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PEST MANAGEMENT

Induction of Insect Plant Resistance to the Spittlebug *Mahanarva fimbriolata* Stål (Hemiptera: Cercopidae) in Sugarcane by Silicon Application

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Abstract

Changes in the agroecosystem with the increase of green cane harvesting in Brazil affected the insect populations associated to this crop, and secondary pests like the spittlebug *Mahanarva fimbriolata* Stål, became much more important. Many studies have demonstrated the active role played by silicon in plant defense against herbivory. The objective of this study was to evaluate the effects of silicon applications on the biology of the spittlebug reared on two resistant (SP79-1011 and SP80-1816) and one susceptible (SP81-3250) sugarcane cultivars. Sugarcane plants were grown under greenhouse conditions and submitted to different treatments: with and without silicon fertilizer in two different soil type (sandy and clay soil). The newly hatched nymphs were transferred to sugarcane roots and placed into boxes with lids, to keep a moistened and dark environment favoring their growth and maintenance of the root system, providing food access to the developing nymphs. After emergence, adult males and females were placed in cages for mating and oviposition. The silicon absorbed and accumulated in the plant caused an increase in nymphal mortality, and depending on the sugarcane cultivar tested this element also provided an increase in the duration of the nymphal stage and a decrease in the longevity of males and females. 'SP79-1011' presented the highest silicon content in leaves, and *M. fimbriolata* had the highest nymph mortality and the shortest female longevity. The pre-oviposition period, fecundity and egg viability were not affected by the silicon content in plants or the cultivar used.

Introduction

The expansion of sugarcane plantings in Brazil and the gradual elimination of the burning practice before harvesting have contributed to the increase in population of the spittlebug *Mahanarva fimbriolata* (Stål). Consequently, this spittlebug, previously with no economic importance, has now became an important sugarcane pest (Dinardo-Miranda *et al* 2002). In addition to productivity losses, *M. fimbriolata* attack directly impairs the photosynthesis and other metabolic processes of the plant, affecting the quality of raw materials for industrial processing, as well as its commercial value (Macedo *et al* 2003).

Chemical control of this pest is widely used in sugarcane, but due to the high costs of pesticides and the possibility of selection of an insecticide-resistant population to the products traditionally used, new control alternatives should be sought (Macedo *et al* 2003, Loureiro *et al* 2005). Biological control (Macedo *et al* 2006), cultural control (Dinardo-Miranda 2002) and the use of resistant cultivars (Garcia 2006) are among the most viable options. However, another technique can be the induction of insect resistance in plants, which can be obtained by the application of chemicals or mineral products. Recent evidence suggests that the deposition of silicon in plants is likely to build resistance to plants against some insect pests, due to physical, chemical and structural barriers (Korndörfer *et al* 2004).

Sugarcane draws silicon from the soil, favorably responding to silicon fertilization, particularly in soils in which this element is poorly available (Korndörfer & Datnoff 1995). In addition to its important role as an inducer of resistance to pests of economic importance, silicon has several other beneficial functions in this crop, such as improving photosynthetic capacity, tolerance to frost, plant architecture, tolerance to water stress, pest control, quality of raw materials and productivity (Savant *et al* 1999).

Despite the numerous studies showing the importance of silicon as a beneficial element for sugarcane and as a promoter of pest resistance in this crop (Keeping & Meyer 2002, Keeping *et al* 2004, Kvedaras & Keeping 2007, Kvedaras et *al* 2007), there are no studies involving silicon application and its effect on resistance to the spittlebug. Therefore, the objective of this study was to evaluate the effect of silicon application to sugarcane cultivars on the biology of the spittlebug *M. fimbriolata*.

Material and Methods

Sugarcane bud setts of the cultivars SP79-1011, SP80-1816 and SP81-3250 were planted in 500 ml containers using two types of soil (clay and sandy). As a initial source of nutrients for sugarcane growth, a 4-14-8 ($N-P_2O_5-K_2O$) + micronutrients (1.5 g / kg substrate) formulation was used. For each cultivar in each type of soil, two other treatments were established: with and without silicon. We used 800 kg/ha of Si (source: K_2SiO_3), corresponding to 2.8 ml of fertilizer dissolved in 50 ml of distilled water for each container. Insects used in the experiments came from a laboratory colony maintained according to Garcia (2007), and experiments were conducted under controlled conditions (25±1ºC; 70 ± 10% RH; 14h photophase).

Each replicate consisted of 10 newly hatched nymphs, which were daily evaluated for mortality and adult emergence, from which we calculated the duration of the nymphal stage. Ten replicates were made for each treatment. Upon adult emergence, 10 couples (one male + one female) per replicate were formed and placed in separate cages with their respective plant per treatment for mating, breeding and feeding, and we assessed female fecundity and fertility and adult longevity (males and females).

Female fertility was determined by collecting all eggs each female produced daily and placing them in Petri dishes (6 cm x 2 cm high) lined with filter paper moistened with distilled water, and the number of nymphs hatching eggs was assessed daily.

The Si content in leaves extracted from the plants in each treatment was determined at the end of the experiments according to Korndörfer (2004).

The data from the effects of Si on the development, reproduction and longevity of the spittlebug were analyzed by a factorial 3 x 2 x 2 (three sugarcane cultivars, two doses of potassium silicate and two soil types). Means were compared by the Tukey´s test at a significance level of 5%.

Results and Discussion

Plants treated with potassium silicate in the soil (+Si) accumulated significantly higher amounts of silicon in leaves (2.6%) than the untreated plants (-Si) (1.05%) (Table 1).

When comparing cultivars, silicon was absorbed in different quantities among cultivars. The highest absorption was recorded for cultivar SP79-1011, whose value (2.31%) was significantly higher than that of 'SP80-1816' (1.60%) and 'SP81-3250' (1.56%) (Table 1). These results reinforce that sugarcane cultivars vary in their ability to accumulate this nutrient, as mentioned

Table 1 Mean (± SE) of foliar silicon content of sugarcane leaves of different cultivars treated (+Si) and untreated (-Si) with silicon grown in two types of soil and its effect on nymph mortality and nymph duration phase of the spittlebug *Mahanarva fimbriolata*. Temp.: 25 ± 1ºC, RH: 70% and photophase: 14h.

	Foliar silicon content (%)	Nymph mortality (%)	Nymph duration (days)^{ns}
Silicon			
- Si	1.05 ± 1.27 a	41.0 ± 2.13 a	48.5 ± 0.58
+ Si	2.60 ± 1.27 b	59.5 ± 2.13 b	49.6 ± 0.58
Cultivar			
SP79-1011	2.31 ± 1.55 a	66.3 ± 2.61 a	50.1 ± 0.72
SP80-1816	1.60 ± 1.55 b	49.5 ± 2.61 b	49.4 ± 0.72
SP81-3250	1.56 ± 1.55 c	35.0 ± 2.61 c	47.8 ± 0.72
Soil			
Sandy	1.68 ± 1.26 a	55.2 ± 2.13 a	48.5 ± 0.58
Clay	1.96 ± 1.26 b	45.3 ± 2.13 b	49.6 ± 0.58
CV (%)	1	32	9

Means followed by the same letter in column are not significantly different by the Tukey´s test (P > 0.05); ^{ns}Non significant.

by Rossetto *et al* (2005).

Regarding the influence of the soil type in this experiment, plants grown in clay soil had higher leaf Si content, unlike the value for plants grown in sandy soil (Table 1), as already reported by others (Raij & Campbell 1973). With a higher concentration of soluble Si in the soil, plants can absorb a greater quantity of this element from the substrate.

The 2.5 fold difference observed in the silicon content of leaves from silicon-treated and untreated plants can be explained by the high solubility of the source (potassium silicate) and the high amount of silicon applied (800 kg/ha), and corroborates the sugarcane capacity to accumulate silicon (Ross *et al* 1974, Datnoff *et al* 2001), which may also constitute a defense strategy against this insect pest.

Analyses indicated that significant interaction was only observed between cultivar and silicon treatment for the parameters nymph mortality, duration of nymphal stage and adult longevity (Table 2). Nymph mortality in untreated plants was higher on 'SP79-1011', while in Si-treated plants nymph mortality was also higher on 'SP79-1011', but in this case not different from the one observed on 'SP80-1816' (Table 2). Comparing Si-treated against untreated plants, mortality on Si-treated plants was always higher than on untreated plants. Differences in nymph mortality between Si-treated and untreated plants was near 75% on 'SP80-1816', 69% on 'SP81-3250', and 17% on 'SP79-1011' (Table 2). This smallest increase in nymph mortality observed on Si-treated 'SP79-1011' as compared to untreated plants can be explained by the fact that leaves of this cultivar naturally carries relatively high values of Si, which is consistent with the fact that it is considered unsuitable to spittlebugs. However, induction of plant resistance due to Si treatment could be clearly observed when comparing nymph mortality on cultivar SP80-1816 to that on SP81-3250. In this case, no differences in nymph mortality were observed between these two cultivars on untreated plants, but silicon application to these cultivars significantly increased nymph mortality on SP80-1816 to a level similar to that observed in the resistant cultivar (SP79-1011) (Table 2).

Nymphs maintained in plants grown in sandy soil had higher mortality than those kept in plants grown in clay soil, regardless of silicon application or the plant cultivar (Table 1). The highest mortality observed in sandy soils might be related to environmental conditions such as humidity and temperature, which are known to affect egg diapause and hatching (Byers 1965). Besides, moisture is a very important factor in the development of this insect, especially for the immature stages, and the higher capacity of clay soils to retain water as compared to sandy soils may provide an environment with the adequate conditions to promote the development of the insect. However, the soil type did not affect the spittlebug's nymph duration.

Silicon treatment also affected nymph development. While no difference in nymph development was observed among the untreated cultivars, nymph development on Si-treated plants was prolonged on SP79-1011 as compared to SP81-3250 (Table 3). This difference occurred because 'SP79-1011' Si-treated plants were the only ones to have an effect on nymph development when exposed to silicon (Table 3).

Male and female longevity in untreated or Si-treated plants was always lower on 'SP79-1011', although male longevity on Si-treated SP80-1816 did not differ from that observed for SP79-1011 (Table 4). Silicon treatment was able to reduce female longevity on SP79-1011 and SP80-1816 and male longevity only on SP80-1816. No differences were observed for male of female longevity between Si-treated and untreated SP81-3250 plants (Table 4). Once again, induction of plant resistance by silicon treatment could be observed on SP80-1816, as this was the only cultivar tested where male and female longevity were affected by silicon treatment (Table 4).

There are evidences in the literature that susceptible cultivars can benefit more than the resistant ones with

Table 2 Nymph mortality (%) (± SE) of the spittlebug *Mahanarva fimbriolata* in three cultivars treated (+Si) and untreated (-Si) with silicon. Temp.: 25 ± 1ºC, RH: 70% and photophase: 14h.

Cultivar	Nymph mortality (%)	
	-Si	+Si
SP81-3250	26.0 ± 3.69 aA	44.0 ± 3.69 aB
SP80-1816	36.0 ± 3.69 aA	63.0 ± 3.69 bB
SP79-1011	61.0 ± 3.69 bA	71.5 ± 3.69 bB

Means followed by the same letter, small in the column and capital in the row, are not significantly different by the Tukey´s test (P > 0.05).

Table 3 Nymph development (± SE) of the spittlebug *Mahanarva fimbriolata* in three cultivars treated (+Si) and untreated (-Si) with silicon. Temp.: 25 ± 1ºC, RH: 70% and photophase: 14h.

Cultivar	Nymph development (days)	
	-Si	+Si
SP79-1011	47.6 ± 1.01 aA	52.5 ± 1.01 bB
SP80-1816	49.3 ± 1.01 aA	49.5 ± 1.01 abA
SP81-3250	48.7 ± 1.01 aA	46.8 ± 1.01 aA
Mean	48.5	49.6

Means followed by the same letter, small in the column and capital in the row, are not significantly different by the Tukey´s test (P > 0.05).

Table 4 Male and female longevity (± SE) of *M. fimbriolata* in different cultivars treated (+Si) and untreated (-Si) with silicon. Temp.: 25 ± 1ºC, RH: 70% and photophase: 14h.

Cultivar	Female longevity (days)		Male longevity (days)	
	-Si	+Si	-Si	+Si
SP79-1011	12.1 ± 0.57 bA	9.9 ± 0.57 bB	11.9 ± 0.58 bA	10.9 ± 0.58 bA
SP80-1816	16.3 ± 0.57 aA	14.0 ± 0.57 aB	14.4 ± 0.58 aA	12.3 ± 0.58 bB
SP81-3250	17.0 ± 0.57 aA	15.5 ± 0.57 aA	15.3 ± 0.58 aA	14.1 ± 0.58 aA

Means followed by the same letter, small in the column and capital in the row, are not significantly different by Tukey's test (P > 0.05).

silicon application (Keeping & Meyer 2002, Kvedaras *et al* 2007).

The lowest male and female longevity of *M. fimbriolata* observed on cultivar SP79-1011 is in accordance to Garcia (2006), indicating the unsuitable traits of this cultivar to the spittlebug may be associated with its high Si absorption capacity.

On average, adults (males and females) fed on plants with high content of Si lived about two days shorter than on plants where there was no Si application. A two-day reduction in longevity for pests such as spittlebugs can result in a significant reduction in the number of eggs laid in the environment, as females can lay an average of 25 eggs/day, as reported in here (Table 5). The decrease in the number of eggs in the environment will decrease the future population of nymphs and, consequently, the damage caused to plants. A reduced longevity will also represent a decreased period of plant feeding, also resulting in a lower injection of the toxic saliva into the plant.

The soil type in which plants were grown did not affect the male or female longevity (Table 6). In spittlebug adults, Si application in the soil did not affect the female pre-oviposition period, fecundity or fertility (Table 5).

The different cultivars tested affected several reproductive traits of *M. fimbriolata* (Table 5). The pre-oviposition period was much longer on 'SP81-3250' than on 'SP80-1816' and 'SP79-1011' (Table 5). The lowest fecundity was observed for females from 'SP79-1011' (Table 5), although no difference was observed for female fertility (egg viability ranged from 84.3% to 98.2%). The soil did not interfere in any of these three parameters.

The nutritional quality of the host plant, as well as, the conditions for consumption, can affect female fecundity. Aphids fed on low quality plants reabsorbed their embryos as a mechanism to extend their life span (Awmack & Leather 2002). In addition, malnourished aphids tend to die before producing their full expected lifetime fecundity (Leather 1985). These effects were confirmed by more recent studies, in which only 33% of *Cinara atlantica* (Wilson) females laid nymphs when exposed to high doses of silicon, in contrast to 46% of females feeding on plants with lower silicon doses (Campbell *et al* 2008).

Physical, morphological and/or chemical characteristics

Table 5 Foliar silicon content (± SE) of sugarcane leaves of different cultivars treated (+Si) and untreated (-Si) with silicon grown in two types of soil and its effect on pre-oviposition, oviposition and egg viability of the spittlebug *Mahanarva fimbriolata*. Temp.: 25 ± 1ºC, RH: 70% and photophase: 14h.

	Foliar silicon content (%)	Pre-oviposition (days)	Fecundity (n^{o} of eggs/female)	Egg viability (%)^{ns}
Silicon				
-Si	1.1 ± 1.27 a	3.9 ± 0.14 a	376.0 ± 18.42 a	88.2 ± 5.22
+Si	2.6 ± 1.27 b	3.7 ± 0.14 a	361.5 ± 18.42 a	77.0 ± 5.22
Cultivar				
SP79-1011	2.3 ± 1.55 a	3.3 ± 0.17 a	242.4 ± 22.56 a	84.3 ± 7.02
SP80-1816	1.6 ± 1.55 b	3.7 ± 0.17 a	368.7 ± 22.56 b	89.0 ± 7.02
SP81-3250	1.6 ± 1.55 c	4.4 ± 0.17 b	495.1 ± 22.56 c	98.2 ± 7.02
Soil				
Sandy	1.7 ± 1.26 a	4.0 ± 0.13 a	362.2 ± 18.42 a	87.6 ± 4.37
Clay	2.0 ± 1.26 b	3.6 ± 0.13 a	375.3 ± 18.42 a	89.6 ± 4.37
CV (%)	1	28	38	23

Means followed by the same letter in column are not significantly different by Tukey's test (P > 0.05); ^{ns}Non significant.

Table 6 Foliar silicon content (± SE) of sugarcane leaves of different cultivars treated (+Si) and untreated (-Si) with silicon and grown in two types of soil, and its effect on male and female longevity of the spittlebug *Mahanarva fimbriolata*. Temp.: 25 ± 1ºC, RH: 70% and photophase: 14h.

	Foliar silicon content (%)	Longevity (days)	
		Male	Female
Silicon			
- Si	1.1 ± 1.27 a	13.9 ± 0.34 a	15.2 ± 0.33 a
+ Si	2.6 ± 1.27 b	12.5 ± 0.34 b	13.2 ± 0.33 b
Cultivar			
SP79-1011	2.3 ± 1.55 a	11.4 ± 0.41 b	11.0 ± 0.40 a
SP80-1816	1.6 ± 1.55 b	13.4 ± 0.41 a	15.2 ± 0.40 b
SP81-3250	1.6 ± 1.55 c	14.7 ± 0.41 a	16.3 ± 0.40 b
Soil			
Sandy	1.7 ± 1.26 a	13.2 ± 0.33 a	14.1 ± 0.32 a
Clay	2.0 ± 1.26 b	13.1 ± 0.33 a	14.2 ± 0.32 a
CV (%)	1	19	17

Means followed by the same letter in column are not significantly different by the Tukey´s test (P > 0.05).

of the plants can alter insect behavior and interfere with insect biology, leading to a reduced fitness and providing protection to plants (Lara 1991, Goussain *et al* 2005). These characteristics may, therefore, be used in the selection of resistant cultivars. In the present study, regardless of the cultivar and soil where sugarcane was grown, the increase in silicon content of these plants after Si provisioning affected the spittlebug nymph survivorship, and male and female longevity, which are all important aspects of plant resistance to insects. However, the resistance of a plant to an insect pest may not be simply related to the total quantity of silicon, but where and how this element is distributed and organized in the plant. Miller *et al* (1960) showed that leaf sheaths of certain wheat and oat cultivars resistant to *Phytophaga destructor* (Say) exhibited a more complete distribution of silicon deposits on the surface than did susceptible cultivars. These authors suggest that the silicon deposition in susceptible plants may allow the larvae of *P. destructor* to feed between the rows of Si. In sugarcane, the accumulation of silicon in the plant has proven to be an important factor of resistance against various insect pests (Keeping & Meyer 2002), increasing the difficulty of penetration in the plant and exposing the insects to natural enemies and abiotic conditions (Kvedaras & Keeping 2007), or decreasing pest survival and favoring a decrease in plant susceptibility to the pest with the increase of Si content (Keeping & Meyer 2002).

In this study, the increased resistance to spittlebug in the three cultivars due to silicon treatment suggests that there may be differences among cultivars in the assimilation/accumulation of silicon. Thus, the application of silicon in sugarcane growing areas, especially where susceptible cultivars are grown, may reduce the chances of the spittlebug population to reach the economic damage level. We can also conclude that SP79-1011 is the most unsuitable cultivar to *M. fimbriolata*, and that SP81-3250 is the cultivar to better respond to resistance induction by silicon application.

Thus, the results obtained on the biological aspects of the spittlebug *M. fimbriolata* in three sugarcane cultivars grown in two soil types with and without silicon, have shown that different cultivars, as well as the presence of silicon and soil type influence the development of the spittlebug. As the results of this study can collaborate with integrated pest management practices in sugarcane fields, further studies on the interactions of plant cultivars, soil types and silicon application affecting the resistance of plants to insects should be developed.

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