et al.* 2016).

Among the inductors of plant resistance to insects, silicon has been used to control several pests in different cultures, including *Scirpophaga incertulas* in rice (Jeer *et al.* 2017), *Spodoptera frugiperda* in corn (Goussain *et al.* 2002), *Schizaphis graminum* in wheat (Gomes *et al.* 2005), *Eldana saccharina* in sugar cane (Kvedaras *et al.* 2009), *Diabrotica speciosa* and *Liriomyz*a spp. in potatoes (Gomes *et al.* 2009), *Aleurocanthus woglumi* in tangerines (Vieira *et al.* 2016), and *Tuta absoluta* in tomatoes (Santos *et al.* 2015). In this sense, silicon is classified as a beneficial element for higher plants, as it promotes defense against pest attacks, and increases productivity as well as resistance to abiotic stress. The present work aimed to evaluate the induction of silicon resistance in tomato plants against *L. sativae*.

Table 1. Details of silicon sources used in the study

Treatment[1]	Substance[2]	Form of application	Recommendation[3]/Concentration of Si soluble
SiO_2/F	SiO_2 + Tween 80[1]	foliar spray	1.18 g · l^{-1} (0.01% solution)/6.5%
SiO_2/S	SiO_2	soil drench	1.18 g · l^{-1} (0.01% solution)/6.5%
K_2SiO_3/F	K_2SiO_3 + Tween 80[1]	foliar spray	20 ml · l^{-1} (2.0 % solution)/5.9%
K_2SiO_3/S	K_2SiO_3	soil drench	20 ml · l^{-1} (2.0 % solution)/5.9%
Control/F	distilled water + Tween 80[1]	foliar spray	–

[1]F – foliar spray, S – soil drench; [2]products used – SiO_2 Agri Sil®, K_2SiO_3 Chelal Si®, nonionic surfactant (Polysorbate) in 0.01% of the solution; [3]dose recommended for tomato culture by manufacturer

Materials and Methods

The experiments were carried out in the of Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário de Pragas e Doenças (NUDEMAFI), at the Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo (CCAE-UFES) in Alegre, Espírito Santo, Brazil.

The adults of *L. sativae* were obtained from the creation of NUDEMAFI stock, based on the methodology described by Araujo *et al.* (2007), raised on cotyledon leaves of pork beans *Canavalia ensiformis* in a protected environment [25.0 ± 1.0°C, relative humidity (RH) of 60 ± 10% and photophase of 12 h].

Cultivation of tomato plants and application of silicon

Commercial tomato seedlings, *Solanum lycopersicum* variety Alambra F1 were produced in 200-cell seed trays containing commercial substrate for Bioplant® kept in a greenhouse, with temperatures ranging from 27.0 ± 1.0 to 32.0 ± 1.0°C, and 70 ± 10% RH. Irrigation was carried out manually and daily, with each plant receiving the same amount of water during irrigation. At 21 days after planting, the seedlings were transferred to closed-bottom pots with 2 dm^3 of soil. Fertilization was performed based on the fertilization recommendations proposed by Novaes *et al.* (1991), with 50% of the dose applied during transplantation and 50% 15 days after transplantation. Nitrogen was supplied at a dose of 139.85 mg · kg^{-1} of soil in the form of $(NH_4)_2SO_4$. Potassium and phosphorus were applied at doses of 115.46 and 91.49 mg · kg^{-1} of soil, respectively, in the form of KH_2PO_4.

At 35 days after transplanting, the plants were transferred to an air-conditioned room under conditions of 25.0 ± 1.0°C, 60 ± 10% RH and 12 h photophase with 40 W and 2,700 lm fluorescent tubes. In view of the role of draining soluble solids from the tomato fruits, the inflorescences were removed to maintain the growing season.

At the end of the acclimatization period (40 days after transplanting) the plants received two commercial products based on silicon: Agrisil Sil® and Chelal Si® in two forms of application, foliar and soil drench. The control was represented by a single leaf application of distilled water. For each treatment used and the control group, four repetitions were performed. However, for the application of the products, the commercial indication for tomato culture was followed (Table 1).

The tests were carried out in two tomato batches (1st and 2nd batch) with an interval of 17 days. The treatments described in Table 1 were applied only once in both batches.

Obtaining the parental generation (P)

Parental generation (P) of *L. sativae,* from the laboratory stock, was obtained from plants treated with silicon (1st batch of tomatoes). First generation individuals were also grown on silicon plants (2nd batch of tomatoes) to evaluate the effects of silicon treatments on the biology of liriomyza for two generations. Only insects of the of *L. sativae* were considered in this study to evaluate the d effect of cultivation with silicon. To carry out the experiment, 10 mating pairs of *L. sativae* were considered. The mating pairs were confined in microtubes (10 ml) for mating for 24 h, and kept in a climatic chamber (25.0 ± 1.0°C, 60 ± 10% RH and photophase 12 h).

After that period, the *L. sativae* couples were confined in cages (22 × 15 cm) made with non-woven fabric (TNT). One day after the treatments were applied, oviposition was allowed for 24 h in two complete sheets of the upper middle third of the tomato plants containing silicon, 1° stage (Fig. 1).

Effect of silicon on first generation

In order to obtain F1 offspring, adults of generation P that grew in the first batch of tomato plants with silicon were confined for mating for 24 h. The survivors were confined in polypropylene microtubes (1.5 ml) with four mating pairs/plant, and four plants per treatment/group. The microtubes were kept in a climatic chamber at 25.0 ± 1.0°C, 60 ± 10% RH and photophase of 12 h. After this period, the first generation mating

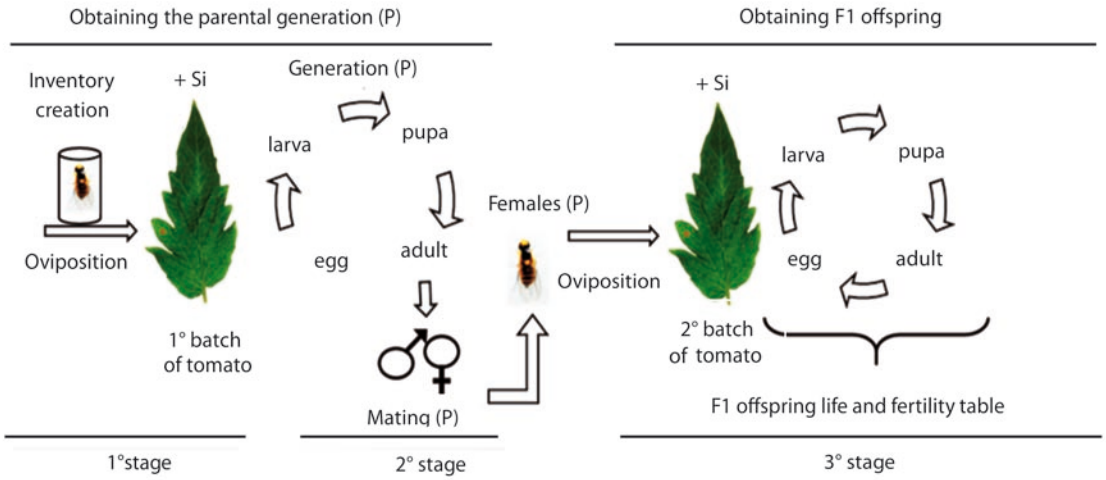

Fig. 1. Diagram of obtaining *Liriomyza sativae* generations in tomato plants subjected to silicon application

pairs were grouped for food and oviposition of the 2nd batch of tomato with silicon (Fig. 1).

After 24 h of oviposition, the P couples were removed. In the 2nd stage, the eggs deposited in the 2nd batch of tomatoes, which constituted the first generation, were counted with the aid of a diascopic LED light coupled to a stereoscope. From the data of the biological parameters of *L. sativae*, such as duration and viability of each stage: egg, larva, pupa and adult, sex ratio (*sr* = number of females/number of females + number of males), longevity of adults and fertility of females that grew consecutively in silicon-containing plants for two generations, and the fertility life table (FVT) of the populations were calculated.

To analyze the fertility of first generation adults observations were made every 24 h, counting the number of eggs deposited in the leaf mesophyll of tomato leaves. During this test, in addition to the leaves, honey was provided daily to feed the mating pairs.

Biochemical analysis of tomato leaves

After the complete development of the immature stages of generation P *L. sativae* in tomato plants (1st cultivated lot), all plants with gall leafminer larvae were divided into two parts, damaged leaves (DL) and undamaged leaves (UL) (Table 2). After dividing the plants, the samples were packed in aluminum foil envelopes and dehydrated at –55°C for 72 h in a bench-top freeze-dryer (Liotop® model l101) coupled to a vacuum pump set to 3.7 μHg.

Data analysis

The tests were arranged in a completely randomized design (DIC). To make comparisons between variables provided in the fertility life table (FVT), the jackknife estimate was used to generate the means and the Tukey test to determine the differences between the groups ($p < 0.05$) (Maia *et al.* 2000; Maia and Luiz 2006).

Table 2. Silicon sources

Treatment	Substance	Product [2]	Form of application
SiO_2/F/DL	SiO_2 + Tween 80[1]	Agri Sil®	foliar spray
SiO_2/F/UL	SiO_2 + Tween 80[1]	Agri Sil®	foliar spray
SiO_2/S/DL	SiO_2	Agri Sil®	soil drench
SiO_2/S/UL	SiO_2	Agri Sil®	soil drench
K_2SiO_3/F/DL	K_2SiO_3 + Tween 80[1]	Chelal Si®	foliar spray
K_2SiO_3/F/UL	K_2SiO_3 + Tween 80[1]	Chelal Si®	foliar spray
K_2SiO_3/S/DL	K_2SiO_3	Chelal Si®	soil drench
K_2SiO_3/S/UL	K_2SiO_3	Chelal Si®	soil drench
Control/F/DL	Distilled water + Tween 80[1]	–	foliar spray
Control/F/UL	Distilled water + Tween 80[1]	–	foliar spray

DL – leaves damaged by leafminer; UL – undamaged leaves; F – foliar spray; S – soil drench; [1]nonionic surfactant (Polysorbate) in 0.01% of the solution; [2]concentration recommended for tomato culture by manufacturer: Agri Sil 1.18 g · l^{-1} (0.01% of the solution), Chelal Si® 20 ml · l^{-1} (2.0% of the solution)

Table 3. Table of life and fertility of *Liriomyza sativae* offspring first generation in tomatoes subjected to the application of silicon

Treatment	R_o ± SEM	r_m ± SEM	λ ± SEM	*IMG* ± SEM	*TD* ± SEM
SiO_2/F	0.54 ± 0.03 b	–0.03 ± 0.00 c	0.97 ± 0.00 b	19.82 ± 0.04 b	–21.6 ± 2.62 c
SiO_2/S	6.95 ± 1.51 a	0.08 ± 0.01 a	1.08 ± 0.01 a	23.60 ± 0.74 a	5.56 ± 3.32 a
K_2SiO_3/F	0.04 ± 0.01 b	–0.16 ± 0.01 b	0.85 ± 0.01 bc	21.00 ± 0.30 b	–4.37 ± 0.41 b
K_2SiO_3/S	5.17 ± 2.18 ab	0.07 ± 0.02 a	1.08 ± 0.02 a	24.18 ± 0.41 a	8.71 ± 3.04 a
Control/F	7.07 ± 1.49 a	0.08 ± 0.01 a	1.09 ± 0.01 a	23.33 ± 0.79 a	8.10 ± 0.83 a

SEM – standard error of the mean; R_o – net reproduction rate; r_m – intrinsic rate of increase in number; λ – finite rate of increase; *IMG* – average time between generations; *TD* – population doubling time (days); SiO_2/F – Agri Sil® foliar spray 1.18 g · l^{-1}; SiO_2/S – Agri Sil® soil drench 1.18 g · l^{-1}; K_2SiO_3/F – Chelal Si® foliar spray 20 ml · l^{-1}; K_2SiO_3/S – Chelal Si® soil drench 20 ml · l^{-1}; control – distilled foliar spray water; temperature – 25.0 ± 1.0°C; relative humidity – 60 ± 10%; photophase – 12 h
Averages followed by the same letter in the same column do not differ by Tukey's test ($p < 0.05$)

The fertility observations were submitted to one way analysis of variance (ANOVA) and the means were compared by the Tukey test ($p < 0.05$).

Survival curves were calculated for F1 adults using the Kaplan-Meier (KM) estimator, the log-rank test, with the significance of paired comparisons adjusted to a treatment-wide level of alpha = 0.05 using the sequential Bonferroni adjustment to group the estimated curves (Colosimo and Giolo 2006).

For chlorophyll A (cla), chlorophyll B (clb), chlorophyll A + B (clt), chlorophyll A/B (clab) ratio, carotenoids (car), chlorophyll A + B/carotenoid ratio (clcar), and phenolic compounds total (fst), a double factor analysis was used, with factor 1 being the inducers and factor 2 being the presence of larvae in the tissues, subsequently subjected to ANOVA analysis of variance ($p < 0.05$). The residues were tested for normality with the Shapiro-Wilk test and homogeneity of variances with the Bartlett test for the variables cla, clb, clt, clab, car, clcar and the Samiuddin test for fst. The averages of clab, clcar were transformed into root (x)/1, and phenols (fst) were transformed into root (x) to meet the premises of the analysis of variance. The means were compared using the Scott-Knott test ($p < 0.05$). Subsequently, the data were submitted to Cluster Kant analysis to identify the treatments with the greatest similarity for the levels of chlorophylls A, B, carotenoids and total phenols found. The Mahalanobis distance (D2) was calculated and the cophenetic correlation was evaluated by the Mantel statistics for the UPGMA method. All analyzes were conducted using the R Development Core Team program (2010) with the aid of ExpDes.pt, vegan, exchange and cluster packages.

Results

After analyzing the data, the results showed a reduction in the net reproduction rate (R_o), in the intrinsic rate of increase in number (r_m), in the finite rate of increase (λ), in the average interval between generations (IMG) and in time duplication (TD) of first generation females of *L. sativae* that grew in tomato treated with SiO_2/F and K_2SiO_3/F ($p < 0.05$) (Table 3).

There was a reduction in the number of eggs/female/day and the accumulated egg laying ($p < 0.05$) of the first generation that developed in tomato plants treated with K_2SiO_3/F (Fig. 2).

The mean of survival probability of the first generation developed in tomato plants with SiO_2/F and K_2SiO_3/F was affected ($p < 0.05$), in comparison with the control. However, the survival of first generation males was not compromised by silicon ($p = 0.730$) (Fig. 3).

The interaction between the inducers and the presence of insects in the leaf tissues was not significant ($p < 0.05$). So, the simple effects were evaluated for chlorophyll A, chlorophyll B, total chlorophyll, carotenoids, chlorophyll/carotenoid ratio, chlorophyll ratio were evaluated A + B and total phenolic compounds (Table 4). Therefore, the SiO_2/F/UL treatment showed a difference the is control, chlorophyll B, carotenoids and total phenolic compounds. The same source of silicon, with the same form of application, however with the presence of damaged leaves SiO_2/F/DL differed control, chlorophyll and carotenoids.

In the cluster analysis, it can be noted that the SiO_2/F/UL treatment presented the greatest dissimilarity between treatments for biochemical levels. It can also be noted that SiO_2/F/UL and control/UL showed the greatest dissimilarity with SiO_2/F/DL and control/DL. The SiO_2/S/DL, K_2SiO_3/S/DL, K_2SiO_3/S/UL, K_2SiO_3/F/DL and SiO_2/S/DL treatments also differed from control/DL. Since the same plant (SiO_2/F/DL and SiO_2/F/UL) had dissimilar biochemical levels (only of chlorophyll B and carotenoids) due to the presence of insects in the tissues (Fig. 4).

Discussion

Changes in net reproduction rates, intrinsic rate of increase, finite rate of increase, average time between generations, population doubling time, survival time and fertility of female *L. sativae* may be related to plant

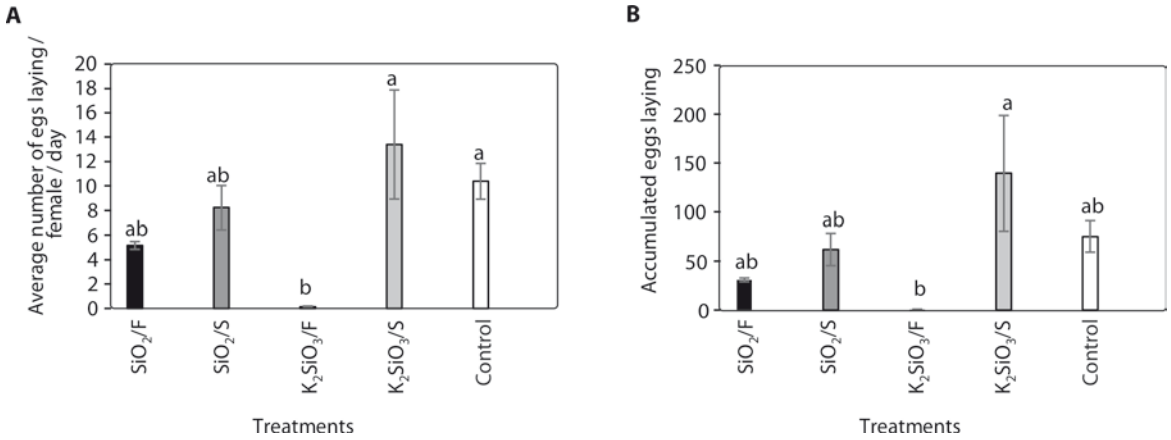

Fig. 2. Oviposition of *Liriomyza sativae* first generation developed in tomato plants submitted to silicon application. Average fecundity per day (A) and accumulated female posture until death (B). Bars followed by the same letter on the same graph do not differ by the Tukey test ($p < 0.05$); SiO_2/F – Agri Sil® foliar spray 1.18 g · l^{-1}; SiO_2/S – Agri Sil® soil drench 1.18 g · l^{-1}; K_2SiO_3/F – Chelal Si® foliar spray 20 ml · l^{-1}; K_2SiO_3/S – Chelal Si® soil drench 20 ml · l^{-1}; control – distilled water; temperature – 25.0 ± 1.0°C; relative humidity – 60 ± 10%; photophase – 12 h

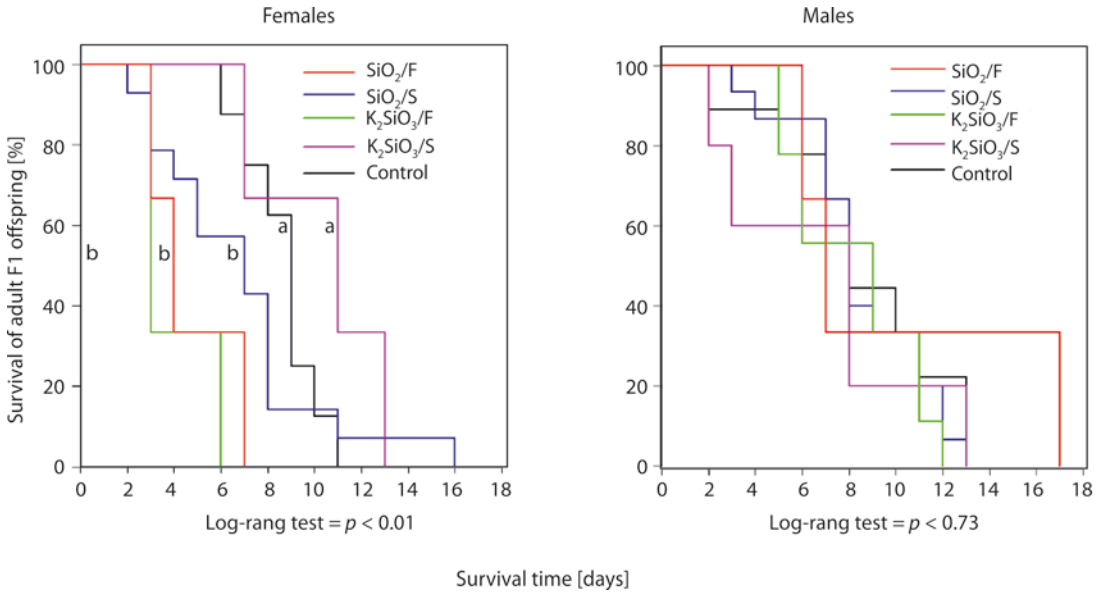

Fig. 3. Survival probability of adults of *Liriomyza sativae* first generation offspring grown in tomato plants subjected to the application of silicon. Different letters near the survival curves indicate a significant difference between the different treatments (survival analysis, log-rank test, with the significance of paired comparisons adjusted to a treatment-wide level of $\alpha = 0.05$ using the sequential Bonferroni adjustment). SiO_2/F – Agri Sil® foliar spray 1.18 g · l^{-1}; SiO_2/S – Agri Sil® soil drench 1.18 g · l^{-1}; K_2SiO_3/F – Chelal Si® foliar spray 20 ml · l^{-1}; K_2SiO_3/S – Chelal Si® soil drench 20 ml · l^{-1}; control – distilled water; temperature – 25.0 ± 1.0°C; relative humidity – 60 ± 10%; photophase – 12 h

nutritional quality of the host since the immature phases of insects need a greater amount of nutrients for adults to have high fertility and longevity (Awmack and Leather 2002).

Several studies have shown that insects that feed on plants that contain silicon have less longevity and low fertility. These factors are correlated with biochemical and morphological changes that silicon causes in plants. As a result of these changes insects cannot absorb the necessary nutrients, and therefore they tend to die before producing the expected fertility (Leather 1985). Changing the morphological and/or biochemical characteristics of plants can also change the behavior of insects and interfere with their biology (Goussain *et al.* 2005). It has been observed that higher levels of silicon in plants reduce digestibility, palatability of plant tissues, and increase the hardness of plant tissues (Reynolds *et al.* 2009). Furthermore, abrasiveness is increased, wearing down the mouth parts of the plants' insects. Consequently, their feeding and development is hindered or prevented (Coskun *et al.* 2019).

In addition to morphological defenses, the biochemical changes caused by silicon in plants increase the production of callose. Callose is a plant

Table 4. Average contents of chlorophyll A (cla), chlorophyll B (clb), chlorophyll A + B (clt), chlorophyll A/B ratio (clab), carotenoids (car), chlorophyll A + B/carotenoid ratio (clcar), and total phenolic compounds (fst) in tomato plants subjected to silicon application with and without injuries to *Liriomyza sativae* larvae

Treatments	Biochemical parameters						
	cla [mg · g^{-1}]	clb [mg · g^{-1}]	clt [mg · g^{-1}]	clab [ratio]	car [mg · g^{-1}]	clcar [ratio]	fst [mg · g^{-1}]
K_2SiO_3/F/DL	10.43 ns	3.16 b	13.59 ns	3.18 ns	1.66 c	7.99 ns	1.30 c
K_2SiO_3/F/UL	9.97 ns	3.73 b	13.70 ns	2.68 ns	2.03 c	6.76 ns	1.53 c
K_2SiO_3/S/DL	11.46 ns	3.42 b	14.87 ns	3.36 ns	1.89 c	8.01 ns	1.55 c
K_2SiO_3/S/UL	10.36 ns	3.29 b	13.64 ns	3.13 ns	1.89 c	7.11 ns	1.69 c
SiO_2/F/DL	13.27 ns	4.37 a	17.64 ns	3.02 ns	2.42 b	7.24 ns	1.55 c
SiO_2/F/UL	14.16 ns	4.72 a	18.88 ns	2.99 ns	2.72 a	6.93 ns	2.22 a
SiO_2/S/DL	10.59 ns	3.45 b	14.04 ns	3.07 ns	1.89 c	7.56 ns	1.46 c
SiO_2/S/UL	12.72 ns	3.51 b	16.23 ns	3.48 ns	1.88 c	8.35 ns	1.42 c
Control/DL	11.23 ns	3.77 b	15.00 ns	2.98 ns	2.17 c	7.08 ns	1.72 c
Control/UL	9.71 ns	3.07 b	12.78 ns	3.07 ns	1.76 c	7.09 ns	1.91 c
CV %	31.46	19.60	27.83	8.64	17.54	10.05	16.61

Means followed by the same letter in the same column do not differ ($p < 0.05$) by the Scott-Knott test; ns – do not differ from each other by the F test ($p < 0.05$); DL – leaves damaged by leafminer; UL – undamaged leaves; control – distilled water; SiO_2/F – Agri Sil® foliar spray 1.18 g · l^{-1}; SiO_2/S – Agri Sil® soil drench 1.18 g · l^{-1}; K_2SiO_3/F – Chelal Si® foliar spray 20 ml · l^{-1}; K_2SiO_3/S – Chelal Si® 20 ml · l^{-1}

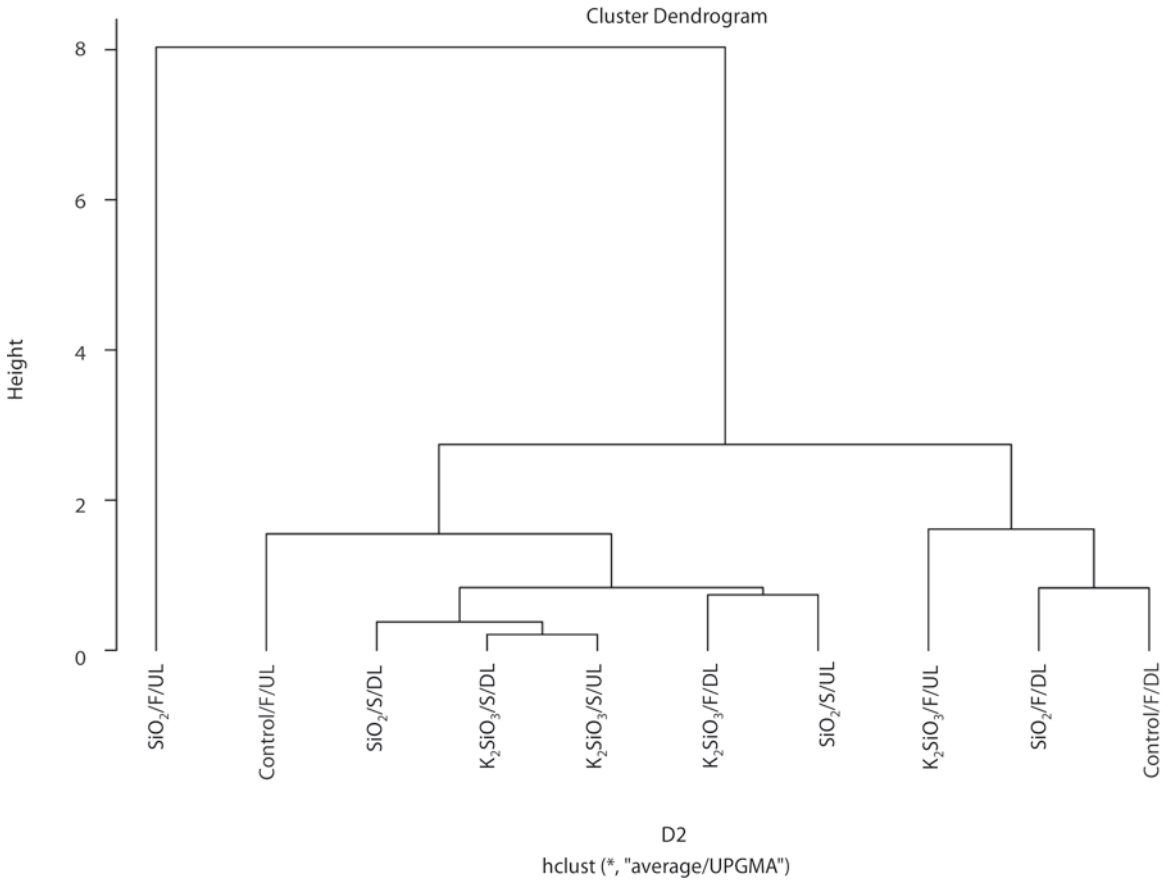

Fig. 4. Dendogram of tomato plants subjected to silicon application by the Mahalanobis distance (D2) as a function of silicon treatments, in the damaged (DL) and undamaged leaves (UL) of *Liriomyza sativae* larvae. Chlorophyll [mg · g^{-1}] contents A (cla), chlorophyll B (clb), chlorophyll A + B (clt), carotenoids (car), relation chlorophyll A + B/carotenoids (clcar), relation chlorophyll A/B (clab) in addition to total phenolic compounds (fst). Control – distilled water; SiO_2/F – Agri Sil® foliar spray 1.18 g · l^{-1}; SiO_2/S – Agri Sil® soil drench1.18 g · l^{-1}; K_2SiO_3/F – Chelal Si® foliar spray 20 ml · l^{-1}; K_2SiO_3/S – Chelal Si® 20 ml · l^{-1}

polysaccharide, produced in response to a stress or damage stimulus (herbivory) that acts as a cell wall. The callose arrangement prevents phloem transport and avoids the ingestion of sap by insects, interfering in their nutrition and biology (Hao *et al.* 2008). Also, the deposition of silicon on the membrane walls, trichomes, lumen and intercellular spaces acts as an abiotic elicitor of signs of systemic stress, mediated by phytohormone pathways, leading to the efficient synthesis of defensive compounds (Fauteux *et al.* 2005). These compounds contribute to the natural defense mechanisms of plants through the biosynthesis of phenolic compounds that interfere in the growth and development of phytophagous insects (Chérif *et al.* 1994).

Although the role of silicon in plants is not completely elucidated, it is known that it has low mobility in tomato plants and has direct and indirect effects on plant defense against herbivores (Alhousari and Greger 2018). In *Tuta absoluta* the ingestion of tomato leaves treated with silicon foliar application caused changes in the epithelial cells of the intestine of caterpillars and higher mortality of caterpillars than silicon applications via soil (Santos *et al.* 2015).

Another important point to be analyzed is that silicon was more efficient foliar application than systemic, because silicon is not very mobile inside the plant (Datnoff *et al.* 2001). The anti-food or protective effects mediated by silicon in the leaf tissues of plants that do not accumulate silicon or with low mobility such as tomato (Andrade *et al.* 2013) or with the supply via soil or application leaf in other plants classified as accumulators of silicon as rice, corn, wheat or sugar (Goussain *et al.* 2002; Gomes *et al.* 2009; Kvedaras *et al.* 2009; Jeer *et al.* 2016). In the tomato, silicon uptake and transport take place in the form of silicic acid that is regulated by transporters found in the plasma membrane, however in rice these transporters are more abundant (Mitani and Ma 2005). In addition, the efficiency of each application form is regulated according to the silicon source, granulometry, purity and application form. Silicon supply via foliar application can supply the plant's needs and stimulate the defensive effects against insects (Buck *et al.* 2008).

Conclusions

SiO_2 and K_2SiO_3 applied by foliar application gave the tomato a localized protective effect, caused antibiosis, affected fertility and the survival of first generation females of *L. sativae*.

In summary, foliar application of SiO_2 and K_2SiO_3 is effective in the management of *L. sativae* plants in tomato.

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