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Resistance in Rice to *Tibraca limbativentris* (Hemiptera: Pentatomidae) Influenced by Plant Silicon Content

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Resistance in rice to *Tibraca limbativentris* (Hemiptera: Pentatomidae) influenced by plant silicon content

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Abstract

Tibraca limbativentris Stål (Hemiptera: Pentatomidae) is a major pest causing economic losses to rice cultivation in Brazil. The objectives of this work were to assess different sources of silicon in the induction of plant resistance, to examine the interaction of rice varieties with silicon-based resistance, and to determine the effects of varieties and silicon on stink bug biology. The interaction of rice cultivars BRS Esmeralda, IRGA 429 (widely cultivated in Brazil), and Canela de Ferro (a resistant cultivar), and 2 sources of silicon (K silicate, and Ca + Mg silicate) were studied. Plant parameters measured were percentage of damaged stems, chlorophyll content at 50 and 65 d after emergence, and silicon content. Insect biological parameters measured were the number of live insects, total dry mass, individual insect dry mass, total body surface area, and individual insect body surface area. The interaction between BRS Esmeralda with K silicate and Ca + Mg silicate provided greater plant silicon content and resulted in a lower level of stink bug-damaged stems. These results show that it is possible to increase resistance in rice plants susceptible to *T. limbativentris* by increasing silicon content.

Key Words: rice stalk stink bug; plant resistance to insects; induced resistance; *Oryza sativa*

Resumen

Tibraca limbativentris Stål (Hemiptera: Pentatomidae) es una plaga importante que causa pérdidas económicas al cultivo de arroz en Brasil. El objetivo de este trabajo fue estudiar la interacción de variedades de arroz con diferentes niveles de resistencia y fuentes de silicio en la inducción de la resistencia de las plantas y el efecto sobre la biología de los chinches. Se estudió la interacción de los cultivares de arroz BRS Esmeralda, IRGA 429 (ampliamente cultivado en Brasil), y Canela de Ferro (Resistente), y 2 fuentes de silicio (silicato de K y silicato de Ca + Mg). Los parámetros de la planta medidos fueron: el porcentaje de tallos dañados, el contenido de clorofila a los 50 y 65 días después de la emergencia y el contenido de Si. Los parámetros biológicos de insectos medidos fueron: el número de insectos vivos, la masa seca total, la masa seca de insecto individual, la superficie corporal total y la superficie corporal de insectos individual. La interacción entre BRS Esmeralda con silicato K y silicato de Ca + Mg proporcionó más contenido de silicio vegetal y resultó en un menor porcentaje de tallos dañados por el chinche hedionda. Las variedades de arroz con diferentes niveles de resistencia y el aumento del contenido de silicio en la planta es una opción prometedora en el control de *T. limbativentris*. Estos resultados muestran que es posible aumentar la resistencia en las plantas de arroz susceptibles a *T. limbativentris* al aumentar el contenido de silicio.

Palabras Clave: chinche de arroz; planta resistencia a insectos; resistencia inducida; *Oryza sativa*

Among the principal factors that contribute to the reduction of productivity in rice cultivation in Brazil are the rice stink bugs that attack the stem and the panicle of the plant (Souza et al. 2009; Quintela et al. 2013). The predominant species in Brazil and other South American countries include the rice stalk stink bug, *Tibraca limbativentris* (Stål) (Hemiptera: Pentatomidae), and the panicle feeding stink bugs, *Oebalus poecilus* (Dallas) and *Oebalus ypsilongriseus* (De Geer) (Hemiptera: Pentatomidae) (Pantoja et al. 2007). The first species is the most economically significant pest, causing a reduction in rice yield of up to 80% (Ferreira et al. 1997).

The adult of *T. limbativentris* is brown with pale yellowish lateral margins of the body, and a long head with piercing-sucking mouthparts (Fernandes & Grazia 1998). Oviposition occurs on the surface of the leaves, the eggs becoming greenish and darker with development (Souza et al. 2009). The insect prefers the lower part of the rice stem for feeding and reproduction (Souza et al. 2009). Damage is first observed beginning with the second instar by feeding at the base of the plant, which causes dead tillers (dead hearts) in the vegetative stage and unfilled panicles (whiteheads) in the reproductive stage (Costa & Link 1992; Pazini et al. 2012).

Control of this insect mostly involves the application of chemical insecticides that cause undesirable effects on the environment, nontarget organisms, and human health (Sosa-Gómez & Silva 2010; Krinski & Foerster 2016). However, with the current high populations of this

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pest in Brazilian rice fields, there is a need to apply sustainable control practices compatible with an Integrated Pest Management (IPM) program. The use of resistant plants is an important strategy in reducing the population of the pest. The induction of resistance by increasing the silicon content in plants is another promising tactic in IPM (Ferreira et al. 2011; Lemes et al. 2011; Cruz et al. 2012).

Silicon confers resistance to insects in plants by its deposition on the cell wall, forming a mechanical barrier to insect penetration and feeding (Datnoff et al. 1991; Savant et al. 1997; Goussain et al. 2002; Sidhu et al. 2013), by being related to specific plant defense reactions (Chérif et al. 1992), or by acting as an elicitor of the induced resistance process (Fawe et al. 2001; Gomes et al. 2005; Reynolds et al. 2016). Plants treated with silicon display resistance to sucking insects (Gomes et al. 2005; Costa et al. 2011; Almeida et al. 2015; Souza et al. 2016). The application of silicon contributed to an increase in non-preference for feeding by the aphid *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) in maize (Almeida et al. 2015). In resistant soybean cultivars IAC 17 and IAC 100, treated with Ca + Mg silicate and K silicate, the life cycle of *Euschistus heros* (F.) (Hemiptera: Pentatomidae) was prolonged (Souza et al. 2016). Silicon amendment reduced densities of the water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), larvae in rice (Villegas et al. 2017). Sidhu et al. (2013) observed a significant increase in the silicon content of rice plants supplemented with Ca silicate, and this silicon amendment led to lower relative growth rates and reduced boring success of *Diatraea saccharalis* (Fabricius), (Lepidoptera: Crambidae) in rice cultivars. Thus, silicon-based plant resistance can be environmentally benign and economically feasible. The objective of this research was to study the interaction of rice varieties with different levels of resistance treated with silicon sources, and to assess the induction on the biology of the rice stalk stink bug, *T. limbativentris*.

Materials and Methods

PLANT GROWTH AND SILICON TREATMENT

Plants for the experiment were grown under greenhouse conditions at the Agricultural Entomology Laboratory of the Goiano Federal Institute, Urutaí, Goiás, Brazil. The experiment consisted of 9 treatments (3 sources of silicon × 3 rice cultivars) and 5 replicates arranged in a completely randomized design. The 3 rice cultivars used were BRS Esmeralda and IRGA 429 cultivars adopted by producers of this crop, and Canela de Ferro, which is reported to resistant to rice pests and is being adopted in breeding programs for plant resistance to insects (Terra et al. 2015).

To assess the induction of resistance in rice cultivars mediated by silicon, the following treatments were applied: T1 – plant sprayed to the point of runoff with a 1% K silicate solution (12% SiO_2 and 12% K_2O) (vol of 20 mL per pot), T2 – application of Ca + Mg silicate (CaMgSiO) to the soil (1.5 g per pot or 600 kg per ha^{-1}), and T3 – control (without application of silicon).

INSECT REARING

The colony of *T. limbativentris* began from the insects obtained from the National Rice and Beans Research Center of the Brazilian Agricultural Research Corporation (EMBRAPA Rice and Beans) located at Santo Antônio de Goiás, Góias, Brazil. The insects were reared on the rice cultivar BR IRGA 409. After oviposition, eggs were collected and transferred to acrylic type 350 mL germination boxes (11.0 × 11.0 × 3.5 cm, CasaLab, Belo Horizonte, Minas Gerais, Brazil) lined with moistened paper towels and kept in the laboratory with a range of 25 ± 2 °C, 70 ± 10% RH, and 14:10 h (L:D) photoperiod until the nymphs hatched. The nymphs remained under these conditions until the second instar. Then the insects were transferred to organdy fabric cages containing 28 to 30 rice stems at 25 to 30 d after sowing. They remained there until oviposition in the adult stage. The plants were inspected every 2 d for removal of dead insects, and eggs.

SOWING OF CULTIVARS

Cultivar seeds were sown in plastic trays containing commercial substrate (Bioplant, Nova Ponte, Minas Gerais, Brazil). At 13 d after sowing the plants were transplanted to 5 L pots containing substrate composed of a mixture of soil, sand, and bovine manure in the proportion of 2:1:1. The pots were kept in a greenhouse and were not sprayed with insecticides. The plants received the recommended fertilization for rice cultivation (Sousa & Lobato 2004) and were irrigated daily according to the water requirement.

PARAMETERS RELATED TO RICE PLANTS

For the rice plant parameters, the following variables were evaluated: percentage of damaged stems, percentage of normal stems, relative chlorophyll index at 50 and 65 d after emergence, and silicon content in the aerial part of the plant.

The percentage of damaged stems was obtained based on the total stems minus the undamaged stems divided by the total stems and multiplied by 100. The non-visible dead heart symptom was detected by making a cut on all plant stems to see if the central leaf was dead inside. The numbers of damaged stems were obtained by adding together the stems with visible and non-visible dead hearts.

To determine the silicon content in the aerial part of the plant, leaves and stems were randomly collected in the treatments. The leaves and stems were removed, packed in a paper bag, and placed in an oven for 48 h at 65 °C for drying. After the drying process the plant material was ground to a powder in a Wiley-type grinder (Tecnal, Piracicaba, São Paulo, Brazil). These samples were sent to the Fertilizer Laboratory of the Federal University of Uberlândia, Minas Gerais, Brazil, for silicon content analysis (Korndörfer 2004).

The Falker relative chlorophyll index was obtained from the average of measurements in 3 leaflets of the second leaf from the apex, using a ChlorofiLog (Model CFL 1030, Falker, Porto Alegre, Rio Grande do Sul, Brazil) (Barbieri Junior et al. 2012).

BIOLOGICAL PARAMETERS OF *TIBRACA LIMBATIVENTRIS*

Rice plants at 35 d after sowing were infested with 10 nymphs of second instar *T. limbativentris* at 48 h after hatching. The nymphs were conditioned on the stem of the rice plant and were protected with an organdy fabric cage to prevent escape and attack by natural enemies. Second instars were used because nymphs of *T. limbativentris* are aggregated until the end of the first instar, and feeding on the plant begins in the second instar. In addition, high natural mortality occurs in the first instar, especially when the nymphs are handled. Each pot containing 1 plant corresponded to 1 replicate, totaling 5 replicates in a completely randomized design.

The experiment was finished at 43 d after infestation, and the following biological parameters were assessed: number of live insects, total dry mass of the insect, individual dry mass, total body surface area, and individual body surface area. The dry mass and the body surface area were obtained by dividing the total of the dry mass and the total of the body surface area by the number of live insects in each cage. To quantify the body size of the insect, an analytical balance (Mars Model

AY220-CQA, Quimica Paulinia, São Paulo, Brazil) was used to determine mass, and the body surface area was estimated with a digital caliper (Zaas Absolute Model 6, Mitutoyo, São Paulo, São Paulo, Brazil) by measuring and multiplying the length and width of each insect.

STATISTICAL ANALYSIS

The data were analyzed as a factoral experiment to assess the effect of the cultivars, and resistance inducers, and the interaction between them. The residual normality and homoscedasticity were checked by applying Shapiro-Wilk and Bartlett tests. When the data did not meet these assumptions, the Box-Cox method was used in order to find an optimal transformation. The transformed data were used to fit the analysis of variance models and the means compared using Tukey's test ($\alpha = 0.05$). The means were back-transformed for presentation purposes. All analyses were performed using R software, version 3.2.2 (R Core Team 2017).

Results

The effects of resistance inducers on the growth and survival of the insects, and damage to the rice cultivars, are presented below. For the cultivars, there was a significant difference for the percentage of damaged stems ($F_{2, 32} = 6.10$; $P = 0.0057$), relative chlorophyll index at 50 d after emergence ($F_{2, 18} = 5.66$; $P = 0.0140$), and silicon content ($F_{2, 32} = 6.74$; $P = 0.0032$). In relation to resistance inducers, there was a significant difference for percentage of damaged stems ($F_{2, 32} = 3.92$; $P = 0.0299$), and silicon content ($F_{2, 32} = 6.51$; $P = 0.0038$). There was a significant interaction between the cultivars and resistance inducers for percentage of damaged stems ($F_{2, 32} = 2.54$; $P = 0.0590$), and silicon content ($F_{2, 32} = 3.13$, $P = 0.0262$) (Table 1).

The level of damaged stems averaged higher in the cultivar IRGA 429 (74.4), which differed statistically from BRS Esmeralda (43.67). Among the resistance inducers, K silicate seemed most effective, producing a significantly lower level of stem damage relative to the control. The relative chlorophyll index at d 50 was higher in BRS Esmeralda (46.52) relative to Canela de Ferro (40.31). The silicon content in the cultivar BRS Esmeralda (2.93) was higher than in Canela de Ferro (2.57). The Ca + Mg silicate produced the highest silicon content in rice plants (2.96).

In the interaction of cultivars and resistance inducers for percentage of damaged stems, the cultivar Canela de Ferro ($F_{2, 32} = 0.84$; $P = 0.4429$) was not influenced by resistance inducers (Fig. 1). However, the cultivars BRS Esmeralda ($F_{2, 32} = 5.56$; $P = 0.0084$) in combination with K silicate (33.33) and Ca + Mg silicate (25.00), and IRGA 429 ($F_{2, 32} = 2.60$; $P = 0.0895$) treated with K silicate (59.05) had the lower percentages of damaged stems compared to controls (72.67 and 93.33, respectively) (Fig. 1).

In the interaction of rice cultivars and resistance inducers in relation to silicon content, the silicone content in the cultivar IRGA 429 ($F_{2, 32} = 0.97$; $P = 0.3900$) was not influenced by the inducers (Fig. 2). The cultivars BRS Esmeralda ($F_{2, 32} = 7.26$; $P = 0.0025$) and Canela de Ferro ($F_{2, 32} = 5.62$; $P = 0.0081$) treated with Ca + Mg silicate had the highest silicon content (3.32 and 2.89, respectively) (Fig. 2).

The number of surviving insects ($F_{2, 32} = 4.11$; $P = 0.0292$), individual dry mass ($F_{2, 32} = 3.05$; $P = 0.0662$), total body surface area ($F_{2, 32} = 2.69$; $P = 0.0884$), and individual body surface area ($F_{2, 32} = 2.68$, $P = 0.0890$) were influenced by the resistance inducers (Table 2). The numbers of surviving insects were significantly greater in the control (5.33), as compared to the K silicate treatment (2.67). Individual dry mass was higher in the insects fed on the control plants (0.12) and lower in the K silicate treatment. Individual body surface area was higher in insects from the control treatment as compared to those fed on plants treated with K silicate, and total body surface area also was higher in the control compared with K silicate (Table 2).

Discussion

The rice stalk stink bug, *T. limbativentris*, occurs in all rice producing regions of Latin America, and causes significant losses in rice production (Ferreira et al. 1997; Krinski & Foerster 2016). Despite the importance of the pest, few studies in the development of IPM management strategies have been conducted, including the induction of resistance of rice plants to insects.

This study was conducted to determine whether silicon, applied as a plant spray or as a soil application, can be used to induce resistance in rice plants to the rice stalk stink bug, *T. limbativentris*, and its potential as a tool to effectively manage field populations of the stink bug in an integrated approach.

Table 1. Percentage of damaged stems (PDS), relative chlorophyll index at 50 and 65 d after emergence (DAE) (RCI), and silicon content (silicon content %) in 3 rice cultivars treated with different silicon sources. Urutaí, Goiás, Brazil.

Cultivar (C)	PDS	RCI (50 DAE)	RCI (65 DAE)	Silicon content (%)
1 – Canela de Ferro	60.7 ± 7.4 ab	40.3 ± 1.5 b	41.0 ± 1.6	2.6 ± 0.1 b
2 – BRS Esmeralda	43.7 ± 9.3 b	46.5 ± 0.9 a	43.8 ± 1.8	2.9 ± 0.1 a
3 – IRGA 429	74.4 ± 6.9 a	43.3 ± 1.4 ab	37.3 ± 1.6	2.7 ± 0.1 ab
F (C)	6.10	5.66	3.05	6.74
P valor (C)	0.0057	0.0140	0.0756	0.0032
Inducers (I)				
1 – Control	73.3 ± 6.3 a	45.09 ± 1.5	41.46 ± 1.9	2.6 ± 0.1 b
2 – K Silicate	49.5 ± 8.6 b	41.14 ± 1.7	39.69 ± 2.2	2.6 ± 0.5 b
3 – Ca + Mg Silicate	55.9 ± 9.5 ab	43.76 ± 1.2	41.00 ± 1.5	2.9 ± 0.1 a
F (I)	3.92	2.36	0.25	6.51
P value (I)	0.0299	0.127	0.7836	0.0038
Interaction (C × I)				
F (C × I)	2.54	0.31	0.45	3.13
P value (C × I)	0.0590	0.8694	0.7694	0.0262
C.V. (%)	40.51	9.05	13.58	11.01

Means followed by the same letter in the column do not differ statistically from each other according to the Tukey test at 0.05% probability. C.V. = coefficient of variation.

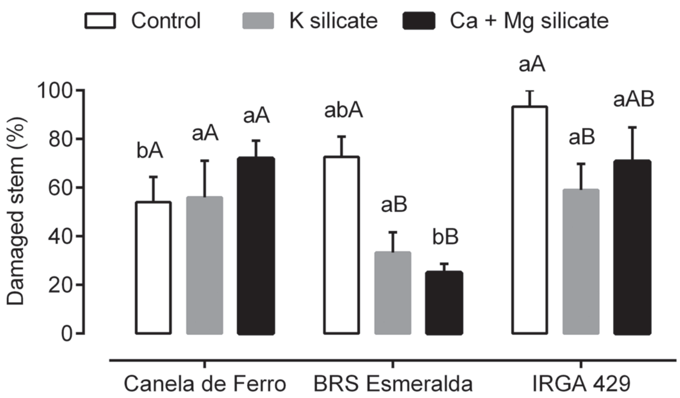

Fig. 1. Percentage of damaged stems (PDS) in 3 rice cultivars treated with different sources of silicon. Urutaí, Goiás, Brazil. 2016. Means followed by the same capital letter (inducers) and lower case letters (cultivars) in the columns do not differ statistically from each other according to the Tukey test at 0.05% probability.

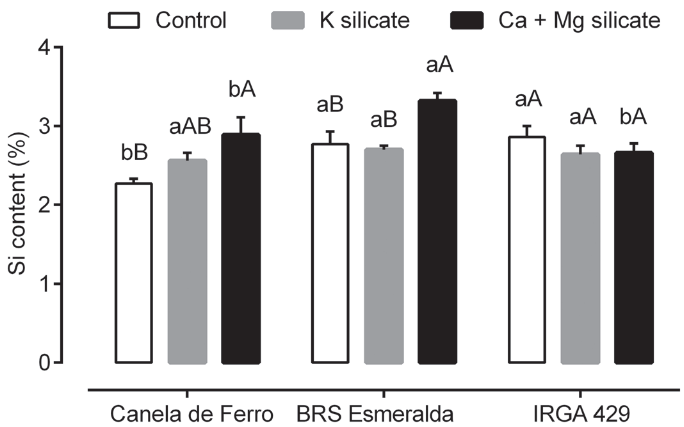

Fig. 2. Percentage of silicon content in 3 rice cultivars treated with different sources of silicon. Urutaí, Goiás, Brazil. 2016. Means followed by the same capital letter (inducers) and lower case letters (cultivars) in the columns do not differ statistically from each other according to the Tukey test at 0.05% probability.

The application of silicon to the soil or as sprayed on the plant increased the silicon content in the rice plant, and consequently plants were more resistant to *T. limbativentris*. This induction of resistance can be observed by the lower value of percentage of damaged stems in the rice cultivars, especially in BRS Esmeralda. The lower percentage of damaged stems in the BRS Esmeralda cultivar may be related to the increase of silicon content in the plant that forms a mechanical barrier, making it difficult for *T. limbativentris* to inject its stylets into the rice stem (Datnoff et al. 1991; Savant et al. 1997; Goussain et al. 2002). Another parameter that can explain the resistance in BRS Esmeralda is associated with the highest chlorophyll index, especially in plants treated with silicon. The increase in the chlorophyll content of plants has been reported to be a mechanism of plant tolerance to insects (Koch et al. 2016; Jesus et al. 2018).

The incorporation of Ca + Mg silicate increased the silicon content in the leaves of the BRS Esmeralda cultivar by 19.9% compared to the control plants. This increase in silicon content is similar to that found by Sidhu et al. (2013), and increase of 32% in the Cocodrie rice cultivar and 17% in XL723. Hou and Han (2010) observed an increase of silicon in rice plants from 15 to 20% in susceptible cultivars, and 15 to 24% in cultivars resistant to *Chilo suppressalis* Walker (Lepidoptera: Crambidae). These variations in silicon content in the rice plant are influenced by factors such as difference in silicon content in the cultivars, method of application, source of silicon used, and the method of analysis used to quantify the silicon content in the plant (Moraes et al. 2005; Chandramani et al. 2010; Sidhu et al. 2014).

Increase of silicon levels in rice plants can increase the level of resistance to important pests in rice, especially the stem borers. Sidhu et al. (2014) observed an increase in silicon content in leaves of rice cultivars Cocodrie and XL723 supplemented with calcium silicate, and reduction in the number of damaged stems by *D. saccharalis*. Hou and Han (2010) observed an increase in silicon content in rice leaves supplemented with calcium silicate and a decreased stem penetration of the Asiatic rice borer, *C. suppressalis*, in the cultivar Yangfeng 47 (moderately resistant).

Tibraca limbativentris feeding on rice plants treated with K silicate and Ca + Mg silicate were negatively influenced relative to the control treatment. Insects feeding on these plants presented the lowest value of the number of live insects, individual dry mass, total body surface area, and individual body surface area. The negative responses of *T.*

Table 2. Average number of live insects (NLI), total insect dry mass (TDM), individual dry mass (IDM), total body surface area (TBS – mm^2), and individual body surface area (IBS – mm^2) of *Tibraca limbativentris* fed on 3 rice cultivars treated with different sources of silicon. Urutaí, Goiás, Brazil.

Cultivar (C)	NLI	TDM	IDM	TBS	IBS
1 – Canela de Ferro	4.4 ± 0.7	0.5 ± 0.1	0.1 ± 0.0	429.1 ± 68.2	97.2 ± 1.7
2 – BRS Esmeralda	3.7 ± 0.8	0.4 ± 0.1	0.1 ± 0.0	334.2 ± 72.1	93.1 ± 1.9
3 – IRGA 429	4.0 ± 0.7	0.4 ± 0.1	0.1 ± 0.0	373.7 ± 63.1	93.6 ± 2.4
F (C)	0.33	0.53	0.14	0.54	1.44
P value (C)	0.7250	0.5965	0.9863	0.5917	0.2575
Inducers (I)					
1 – Control	5.3 ± 0.6 a	0.5 ± 0.1	0.1 ± 0.1 a	483.1 ± 63.1 a	98.1 ± 2.3 a
2 – K Silicate	2.7 ± 0.6 b	0.3 ± 0.1	0.1 ± 0.1 b	270.6 ± 58.1 b	92.8 ± 2.1 b
3 – Ca + Mg Silicate	4.1 ± 0.7 ab	0.5 ± 0.1	0.1 ± 0.1 ab	383.5 ± 69.1 ab	93.1 ± 1.3 ab
F (I)	4.11	1.22	3.05	2.69	2.68
P value (I)	0.0292	0.3126	0.0662	0.0884	0.0890
Interaction (C × I)					
F (C × I)	1.98	1.12	1.66	1.50	1.50
P value (C × I)	0.1303	0.3717	0.1927	0.2321	0.2345
C.V. (%)	56.61	65.05	23.88	59.26	06.82

Means followed by the same letter in the column do not differ statistically from each other according to the Tukey test at 0.10% probability.

limbativentris to the silicon treatments may be related to reduction of the feeding period due to the silicon accumulation in the cell wall of the plants, and the formation of the silica protective layer (Ma et al. 2001; Massey et al. 2006; Massey & Hartley 2009). Korndörfer et al. (2011) observed higher mortality of *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) nymphs in the sugarcane cultivar SP79-1011 that also presented the highest silicon content in the leaves. Moraes et al. (2005) observed a reduction in the feeding preference of *R. maidis* for maize treated with silicon applied through soil and foliar application.

The induction of resistance is a promising option in the management of *T. limbativentris*. This approach should be further evaluated under field conditions with natural populations of *T. limbativentris*.

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