CROP PROTECTION

Silicon Influence on the Tritrophic Interaction: Wheat Plants, the Greenbug *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), and Its Natural Enemies, *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) and *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae)

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Influência do Silício na Interação Tritrófica: Plantas de Trigo, Pulgão-Verde *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) e seus Inimigos Naturais *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) e *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae)

RESUMO - Estudou-se a interação tritrófica: trigo, pulgão-verde *Schizaphis graminum* (Rondani) e seus inimigos naturais-chave *Chrysoperla externa* (Hagen) e *Aphidius colemani* Viereck, em plantas com ou sem adubação de silício. Os tratamentos consistiram em: 1) aplicação de silício via foliar; 2) aplicação de silício no solo e 3) testemunha (sem aplicação). As avaliações consistiram: a) teste de preferência do pulgão com chance de escolha em laboratório e b) aspectos biológicos das fases imaturas do predador *C. externa*, alimentado com pulgões criados em plantas dos diferentes tratamentos. Para a realização dos bioensaios com *A. colemani* foram realizados dois tratamentos: 1) aplicação de silício no solo e 2) testemunha (sem aplicação). As características biológicas avaliadas foram: duração da fase imatura, longevidade, razão sexual, ciclo total e porcentagem de parasitismo. Verificou-se que a aplicação de silício aumentou o grau de resistência das plantas de trigo diminuindo a preferência do pulgão-verde em relação à testemunha. Esse resultado pode estar relacionado à barreira mecânica proporcionada pela deposição de sílica na parede celular, o que dificultaria a penetração do estilete no tecido da planta, como também ao aumento na síntese de compostos de defesa da planta. Entretanto, não foi observado nenhum efeito indireto da aplicação de silício nas características biológicas tanto do predador como do parasitóide.

PALAVRAS-CHAVE: Insecta, *Triticum aestivum*, resistência, predador, parasitóide

ABSTRACT - We studied the tritrophic interaction: wheat, greenbug *Schizaphis graminum* (Rondani) and its key natural enemies, *Chrysoperla externa* (Hagen) and *Aphidius colemani* Viereck, in plants with or without silicon fertilization. Treatments consisted of: 1) silicon application via the leaves; 2) silicon application in the soil 3) control (no application). The evaluations consisted of: a) free-choice aphid preference test in the laboratory, from the 35th day after plant emergence, and b) biological aspects of the immature stages of predator *C. externa*, feeding on aphids reared on plants from the different treatments. Two treatments were tried in the bioassays involving *A. colemani*: 1) silicon application in the soil, and 2) control (no application). The biological traits evaluated were: duration of the immature stage, longevity, sex ratio, total developmental time, and percentage parasitism. Silicon application increased the degree of resistance in wheat plants, decreasing greenbug preference in relation to the control. This result could be related to the mechanical barrier provided by silica deposition in the cell wall, which would make it difficult for the stylet to penetrate the plant tissue, as well as an increase in the synthesis of plant defense compounds. However, no indirect effect of silicon application was observed on the biological traits of either the predator or the parasitoid.

KEY WORDS: Insect, *Triticum aestivum*, resistance, predator, parasitoid

In integrated pest management programs, the combination of population regulation strategies, such as plant resistance to insects and biological control, can potentialize the control of insect pests, keeping them below the control level, with little impact on the environment. In the tritrophic interaction between plants, aphids, and their natural enemies, growing resistant varieties can affect natural enemies directly, by modifying their behavior, or indirectly, by changing the nutritional quality of the prey and/or host (van Endem 1995). The impact of this interaction could be either negative, thus demonstrating the incompatibility between the two control methods (Obrycki & Tauber 1984, Gamarra *et al.* 1997), or positive, having cummulative effects on the reduction of aphid populations (Farid *et al.* 1998, Messina & Soureson 2001).

Biological control is an important component in integrated management of wheat aphids (Tanigoshi *et al.* 1995, Mohamed *et al.* 2000). Chrysopids are important predators in several crops of economic interest (Tauber *et al.* 2000) and are considered, together with aphidiid wasps, as key natural enemies of aphids. The first, and among them *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae), are predators with high biological agressiveness (Fonseca *et al.* 2000, Messina & Sorenson 2001), high biotic potential and great voracity, feeding on a variety of insects (aphids, scale insects and mealybugs, eggs, caterpillars and lepidopteran pupae) during their biological cycle, and occur in several crops of economic interest (Berti Filho *et al.* 2000, Fonseca *et al.* 2000). The small parasitoid wasp *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) can utilize several aphid species as hosts, controlling them effectively (Starý *et al.* 1993, Tanigoshi *et al.* 1995).

On the other hand, the application of silicon has increased the degree of resistance of plants against insects, with special emphasis on grasses. Several papers have demonstrated that silicon application provides greater plant protection against abiotic and biotic stresses, due to the mechanical barrier provided by the deposition of silica in the leaf tissues and trichomes, and to the production of phenolic defense compounds (Chérif *et al.* 1992, Savant *et al.* 1997, Carvalho *et al.* 1999, Goussain *et al.* 2002).

Even though the induced resistance of wheat plants to the greenbug is an insect-pest-population-reduction technique, no research information is available concerning its effect on the tritrophic interaction (plant/herbivore insect/natural enemy). Resistant plants can reduce or increase the natural enemies' ability to control insect pests. Thus, the objective of this work was to study the effect of silicon application on wheat plants, on the preference of the greenbug *S. graminum*, and on the development of key natural enemies *C. externa* and *A. colemani*.

Material and Methods

Resistance of Wheat Plants to the Greenbug through Application of Sodium Silicate and its Interaction with *C. externa*. In the greenhouse, 20 wheat seeds, cv. Embrapa-21 (dryland) were planted per pot with capacity for 4 kg substrate, consisting of sifted hillside soil fertilized with 0.6 g 4-14-8 rate . kg^{-1} soil, and irrigated daily with 200 ml water. After seedling emergence, a thinning was performed, leaving only four seedlings/pot.

The pots were randomly arranged on the benches, distributed in three treatments: 1) foliar application of silicon; 2) silicon applied directly to the soil, around the seedlings; and 3) control (no application). In treatment 1, a 1% SiO_2 sodium silicate solution was applied at a dose of 100 ml/pot, 15 days after emergence of plants (Goussain *et al.* 2002, Moraes & Carvalho 2002). In treatment 2, two applications were performed, and the same source and concentration were utilized. The first application was performed 15 days after plant emergence and the second occurred six days after the first, with an application volume of 65 ml/pot/application.

In the free-choice preference test conducted in the laboratory, two juxtaposed sections of wheat leaves for each treatment were placed in 50-ml plastic cups, totaling three groups/container (three treatments). The 5-cm long leaf sections were arranged in vertical position, forming a 90º angle with the container, and were secured in place by a styrofoam cylinder that filled the entire container diameter.

The styrofoam was placed in the upper third of each container, and the part below the styrofoam was filled with water. Ten *S. graminum* adults were distributed at the center of the styrofoam piece and placed on two square trays, measuring 50 cm × 30 cm. The trays were filled with water and liquid dishwashing soap to prevent the aphids from escaping. The trays were maintained inside an incubator at 25 ± 1°C, 70 ± 10% relative humidity and 12h photophase. Evaluations were performed at 24, 48 and 72h after aphids releasing, by counting adults and nymphs present in their respective treatments. After counting, the nymphs were removed with a fine-hair brush. This assay was performed on the 35^{th} day after the plants had emerged.

For the tritrophic interaction study involving wheat plants, greenbugs, and the predator *C. externa*, a stock rearing of this natural enemy was developed, according to Ribeiro *et al.* (1991) and Fonseca *et al.* (2001). In the bioassays, eggs of the predator, all at the same age, were individualized in glass vials (2.5 × 8.5 cm) sealed with PVC film that was pierced with a pin to allow aeration, and maintained in an incubator at 25 ± 1°C, 70 ± 10% relative humidity and 12h photophase. Adult aphids stayed on treated plants for at least 30 days before being used as preys. They were offered to newly hatched chrysopid larvae until the end of the larval stage, assuring a permanent supply.

Duration and survival of each instar and of the larval and pupal stages were evaluated, as well as the sex ratio of individuals that reached the adult stage.

Biological Aspects of the Parasitoid *A. colemani* in Aphids Reared on Wheat Plants Fertilized with Calcium Silicate. Wheat plants cv Embrapa 21 were grown in 500-ml plastic cups where 2.5 g calcium silicate (38% SiO_2) . kg^{-1} soil were added to the substrate, and control plants were also grown in identical cups without silicate application. The pots were irrigated daily and, after plant emergence, thinning was performed leaving four seedlings/pot. After 30 days, each pot was covered with a cage, constructed with an inverted cup of the same type as utilized for planting, which contained three cut-out areas protected with organza fabric on two sides and on the top. Plants were pruned to a 10-cm height in order to fit the cages.

A. colemani from a rearing colony that used the aphid *Myzus persicae* (Sulzer) on green pepper plants as host, maintained in an air-conditioned room at a temperature of 22 ± 1ºC and 12h photophase, were utilized in the bioassays. The parasitoid females used in the tests had no prior oviposition experience, and were mated 24h to 48h after emergence.

Ten third-instar greenbug nymphs were released in each cage, and 4h later one mated parasitoid female was also released and remained there for 2h in order to oviposit on the aphids. Eight days later the cages were opened and the parasitoid mummies were individualized in test tubes, remaining in an incubator at 22 ± 1ºC and 12h photophase. Upon emergence, the parasitoid adults were fed honey and water. The biological traits evaluated were: duration of the immature stage, longevity, sex ratio, total cycle and percentage parasitism.

Experimental Design and Statistical Analysis. In the greenbug preference evaluation, the treatments were designed as random blocks, in a split-plot scheme. The three main treatments were allocated to plots and the variable "evaluation time" to subplots, with eight replicates. In the assay involving the predator *C. externa*, a completely randomized design was adopted, with three treatments and 15 replicates. In the parasitism study, however, we used a completely randomized design, but only two treatments were set up (aphids from plants treated with calcium silicate and from non-treated plants), with 10 replicates; each experimental plot consisted of three cages.

The data were submitted to analysis of variance, and the means were compared by the Scott & Knott test ($P \leq 0.05$) for grouping means, in the assays involving more than two treatments (Scott & Knott 1974).

Results

Resistance of Wheat Plants to the Greenbug through Application of Sodium Silicate and its Interaction with *C. externa*. No significant interaction was detected between the time of evaluation and silicon application for number of nymphs and adults of *S. graminum* present in leaf sections. However, a clear preference of the aphid for leaf sections of plants that had not been fertilized with sodium silicate occurred ($P \leq 0.05$) (Tables 1 and 2). The production of nymphs was constant during the 72h used as maximum observation time. However, the mean number of nymphs produced on silicon treated plants were 41,9% and 47,1% less than on untreated plants (Table 2). The number of nymphs showed a pattern similar to that of adults. An effect of silicon was observed in the aphid's preference, indistinct of its form of application. (Tables 1 and 2). Thus, wheat leaf sections from plants treated with sodium silicate via the leaves or via the soil showed the same degree of resistance to nymphs and adult aphids, and were different only from those that did not receive the nutrient (Tables 1 and 2).

Table 1. Number of *S. graminum* adults (Mean ± SE) on leaf sections from wheat plants in different treatments, 24, 48 and 72h after releasing.

Treatments	Number of adults/leaf section			Mean
	24h	48h	72h	
Control (no silicon)	5.6 ± 0.30	5.2 ± 0.20	5.5 ± 0.40	5.5 a
Silicon via the soil	2.0 ± 0.20	2.5 ± 0.20	2.4 ± 0.40	2.3 b
Silicon via the leaves	2.4 ± 0.30	2.2 ± 0.20	2.2 ± 0.30	2.3 b
Mean	3.1 A	3.1 A	3.4 A	-

Means followed by a common lower case letter, in the column, and by a common upper case letter, in the row, do not differ among themselves by Scott & Knott test ($P \leq 0.05$).

Table 2. Number of *S. graminum* nymphs (Mean ± SE) on leaf sections from wheat plants in different treatments, 24, 48 and 72h after releasing.

Treatments	Number of nymphs/leaf section			Mean
	24h	48h	72h	
Control (no silicon)	12.5 ± 0.50	13.5 ± 0.80	14.9 ± 0.80	13.6 a
Silicon via the soil	7.6 ± 0.60	7.9 ± 0.70	8.4 ± 0.90	7.9 b
Silicon via the leaves	7.2 ± 0.60	6.7 ± 0.40	7.8 ± 0.90	7.2 b
Mean	9.1 A	9.4 A	10.3 A	-

Means followed by a common lower case letter, in the column, and by a common upper case letter, in the row, do not differ among themselves by Scott & Knott test ($P \leq 0.05$).

No significant effect of silicon ($P \geq 0.05$) was observed on the development of the predator *C. externa* fed aphids from plants treated with sodium silicate or from control plants (without silicon application), for duration and survival of instars. The duration and survival of the predator in the three instars were statistically similar, either when the predator was fed greenbugs reared on plants fertilized with sodium silicate or when *C. externa* was given aphids from plants that did not receive this nutrient to induce resistance to the aphid (Table 3).

The duration of the larval and pupal stages, as well as the sex ratio of *C. externa* were not influenced by the type of food received by the prey. The predator larvae developed in thee days on the first and third instars, and it took 3.4 days for second-instar larvae to develop. The predator's larval survival was 100% for all instars and stages, showing that the greenbugs, regardless of being reared on wheat plants that received silicon or not, provided adequate nutrition (Tables 3 and 4).

Biological Aspects of the Parasitoid *A. colemani* in Aphids Reared on Wheat Plants Fertilized with Calcium Silicate. No significant difference was observed with respect to calcium silicate on the different biological traits of *A. colemani* under study, relative to the control. Thus, duration of the young form of the parasitoid was, on average, 12.2 days. Adults lived for about 7.5 days, with mean longevity of 19.8 days, sex ratio of 0.45 and parasitism rate of 33.5% (Table 5).

Discussion

The small number of nymphs and adults of *S. graminum* on leaf sections from plants that received silicon fertilization confirms the possibility that the silicon absorbed by the plants, either through the soil or by the leaves, was deposited in the leaves, providing a mechanical barrier that interferes with feeding in insect pests, especially in sucking insects such as the greenbug. Similar results were obtained by Carvalho *et al*. (1999) and Moraes & Carvalho (2002) with *S. graminum* in free-choice preference tests with sorghum plants treated or not with silicon. According to Jones & Handreck (1967), and Raven (1983), silicon application provides greater transport of this element to the above-ground part of plants, as it is deposited in the epidermal cells as amorphous silica ($SiO_2.nH_2O$), making the leaf tissues more rigid. The deposition of silicon and its solubility increase in wheat plant leaves, after this element was applied via the leaves in a 1% Na_2SiO_2 solution, were the cause of this cereal's resistance to two important aphids, *Metopolophium dirhodum* (Walk) and *Sitobion avenae* (Fabricius) (Hanisch 1980).

The predator *C. externa* was not affected when fed aphids from wheat plants previously fertilized with sodium silicate. This predator continued effective and, under field conditions, a positive interaction between resistance and biological control is likely to occur. The biological parameters observed for *C. externa* were similar to those found by Ribeiro *et al*. (1991), Zheng *et al*. (1993) and Figueira *et al*. (2002). Under similar conditions to those in this bioassay, the predator showed biological parameters for the immature stages very close to those verified by Fonseca *et al*. (2000), Maia *et al*. (2000), and Fonseca *et al*. (2001), who studied the biology of *C. externa* on *S. graminum*. Messina & Sorenson (2001) observed greater effectiveness of the predator *Chrysoperla plorabunda* (Fitch) on resistant wheat plants, where low populations of the aphid *Diuraphis noxia* (Mordvilko) would normally be found, which could increase even more the cummulative effect of the two management tactics used against the aphid.

Also the development and longevity of *A. colemani* were not affected when the insect developed on aphids reared on plants treated with silicon, thus showing its compatibility with biological control. Differences in host quality affected the development period, size, fecundity

Table 3. Duration (days) (Mean ± SE) of the larval stage of *C. externa* fed *S. graminum* reared on wheat plants treated with silicon.

Treatments	1st instar	2nd instar	3rd instar
Control (no silicon)	3.0 ± 0.30	3.3 ± 0.30	3.0 ± 0.90
Silicon via the soil	3.1 ± 0.10	3.5 ± 0.50	3.0 ± 0.60
Silicon via the leaves	3.1 ± 0.30	3.4 ± 0.80	3.0 ± 0.90

Means are similar by F test ($P \geq 0.05$).

Table 4. Duration (days) (Mean ± SE) of the larval and pupal stages, and sex ratio of the predator C. *externa* fed S. *graminum* reared on wheat plants treated with silicon.

Treatments	Larval stage	Pupal stage	SR
Control (no silicon)	9.6 ± 1.30	10.0 ± 1.50	0.47
Silicon via the soil	9.5 ± 1.00	10.0 ± 0.90	0.53
Silicon via the leaves	9.5 ± 0.90	9.9 ± 0.80	0.53

Means are similar by F test ($P \geq 0.05$).

Table 5. Duration (days) of the immature stage, longevity, total cycle, sex ratio and percentage parasitism (Mean ± SE) of *A. colemani* reared on the aphid *S. graminum* raised on wheat plants treated with silicon.

Treatments	Duration/days			Sex ratio	Parasitism (%)
	Immature stage	Longevity	Total cycle		
Treated plants	12.2 ± 0.50	7.6 ± 1.30	19.9 ± 1.50	0.5 ± 0.30	33.0 ± 23.60
Non-treated plants	12.2 ± 0.50	7.5 ± 1.30	19.7 ± 1.50	0.4 ± 0.30	34.0 ± 28.40

Means are similar by F test ($P \geq 0.05$).

and longevity of parasitoids (Vinson & Iwantsch 1980), allowing host quality to be directly evaluated, since the shortest development period can be considered as the best growth trajectory (Sequeira & Mackauer 1992). In recent studies, many authors have observed durations of the development period of *A. colemani* higher than 12.2 days, different from those observed in this work for that developmental stage (Gonçalves-Gervásio *et al.* 2001).

The observed sex ratio and parasitism rate were similar when parasitoids utilized aphids from wheat plants treated with silicon or from non-treated plants as hosts. Sex ratio is strongly influenced by host quality, and a greater proportion of males is associated with the low quality of the host (Mackauer *et al.* 1996). The parasitoid's sex ratio was comparable to those observed by Heimpel & Lundgren (2000). The parasitism rate of aphids by *A. colemani* is variable according to host species (Sampaio *et al.* 2001); however, the mean values obtained (33% and 34%) can be considered satisfactory, in view of the short period of exposure of the host to the parasitoid.

In spite of the fact that the great modifications in plant and aphid quality which occurred under resistance induction conditions could be detrimental to natural enemies (Stadler & Mackauer 1996), our results demonstrated that the increase in the degree of resistance of wheat plants to the greenbug, by non-preference mechanisms, does not significantly affect its key natural enemies from a nutritional standpoint. However, alterations in the population dynamics of these insects are expected to occur in the field, with a consequent reduction in the populations of aphids on cultivated wheat.

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