CROP PROTECTION

Effect of Silicon Applied to Wheat Plants on the Biology and Probing Behaviour of the Greenbug *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae)

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Efeito do Silício, Aplicado em Plantas de Trigo, na Biologia e Comportamento Alimentar do Pulgão-Verde *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae)

RESUMO - Foi avaliado o efeito do silício, aplicado em plantas de trigo (*Triticum aestivum* L.) sobre o pulgão-verde *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae). Plantas de trigo foram tratadas com silício incorporado no solo e aplicado nas folhas. Foram avaliadas as durações dos períodos pré-reprodutivo, reprodutivo e pós-reprodutivo, como também a fecundidade e a longevidade do pulgão-verde. O comportamento alimentar foi investigado utilizando-se a técnica de Electrical Penetration Graphs (EPG-DC) e "*honeydew clock*". Plantas tratadas com silício mostraram efeito adverso sobre o desenvolvimento do pulgão. A penetração dos estiletes não foi afetada pelos tratamentos com silício. Contudo, os estiletes foram retirados mais freqüentemente das plantas tratadas com silício, o que reduziu o tempo de prova. O xilema e o floema foram igualmente alcançados em todos os tratamentos e os pulgões permanecerem alimentando-se no floema por períodos similares. Entretanto, a excreção de *honeydew* foi reduzida no tratamento com silício, indicando menor taxa de ingestão ou maior retenção de seiva no corpo do pulgão. Portanto, alterações químicas e indução de resistência estão provavelmente envolvidas na redução da performance do pulgão, sem, entretanto, alterar seu período de alimentação.

PALAVRAS-CHAVE: Insecta, resistência, biologia, EPG

ABSTRACT - The effect of silicon-treated wheat plants (*Triticum aestivum* L.) on the greenbug, *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae) was evaluated. Plants were treated with silicon incorporated to the soil and by foliar spraying. Aphid development was evaluated by observing the duration of the pre-reproductive, reproductive and post-reproductive periods, as well as fecundity and longevity. Probing behaviour was investigated by using the DC electrical penetration graphs (EPG) technique and a "honeydew clock". Silica treated plants had a clear adverse effect on aphid development. Stylet penetration was not affected by treatments showing no physical barriers by the plant tissue. However, stylet was withdrawn more often on plants treated with silica, resulting in reduction of probing time. Sieve elements were reached equally by aphids in all treatments and the insects remained ingesting phloem sap for similar periods. However, honeydew excretion was highly reduced indicating lower sap ingestion rate or higher sap retention inside the body. Chemical changes and induced resistance are possibly related to the reduction of aphid performance.

KEY WORDS: Insecta, resistance, honeydew, biology, EPG

The greenbug *Schizaphis graminum* (Rond.) is an important pest on cereal crops and causes direct damage by phloem sap ingestion, and indirect by transmitting virus and other pathogens. Photosynthesis is reduced as a result of sooty mould developing on the honeydew excreted by the insect (Ryan *et al*. 1990). Host plant resistance has proven to be an effective tool against insects in many crops. Nonetheless, the value of this approach in

integrated pest management programs has been understated. Resistant plants have reduced pesticide inputs, diminishing worker risks and minimizing potential environmental contamination. There is a renewed interest in plant breeding against insects and diseases as a result of increasing pressure to reduce pesticide use and restrictions to insecticides (organophosphate) (Eigenbrode & Trumble 1994).

Host plant selection by aphids comprises a sequence of several steps where physical and chemical factors of plant tissues act during stylet penetration (probing) (Klingauf 1987). The study of stylet penetration can therefore help to locate resistance factors at different levels into the tissues (Harrewijn 1990, Niemeyer 1990).

The electrical monitoring system (AC-system) was first introduced by McLean & Kinsey (1964) to study plant penetration by the stylets ('probing') of homopteran insects. This system was modified and improved (DC-system) by Tjallingii (1988) who named it as 'Electrical Penetration Graphs' (EPG). This technique has been used to investigate virus transmission (Prado & Tjallingii 1994, Martin *et al.* 1997), host plant resistance to aphids and whiteflies (Helden & Tjallingii 1993, Lei *et al.* 1998), and other fundamental aspects (Tjallingii & Hogen Esch 1993).

The application of silicon (Si) has shown to stimulate growth and production in many vegetables, mainly plants of the family Poaceae (grasses). It protects against abiotic stress and decreases the incidence of insects and diseases (Epstein 1994, 1999;Marschener 1995; Savant *et al.* 1997). Several economic insect pests have been suppressed by improving Si concentration in the plants, including several sucking insects (Savant *et al.* 1997). A reduction in the preference and reproduction of the greenbug, *S. graminu*m was obtained after application of silicon on sorghum (Carvalho *et al.* 1999). Most of Si studies refer to the effect of silicon in plant physiology and mainly to its association to fungi (Chérif *et al.* 1994). Sucking insects are mainly phloem feeders and locating the sieve elements implies to avoid physical and chemical barriers. Rigidity and pectin of middle lamella seem to play a role as a physical barrier to stylet penetration (Dreyer & Campbell 1987). Silicon deposits on cell walls would act as a mechanical barrier to stylet penetration. Silicon is not only involved in mechanical restraints against fungi infection and insect damage, but also with biochemical changes related to plant defences in general (Epstein 1999, Fawe *et al.* 1998). Silicon induced defence mechanisms include also changes in the trichome morphology, lignin accumulation, phenolics, chitinases and peroxidases production (Hodson & Sangster 1988, Samuels *et al.* 1993, Cherif *et al.* 1994). Many of these factors are also connected to plant resistance against sucking insects modifying also their probing behaviour (Ramirez & Niemeyer 1999).

The objective of the present study was to evaluate the effect of silicon-treated wheat plants on the greenbug development and probing behaviour, testing the hypothesis of restraints to the stylet penetration produced by silica in plant tissues.

Material and Methods

A culture of greenbug was maintained on sorghum, *Sorghum bicolor* cv. Br 303, under controlled environment room (22 ± 1°C, 70 ± 10% R.H. and L14: D10 photoperiod). The wheat plants, *Triticum aestivum* L. cv. Lorini, used in the experiments, were maintained in 500 ml plastic pots, on soil, sand and manure in equal proportions.

Soil application was made with calcium silicate from furnace slag containing 38% of SiO_2 (Recmix Co.). This material also contains CaO and MgO, and a minimum amount of heavy metals, assuring its safe use in agriculture. No other material considered toxic to insects or plants is found in this material (Korndörfer *et al.* 2003). A sodium silicate solution of Merck™, containing 25.5 – 28.5% SiO_2 was used for foliar sprays.

The following treatments were used in the studies on biology, EPG and honeydew recording: (1) Plants grown on soil treated with silicon at rate of 2.5 g of calcium silicate (furnace slag) per kg of soil; (2) Plants grown on soil treated with silicon at rate of 2.5 g of calcium silicate (furnace slag) per kg of soil plus foliar application with 0.5% rate of sodium silicate solution (SiO_2, Merck™), 15 days after plant emergence; (3) Untreated plants.

Biology of *S. graminum*. According to the previously described treatments 24h-old nymphs were placed on 4-week old *T. aestivum* plants. The nymphs were kept inside clipcages (8 mm diameter and 10 mm height) and observed daily. Newborn nymphs were removed and counted. Three clipcages per plant were used, each containing one nymph. The average value of these cages represented a replicate with the total of 10 replicates per treatment. Longevity, pre-reproductive, reproductive and post-reproductive periods were noted. Dead nymphs were removed and replaced, so the data only reflect the effect of silicon on those insects that reached the adult stage.

EPG. The Electrical Penetration Graph (EPG, DC system) was recorded on 17-20-day-old plants with a fully expanded second leaf. Virginoparous adults (2-4 days after moulting) were placed on the inverted abaxial side of the youngest fully grown leaf. Aphids were wired with a 20 mm long, 20 µm diameter gold wire, attached to the dorsum of the aphid, using water based (non toxic solvent) silver paint. They were connected to the amplifier (1 Giga-Ohm input resistance and gain of 50x; Tjallingii 1985, 1988). The other electrode was inserted in the soil of the potted plant. The whole setup was placed in a Faraday cage. All signals were recorded on PC hard disc using Stylet 3.0 software (Tjallingii, pers. comm.). All recordings lasted 8h and about 25 replicates were made per treatment. Aphids were starved for 1h prior to the recording. A more detailed description of this technique and setup can be found in Tjallingii (1988, 1990) and Walker (2000). Waveform analyses comprised patterns A, B and C, which were lumped together and considered as a pathway phase. Many variables were analysed but only those which give useful information are presented here. Some variables considered only those aphids reaching an

event, i.e. phloem phase, and others considered all aphids, which are indicated in the tables.

Honeydew Excretion. The number of honeydew droplets excreted by aphids was compared between treatments during 12h. Aphids were starved for 1h prior to the honeydew recording. The frequency of honeydew production was measured on a 12h honeydew clock using a filter paper impregnated with ninhydrin (in butanol and acetic acid solution), which stains the amino acid content (Mittler 1958). Aphids were placed on the abaxial side of the leaf and the honeydew clock was placed at about 2 mm under the aphids. Nine aphids were recorded for each treatment. Longer honeydew clock recording are usually unviable because a large proportion of aphids move outside the recording area. To avoid this constraint, honeydew excretion was also measured in confined aphids inside a 5 cm diameter petri dish during 48h. A filter paper impregnated with ninhydrin was placed on the bottom of the dish and two wheat leaves were placed on the top. Dishes were sealed with paraffin film. One aphid per dish was placed on the leaves and 14 replicates were used. The filter paper was changed at 24h. All honeydew droplets were counted.

Statistical Analysis. EPG variables did not always meet the assumptions for ANOVA so they were analysed using the Kruskal-Wallis analysis followed by multiple comparisons for this test (Conover 1980). Honeydew excretion and development variables were analysed by ANOVA and differences between treatments were compared by the Tukey test. Data was transformed when needed. For some percentage data the Chi square test was performed.

Results

Biology of *S. graminum*. The duration of the pre-reproductive and post-reproductive periods were unaffected by silicon treatments. However, the treatment by silicon in the soil plus foliar application significantly decreased the reproductive period and longevity in about seven days. Silicon affected aphid fecundity especially when applied in the soil followed by foliar spraying and resulted in an average of 59.4 nymphs/female. In soil treated plants 72.2 nymphs/female were obtained, which was not significantly different from untreated plants (89.4 nymphs/female) (Table 1).

Electrical Penetration Graphs (EPG). Many variables were worked out but only the most relevant for this experiment are presented in Table 2. Time to first probe, duration of first probe and non probing time after first probe showed no differences between treatments, thus indicating that silicon application did not affect the initial stylet penetration (variables 1, 2 and 3).

Total probing time was affected by silicon showing the longest probing period in untreated plants (variable 4). The percentage of non-probing period before reaching phloem phase (variable 5) increased in silicon-treated plants with similar result obtained when the phloem phase period was subtracted (variable 6). The time to reach the first phloem phase showed similarities between soil treated plants and untreated but it was delayed (3.6h) in plants receiving soil and foliar applications (variable 7). However, this period was similar when considered only the probe that reached the phloem (variable 8). Most of the insects could reach the phloem and ingested sap for similar periods irrespective of the treatment (variables 9 and 10). The duration of xylem phase (variable 11) showed no differences between treatments.

Honeydew Excretion. Applications of silicon reduced the number of honeydew droplets during the 12h experiment, especially in soil and foliar treated plants. The first droplet appeared also to be delayed in this treatment (Table 3). In a 48h recording a reduced honeydew excretion was also observed (Table 3).

Discussion

A clear deleterious effect of silicon on the greenbug development was obtained only with silicon applied in the soil followed by foliar spraying. Longevity and fecundity was reduced mainly by a reduction of the reproductive period. Reduction of the honeydew excretion and delay in the first droplet indicate that sap ingestion was affected. A direct or

Table 1. Development variables of greenbug *S. graminum* on wheat plants (means and standard error).

Treatments	Duration (days)				Fecundity (n. of nymphs/aphid)
	Pre-reproductive	Reproductive	Post- reproductive	Longevity	
Silicon in soil	6.6 ± 0.1	22.1 ± 1.3 a	9.1 ± 1.4	37.6 ± 1.7 a	72.2 ± 6.7 ab
Silicon in soil plus foliar	6.8 ± 0.5	15.1 ± 1.9 b	8.7 ± 2.0	30.3 ± 1.6 b	59.4 ± 8.9 b
Untreated	6.4 ± 0.2	21.7 ± 1.6 a	9.5 ± 1.3	37.6 ± 0.8 a	89.4 ± 6.5 a
ANOVA P-value	0.714 $^{n.s.}$	0.008	0.942 $^{n.s.}$	0.001	0.028

Means followed by different letters within column are significantly different according to ANOVA test followed by the multiple comparison test of Tukey ($\alpha = 0.05$); $^{n.s.}$ non significant.

Table 2. Probing behaviour of *S. graminum* during 8h recording [means ± (standard error)].

Variable (unit)	Treatments			Kruskal-Wallis P-value
	Silicon in the soil	Silicon in the soil plus foliar	Untreated	
1. Time to 1st probe (min)	9.3 (2.0) a n = 25	5.0 (2.5) a n = 25	2.8 (0.9) a n = 25	0.104
2. Duration of first probe (min)	28.4 (7.0) a n = 25	21.4 (7.6) a n = 25	34.4 (8.1) a n = 25	0.225
3. Non probing time after first probe (min)	6.4 (1.5) a n = 25	8.2 (2.9) a n = 25	3.3 (0.9) a n = 25	0.095
4. Probing duration (h)	6.8 (0.2) b n = 25	6.6 (0.2) b n = 25	7.3 (0.1) a n = 25	0.024
5. % of non probing time until 1st phloem phase[1]	13 (3.0) a b n = 21	21 (3.4) a n = 23	10 (2.3) b n = 23	0.045
6. % of non probing after subtraction of phloem phase[1]	21 (3.3) a n = 21	25 (3.7) a n = 23	13 (2.5) b n = 23	0.021
7. Time to reach first phloem phase (h)[1]	2.3 (0.4) b n = 21	3.6 (0.4) a n = 23	2.9 (0.4) a b n = 23	0.038
8. Time to 1st phloem phase within probe (min)[1]	33 (4.4) a n = 21	33 (5.9) a n = 23	34 (4.5) a n = 23	0.818
9. % of aphids showing phloem phase	84	92	92	Chi square test: 0.571
10. Total phloem phase (h)[1]	3.8 (0.3) a n = 21	3.3 (0.4) a n = 23	3.6 (0.4) a n = 23	0.582
11. Total xylem phase (min)	1.7 (5.7) a n = 25	5.7 (3.8) a n = 25	5.9 (3.3) a n = 25	0.381

Means followed by different letters within lines are significantly different according to the Kruskal-Wallis test followed by multiple comparisons.
n = number of replicates; [1]only aphids reaching the phloem were considered.

indirect effect of silicon can only be speculated. Physical barrier to fungal hyphae penetration has been suggested but not corroborated (Chérif *et al.* 1992, Menzies *et al.* 1992). References indicate that silica could accumulate in tissue spaces, cell wall matrix and inside cells (Kaufman *et al.* 1985) even reaching the vascular bundle (Hayward & Parry 1973). These deposits could make more rigid cell walls interfering with stylet penetration and this was the hypothesis evaluated. However, the results did not support this assumption. Addition of silicate to plants has shown to change trichome development and leaf surface (Samuels *et al.* 1993), together with other changes detected in epidermal and more internal tissues of plants (Hodson & Sangster 1988). Surface and epidermis restraints to stylet penetration were not affected by silicon application as demonstrated by the initial probing behaviour variables (variables 1, 2 and 3). However the EPG technique is not completely appropriated to detect differences at this level due to the aphid manipulation previous to the monitoring, which could modify aphid behaviour at the beginning of probes. Free aphid and direct observation detect better any difference.

Silicon application induced aphids to withdraw the stylet from plants. The significant differences obtained with percentage of non-probing period before reaching the phloem phase suggest that restraints to stylet penetration could be found in the tissue before phloem vessels i.e. epidermal and

Table 3. Honeydew excretion of *S. graminum* on *T. aestivum*.

Treatments	12h recording in a honeydew clock (n = 9 aphids/treatment)			48h recording in petri dish (n = 14 aphids)
	Honeydew droplets	Hour of 1st droplet		Number of honeydew droplets
	(Means ± SE)	Means	Range	Means ± SE
Silicon in the soil	3.3 ± 0.8 a b	3	2 – 5	19,7 ± 3.1ab
Silicon in the soil plus foliar	2.3 ± 0.9 b	5	2 – 10	13.5 ± 2.1b
Untreated	5.3 ± 0.6 a	3	1 - 6	28.1 ± 4.5 a

Means followed by different letters within column are significantly different according to ANOVA test followed by multiple comparisons Tukey test ($\alpha = 0.05$). Analysis with data transformed to $\sqrt{x} + 0.5$.

mesophyll tissue, despite eventually all aphids were able to ingest from the phloem. This also reflected in the longer time to reach the phloem in soil and foliar treated plants (variable 7). Despite this delay, aphids were finally able to reach the phloem. On the other hand, the time to reach the phloem phase in the probe that reached the vessels (variable 8) showed that physically no restraint to stylet penetration was present. Phloem ingestion period was not affected indicating that at least silicon did not modify the time spent by aphids in the sieve elements.

Induced defences could also be involved after silicon absorption. A series of biochemical changes has been reported in silicon-treated plants, including stimulation of chitinase activity, activation of peroxidases and polyphenoloxidases, after fungal infection (Chérif *et al.* 1994). However, no study has been reported, to our knowledge, following insect infection, but many mechanisms of defences are shared with fungi. The results suggest that silicon induced some changes in the tissues, like epidermis and/or mesophyll, that cause stylet withdrawn. These changes do not seem to be physical and impede stylet insertion, because the stylet eventually reached the phloem vessel in similar time than untreated plants. So, chemical changes are more likely to happen after silicon absorption.

Honeydew excretion is given as a measure of sap ingestion showing indirectly the rate of sap intake (Mittler 1958). Silicon-treated plants reduced honeydew excretion by aphids indicating a reduction in sap ingestion. EPG recording showed no reduction of the ingestion period, thus suggesting that silicon could have affected the ingestion rate. Aphids could also be able to retain sap inside the body and not totally excrete it as honeydew. This is an evidence that certain gustatory factor or mechanical blocking (i.e. p-protein or callose formation) impedes a continuous or sustained sucking in silicon-treated plants. Sap ingestion has been believed to be regulated by the period of sap ingestion rather than by changes in the ingestion rate; however this is not supported here (Tjallingii 1995, Prado & Tjallingii 1997). Most of the studies showed that a more suitable host or physiological plant stage increase sap intake and excretion (Auclair 1959, Spiller & Llewellyn 1987). Silica treated plants had a clear adverse effect on aphid development (poor host) and less honeydew excretion. Feeding delay and, by consequence, the time to the first droplet could not be completely responsible for the reduced honeydew excretion because longer honeydew record periods showed also a large difference between treatments (Table 3).

Probing behaviour studies showed a similar time spent by aphids in the sieve elements, but the biology studies indicated that development was strongly influenced by silicon application. These results suggest that silicon could also diminish the quality of phloem sap and affect aphid development. Studies considering induced resistance elicited by silicon could show new approaches to understand silicon physiological effect on plants and insects.

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