Effects of Silicon Fertilizer Application to Rice on Larval Feeding and Adult Oviposition Preference of the Rice Leaf Folder (Cnaphalocrocis medinalis): Postprint

Authors: Han Yongqiang, Gong Shaolong, Wen Lizhang, Hou Maolin

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Abstract

Using the insect-susceptible rice variety TN1, three silicon application levels were established: high silicon (0.32 g Si/kg soil), low silicon (0.16 g Si/kg soil), and a no-silicon control (0 g Si/kg soil), to investigate the effects of silicon fertilizer application on oviposition and feeding preference of the rice leaf folder (Cnaphalocrocis medinalis). The results demonstrated that the feeding preference of rice leaf folder larvae for silicon-treated rice leaves, as well as the egg load and oviposition rate of adults on silicon-treated rice, were significantly lower than those on control rice. The silicon content, soluble sugar content, and carbon-to-nitrogen ratio in leaves of high-silicon-treated rice were higher than those in the control, whereas nitrogen content was lower than the control; the carbon-to-nitrogen ratio in leaves of low-silicon-treated rice was higher than the control, and nitrogen content was lower than the control. Concurrently, silicon treatment significantly reduced both the percentage of plants with rolled leaves and the leaf rolling rate in rice. These results indicate that silicon application can enhance the non-preference of rice leaf folder for rice, thereby enhancing rice resistance to rice leaf folder.

Full Text

Effects of Silicon Fertilizer Application to Rice Plants on Feeding and Oviposition Preference of the Rice Leaf Folder, *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae)

Yongqiang Han[1], Shaolong Gong[2], Lizhang Wen[2], Maolin Hou[1]

[1]State Key Laboratory of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

[2]College of Plant Protection, Hunan Agricultural University / Southern Regional Collaborative Innovation Center for Grain and Oil Crops, Changsha 410128, China

Abstract

Using the susceptible rice variety Taichung Native 1 (TN1), we investigated the effects of silicon amendment on oviposition and feeding preference of the rice leaf folder at three silicon application rates: 0.32 g Si/kg soil (high rate), 0.16 g Si/kg soil (low rate), and 0 g Si/kg soil (control). In preference tests, larval settlement and egg deposition on silicon-amended rice plants were significantly reduced at both low and high silicon rates compared with the control. High-rate silicon amendment significantly increased silicon and soluble sugar contents and the C/N ratio while decreasing nitrogen content in rice leaves compared with the control; low-rate silicon addition significantly increased the C/N ratio and decreased nitrogen content. Damage rates were significantly lower in rice plants amended with silicon at the high rate than in control plants. These results indicate that silicon addition to susceptible rice plants could enhance resistance to the rice leaf folder through reduced feeding and oviposition preference.

Keywords: rice leaf folder; rice; silicon; feeding preference; oviposition preference

Introduction

The rice leaf folder (LF), *Cnaphalocrocis medinalis* (Guenée), is one of the most destructive rice pests in Asian rice-producing countries. Recently, major outbreaks of LF have been reported in many Asian countries including China, severely threatening rice production. Rice varieties resistant to LF exhibit either high silicon cell density or high silicon content in the leaf blade. Currently, LF populations are principally managed with chemical insecticides that have caused high residual toxicity, insecticide resistance, and pest resurgence. Alternatively, cultivar resistance and crop management practices including silicon amendment are being developed.

There is increasing evidence showing a positive association between high plant silicon content and resistance to insect herbivory in both monocots and dicots. Silicon amendment can influence insect herbivores in several ways, including acting as a physical barrier, deterring feeding behavior, reducing food consumption efficiency and performance, and affecting herbivores at a transcriptional level through differential regulation of plant genes. In the present paper, we report the effects of silicon addition to rice plants on feeding and oviposition preference and damage by the LF.

Rice is a silicon-accumulating plant species. It is estimated that a rice crop producing 1,000 kg yield will remove 130 kg Si from the soil, twice as much as the combined absorption of nitrogen, phosphorus, and potassium. Although silicon is not an essential nutrient for plants, it plays an important role in enhancing plant disease and insect resistance. Most studies have shown that silicon fertilizer application can increase plant silicon content and thereby enhance plant resistance to herbivorous insects. For example, silicon application increases leaf roughness in grasses, hinders leaf-feeding insects, reduces food assimilation efficiency, changes leaf surface characteristics, and promotes the synthesis and release of plant volatile secondary substances, thereby affecting herbivorous insect oviposition and feeding preference. A notable characteristic of rice varieties resistant to LF is high leaf silicon content or high silicon cell density. Rice has the ability to actively absorb and accumulate silicon, suggesting that silicon fertilizer application could enhance rice resistance to insects. This study investigated the effects of silicon amendment on LF oviposition and feeding preference to provide a theoretical basis for regulating LF populations through silicon fertilization and integrated pest management.

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1. Rice Cultivation and Silicon Treatment

The test rice variety was Taichung Native 1 (TN1). Seeds were soaked in warm water for 72 h, then placed in an artificial climate chamber for germination before being sown in a seedbed. Seedlings were transplanted in pairs into plastic buckets (24.5 cm × 20 cm) containing 4.2 kg of homogenized, oven-dried sandy loam soil per bucket. The soil had the following properties: organic matter content 21.9 g/kg, available phosphorus 98.16 mg/kg, available potassium 322 mg/kg, and available silicon 0.21 g/kg.

Based on soil available silicon content and rice silicon requirements, silicon fertilizer (silicon-calcium-potassium fertilizer produced by Shanxi Fubang Fertilizer Industry Co., Ltd.; soluble silicon content 11.7%, CaO 25.0%, K O 4.0%, MgO 2.0%) was applied at three rates: (1) 0 g Si/kg soil (control); (2) 0.16 g Si/kg soil (low rate); and (3) 0.32 g Si/kg soil (high rate). All treatments received nitrogen at 0.35 g N/kg soil (including pure nitrogen from diammonium phosphate and urea) and phosphorus at 0.25 g P O /kg soil. Potassium application rates were identical across silicon treatments (including potassium from silicon-calcium-potassium fertilizer and potassium chloride). Diammonium phosphate was applied once as base fertilizer before transplanting (2/10 of total nitrogen). Silicon-calcium-potassium fertilizer was mixed with base fertilizer and applied once before transplanting (1/10 of total nitrogen). Potassium chloride was applied in two splits (4/10 and 3/10 of total nitrogen at respective growth stages), and urea was applied in four splits (2/10, 3/10, 1/10, and 1/3 of total nitrogen at respective growth stages). After fertilizer application, the soil was thoroughly mixed.

Potted rice seedlings were divided into two groups: one group was placed outdoors for natural infestation (without insecticide application), and the other was placed in a greenhouse. All potted seedlings were arranged in a completely randomized design and watered according to rice water requirements at different growth stages, with maximum water level not exceeding the bucket rim.

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2. Insect Source

Adult LF moths were captured with insect nets during peak emergence in late May at the Guilin Field Scientific Observation and Research Station for Pests (Xing' an County, Guangxi, China). Moths were placed in cylindrical mesh cages (120 cm × 100 cm) for mass mating. Rice plants with egg masses were selected from control potted rice at 30 days after transplanting. Leaves with eggs were cut and placed in Petri dishes lined with moist filter paper, with one end wrapped in absorbent cotton to maintain moisture. Dishes were placed in an artificial climate chamber at (28±1)°C, RH (70±5)%, and L:D = 16:8 h. Egg hatching was observed daily. Neonate larvae or 3rd-instar larvae were used for experiments; remaining larvae were reared on control rice leaves until pupation. Emerged adult males and females were reserved for subsequent experiments.

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3. Effects of Silicon Treatment on Soil Silicon Content

Soil samples were collected before transplanting and after transplanting for silicon content determination. After transplanting, three potted rice plants were randomly selected per treatment, and soil from different pots within the same treatment was combined after sampling. Soil samples were oven-dried at 105°C to constant weight, then passed through a 100-mesh sieve. Soil silicon content was determined using the acetic acid buffer extraction method [14].

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4. Effects of Silicon Treatment on Rice Leaf Silicon Content and C/N Ratio

Leaves at the same leaf position were cut from potted rice at 30 days after transplanting (the fourth leaf from the top after leaf differentiation was complete, including the heart leaf). Leaf samples were oven-dried at 105°C for 15 min, then at 70°C to constant weight. Dried leaves were ground with a food grinder and passed through a 100-mesh sieve for determination of silicon content [15], total nitrogen content, and soluble sugar content. Leaf total nitrogen and soluble sugar contents were determined using the Kjeldahl method and anthrone colorimetric method [16], respectively. The C/N ratio was calculated based on total nitrogen and soluble sugar contents.

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5. Preference of Neonate and 3rd-Instar LF Larvae for Silicon-Treated Rice

Different silicon treatments at 30 days after transplanting were selected. For each treatment, the fourth leaf from the top was cut into 30 cm lengths. One end of each leaf was wrapped with absorbent cotton to maintain moisture. Ten neonate larvae were placed in the center of a white circular plate (bottom lined with moist filter paper) with three leaves from different treatments arranged equidistantly. After 3 h, the number of larvae on each leaf was counted. The experimental method for 3rd-instar larvae was similar, except that larvae were placed at the center of the plate and observed until they settled on a rice leaf and began feeding, or observation was terminated after 30 min.

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6. Oviposition Preference of LF Adults for Silicon-Treated Rice

The oviposition preference of LF adults for silicon-treated rice was determined using the cage method. At 30 days after transplanting, robust tillers were selected from different silicon treatments. Three potted rice plants per treatment were arranged equidistantly in a circle. After carefully removing all other insects, plants were covered with cylindrical nylon mesh cages (120 cm $\times$ 100 cm) lined with cylindrical wire frames (123 cm $\times$ 103 cm). Ten pairs of LF adults were released into each cage and allowed to oviposit freely. The experiment was conducted under natural outdoor temperature and light conditions. After 72 h, egg numbers on each treatment were counted to calculate oviposition quantity and oviposition rate. The relative positions of rice plants within cages were rotated between replicates.

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7. Effects of Silicon Treatment on LF Damage

Potted rice plants at 30 days after transplanting were placed outdoors for natural infestation. Ten potted rice plants per treatment were randomly selected. The number of tillers and rolled leaves per pot were counted to calculate the percentage of leaf-rolled plants and leaf-rolling rate:
Percentage of leaf-rolled plants (%) = (Number of plants with rolled leaves / Total number of plants) $\times$ 100;
Leaf-rolling rate (%) = (Number of rolled leaves / Total number of leaves) $\times$ 100.

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8. Data Analysis

All experimental data were analyzed using SPSS 16.0 statistical software. One-way ANOVA was performed, and differences between treatments were compared using Tukey's test. Percentage data were arcsine square-root transformed before ANOVA to meet analysis requirements.

Results

1. Effects of Silicon Treatment on Soil Silicon Content There were significant differences in soil silicon content between pre-transplanting and post-transplanting samples ($F = 124.532$, $df = 3, 11$, $P < 0.001$). Compared with pre-treatment levels, soil silicon content in high-silicon and low-silicon treatments increased by 48.6% and 22.7%, respectively, while silicon content in control pots decreased by 9.3%.

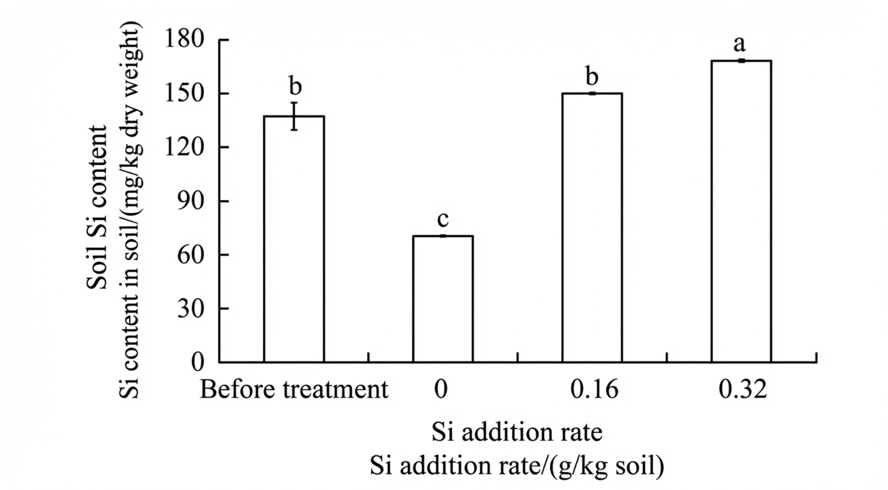

Figure 1: Figure 1

Soil silicon contents before and 60 d after Si addition

2. Effects of Silicon Treatment on Rice Leaf Silicon Content and C/N Ratio Silicon treatment significantly affected rice leaf silicon content, nitrogen content, soluble sugar content, and C/N ratio. High-silicon treatment increased leaf silicon content by 30.9% and soluble sugar content by 26.7% compared with the control ($F = 10.185$, $df = 2, 8$, $P = 0.012$; $F = 13.255$, $df = 2, 8$, $P = 0.006$, respectively). Both high- and low-silicon treatments significantly decreased leaf nitrogen content by 15.2% and 8.3%, respectively ($F = 27.257$, $df = 2, 8$, $P = 0.001$). Silicon amendment significantly increased the C/N ratio, with high- and low-silicon treatments increasing it by 42.8% and 39.6%, respectively, compared with the control ($F = 23.703$, $df = 2, 8$, $P = 0.001$).

[FIGURE:2] Effects of silicon addition to rice plants on contents of silicon, soluble sugar, nitrogen and C N ratio in rice leaves

3. Preference of LF Larvae for Different Silicon-Treated Rice The preference of neonate and 3rd-instar LF larvae for silicon-treated rice leaves decreased gradually with increasing silicon application rate, with significant differences among treatments (neonate: $F = 89.16$, $df = 2, 47$, $P < 0.001$; 3rd-instar: $F = 64.866$, $df = 2, 50$, $P < 0.001$). The selection proportion of neonate larvae for high-silicon and low-silicon treated leaves was 33.1% and 26.7% lower than for control leaves, respectively. For 3rd-instar larvae, the selection proportion for high-silicon and low-silicon treated leaves was 30.9% and 20.8% lower than for control leaves, respectively.

[FIGURE:3] Preference of C. medinalis larvae for rice leaves from plants with or without Si addition

4. Oviposition Preference of LF Adults for Different Silicon-Treated Rice Egg numbers and oviposition rates on silicon-treated rice were significantly reduced (egg number: $F = 17.936$, $df = 2, 26$, $P < 0.001$; oviposition rate: $F = 7.137$, $df = 2, 26$, $P = 0.004$). LF adult oviposition on high-silicon and low-silicon treated rice decreased by 45.3% and 27.6%, respectively, compared with the control. Oviposition rates on high-silicon and low-silicon treated rice decreased by 18.6% and 11.0%, respectively, compared with the control.

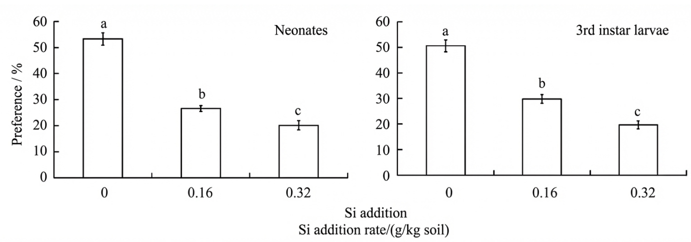

Figure 2: Figure 4

C. medinalis oviposition preference for silicon-treated and control rice plants

5. Effects of Silicon Treatment on LF Damage Silicon treatment significantly affected the percentage of leaf-rolled plants and leaf-rolling rate ($F = 7.716$, $df = 2, 44$, $P = 0.001$; $F = 6.215$, $df = 2, 44$, $P = 0.004$). The percentage of leaf-rolled plants and leaf-rolling rate in high-silicon treated rice were significantly reduced by 24.3% and 10.8%, respectively, compared with the control.

[FIGURE:5] Effects of silicon addition to rice plants on percentage of leaf-rolled plants and rolled leaves

Discussion

Plant resistance to insects is the manifestation of interactions between pests and plants under certain conditions. Plant resistance mechanisms include antibiosis, antixenosis (non-preference), and tolerance. Antixenosis refers to plant characteristics that deter insects from settling, ovipositing, or feeding. Plant surface morphology, chemical characteristics, and released volatile secondary substances can prevent insects from ovipositing or feeding on plants, thereby avoiding or reducing pest damage.

In this study, the selection proportion of neonate and 3rd-instar LF larvae for silicon-treated rice leaves was significantly lower than for control leaves. Meanwhile, silicon treatment significantly reduced the percentage of leaf-rolled plants, leaf-rolling rate, and LF oviposition quantity and rate. Rice is a typical silicon-accumulating plant that can actively absorb and concentrate silicon [13]. Silicon accumulates in epidermal cells of rice stems, leaf sheaths, and leaves, increasing tissue hardness and roughness and reducing plant digestibility [3, 17], which helps reduce leaf-rolling rates in silicon-treated rice.

Previous studies have investigated selection behavior of LF on different resistant rice varieties, showing that 3rd-instar larvae prefer susceptible varieties significantly more than resistant varieties [12, 18], and that LF adults oviposit significantly more on susceptible varieties than on resistant ones [18]. The mechanism by which silicon fertilizer enhances LF antixenosis to rice remains unclear. Some studies have shown that silicon deposition on leaf surface trichomes and other appendages changes leaf surface morphological characteristics [9] and increases the release of plant volatile secondary substances [10]. In this study, silicon amendment increased leaf silicon content, which may have altered leaf surface morphology and volatile secondary substance release, causing LF to avoid silicon-treated rice. This may be one reason for the reduced damage in silicon-treated rice.

Some studies have also indicated that silicon arrangement and deposition sites are more important than silicon content itself for impeding herbivore feeding. For example, wild rice has densely arranged epidermal silicon cells, while hybrid rice has loosely arranged silicon cells, with wild rice showing higher resistance to LF [19].

Silicon treatment significantly increased leaf soluble sugar content. Soluble sugars are major photosynthetic products in higher plants and the primary form of carbohydrate metabolism and temporary storage. They play important roles in plant metabolism and are important stress-regulating substances closely related to plant insect resistance [20-21]. Both excessively high and low sugar content in plant tissues are detrimental to insect growth and development, and high soluble sugar content can enhance plant resistance levels [22]. Silicon fertilizer application can promote photosynthate transport in rice leaves, significantly increasing leaf soluble sugar content, which may have a compensatory effect against pest feeding damage.

Silicon treatment significantly reduced leaf nitrogen content and increased the C/N ratio, thereby reducing the nutritional value of plants to herbivorous insects. High C/N ratios and low nitrogen content in plant tissues are detrimental to most herbivorous insect populations, leading to increased feeding, decreased fecundity, and other adverse effects [23]. The pathways through which silicon changes plant nutritional value to herbivorous insects require further research. Silicon amendment can also enhance rice antibiosis to LF by reducing food conversion efficiency [8].

In conclusion, silicon-treated rice leaves had increased silicon content, soluble sugar content, and C/N ratio, but decreased nitrogen content. Silicon amendment reduced LF larval feeding preference and adult oviposition quantity and rate, thereby decreasing the percentage of leaf-rolled plants and leaf-rolling rate. Silicon fertilizer application can enhance rice resistance to LF by reducing both larval and adult preference for silicon-treated plants. Silicon fertilizer application has multiple beneficial physiological effects on soil and rice, including enhanced disease resistance [24], lodging resistance [25], tolerance to extreme temperatures [26], and UV radiation resistance [27], improved water use efficiency [28], and promoted panicle development with increased rice yield and quality [17, 29]. Therefore, silicon fertilization can be used to comprehensively regulate rice growth and enhance resistance to LF.

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