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Induction of Caterpillar Resistance in Sunflower Using Silicon and Acibenzolar-S-Methyl

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ABSTRACT

The sunflower caterpillar *Chlosyne lacinia saundersii* (Lepidoptera: Nymphalidae) is considered a major pest in sunflower, causing severe defoliation and, thus, threatening production of oilseed. Chemical control remains the most widely used method for the management of this defoliator. To manage insect pests, there is a need to use substances of low toxicity that are able to stimulate the plant to use its own defense mechanisms. Therefore, in this study, we evaluated the induction of resistance by silicon (Si) and acibenzolar-S-methyl (ASM) against *C. lacinia saundersii* in sunflower plants. The bioassay was conducted in a completely randomized design using 4 treatments and 10 replicates. The treatments were: (1) Si; (2) ASM; (3) Si+ASM, and (4) the control. The biological parameters of the caterpillar and the accumulation of Si and lignin in plants were evaluated. The application of resistance inducers reduced weight of the caterpillars 10 and 15 days of age. The use of Si alone or Si+ASM promoted the accumulation of this element; however, only ASM increased lignin content in plants. Negative correlations between the silicon content and larval weight and between the silicon content and lignin content were observed. Therefore, the application of silicic acid or ASM can inhibit the development of *C. lacinia saundersii*, conferring a resistance in plants attributable to the accumulation of silicon and lignin, thus serving as an alternative approach that may potentially be integrated into the management of this key pest in sunflower crops.

Keywords: Benzothiadiazole, *Chlosyne lacinia saundersii*, *Helianthus annuus* L., Integrated pest management, Silicic acid.

INTRODUCTION

The economic significance of sunflower has recently increased at the global level due to its possibility of producing renewable and cleaner fuel and providing essential oils for human consumption (Silva and Freitas, 2008; Ghobadian *et al*., 2008). Farmers have also increased their efforts in controlling insect pests in the field, including the sunflower caterpillar *Chlosyne lacinia saundersii* (Doubleday and Hewitson, 1849) (Lepidoptera: Nymphalidae).

The species *C. lacinia saundersii*, considered a key sunflower pest in Brazil, is a polyphagous insect that can be found in several countries. Further, it is associated with many plant species, especially those of the Asteraceae family, which includes the Mexican sunflower *Tithonia diversifolia* (Ambrósio *et al*., 2008), Cobbler's pegs *Bidens pilosa* L., and the potato weed *Galinsoga parviflora* Cav. (Campos Farinha *et al*., 1997). Previous studies have demonstrated the preference of this defoliator for the sunflower *Helianthus annuus* L., over other plants such as corn, *Zea mays* L. (Antunes *et al*., 2010), and whitetop weed, *Parthenium hysterophorus* L. (Justus *et al.,* 2003). This caterpillar causes considerable damage to plants,

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particularly the leaves, and when the attack occurs within the plant age range of 50 to 70 days, production can be reduced by up to 80% (Gallo *et al*., 2002). It has been reported earlier that at stage R5.5, when 50% of the disc flowers are fertilized, plants are more sensitive to defoliation, with a reduction in the diameter of the capitulum, total seed biomass, and biomass of 100 achenes (Lima Júnior *et al*., 2010).

Although predators and parasitoids of *C. lacinia saundersii* can be naturally found in sunflower fields (Campos Farinha and Pinto, 1996), their population density is still considered too low to efficiently regulate the caterpillar population. Therefore, its control is still restricted to the use of harmful chemicals such as synthetic carbamate-based insecticides and commercial mixtures of benzoylurea+phosphorus or pyrethroid+neonicotinoid (Mapa, 2014).

Plant resistance to insects should be used in integrated pest management to reduce the impact of insect pests. However, no commercial sunflower variety resistant to insect pests has been established. Thus, induced systemic resistance may serve as a potential alternative that may be integrated into pest management schemes to minimize the use of insecticides. Induced systemic resistance is the phenomenon by which plants activate their defense mechanisms after exposure to an inducing agent (or elicitor) in a more or less generalized way and not just at the site of induction (Gozzo and Faoro, 2013). The elicitors of the induction process can be chemical activators, such as benzothiadiazole derivatives (Pereira *et al*., 2010) and silicic acid (Antunes *et al*., 2010), although there is evidence that silicon acts more as a resistance primer than as an elicitor itself (Kvedaras *et al*., 2010).

Research reports have described the effectiveness of acibenzolar-S-methyl (ASM) and silicon (Si) in inducing resistance to pests by causing physically- or chemically-based negative interference, among other effects, on the biology of the silverleaf whitefly, *Bemisia tabaci* biotype *B* (Gennadius, 1889) (Hemiptera: Aleyrodidae) on cucumber *Cucumis sativus* L. (Correa *et al*., 2005), on the feeding behavior of the cucurbit beetle *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae), on potato *Solanum tuberosum* L. (Assis *et al*., 2012), and on the reproduction of the aphid *Schizaphis graminum* (Rondani, 1852) (Hemiptera: Aphididae) on wheat *Triticum aestivum* L. (Pereira *et al*., 2010).

Thus, in the management of insect pests, based on the need for the use of substances with low toxicity and ability to stimulate the plant to use its own defense mechanisms, the aim of this work was to evaluate the induction of resistance in sunflower against *C. lacinia saundersii* by using Si and ASM.

MATERIALS AND METHODS

Bioassay

The experiments were conducted in a greenhouse and in the Laboratory of Plant Resistance to Insects, Department of Entomology, Universidade Federal de Lavras (UFLA), Lavras (latitude 21º45′S, longitude 45°00′W, and altitude 918 m), Minas Gerais, Brazil.

Plant Material

Sunflower triple hybrid DAS 735 seeds were planted in 5-kg polyethylene pots containing C horizon soil (Dark Red Latosol, LVe) fertilized with 1.25 g of NPK fertilizer (4:14:8)/pot, which was equivalent to 500 kg ha^{-1}. Four seeds were sown in each pot. Approximately 30 days after planting, top-dressing was performed using 0.5 g of ammonium sulfate/pot, which was equivalent to 200 kg ha^{-1}. Plants were arranged randomly on benches and watered daily.

Leaves with egg masses of *C. lacinia saundersii* were collected in sunflower fields, placed in Petri dishes (10-cm diameter), and kept in an incubator adjusted to a temperature of 25±2ºC, relative humidity of 70±10%, and photoperiod of 12 hours until hatching. The newly hatched caterpillars were transferred to a Petri dish (5-cm diameter) equipped with a lid and lined with filter paper. Two caterpillars were placed on each plate and for feeding, received sunflower leaf sections (2×2 cm) taken from the middle region of the plants, approximately 10 days after the last treatment application. Before being fed to caterpillars, the leaf sections were washed in distilled water, with any excess moisture blotted out using paper towels. The feed was changed every 2 days, with the leaf area measured before and after delivery to the caterpillars.

Experimental Design

We used a completely randomized design using 4 treatments and 10 replicates, with each replicate consisting of 5 plates. The treatments were as follows: (1) Si; (2) ASM; (3) Si+ASM; and (4) the control. Si was supplied in the form of silicic acid solution (1% $SiO_2·XH_2O$) (Vetec Química Fina, Duque de Caxias, Brazil) at a dose equivalent to 2 t ha^{-1} SiO_2. The commercial product Bion® 500 WG (Benzo [1,2,3] thiadiazole-7-carbothioic acid S-methyl ester, 0.1%) was used as a source of ASM (Syngenta Crop Protection, São Paulo, Brazil). The silicic acid and ASM were applied 15 days after plant emergence and reapplied after 5 days. The Si solution was prepared by diluting 5 g of silicic acid in 500 mL water pot^{-1}. The solution was poured around the stem of the plant to drench the soil. ASM was liberally sprayed until it was dripping from the plant using a 1.5 L manual sprayer with cumulative pressure and a tapered jet nozzle at a concentration of 1 g commercial product L^{-1} of water, which was equivalent to 0.45 mL $plant^{-1}$. The control pots received water of the same amount.

Parameters Evaluated

The following biological parameters were evaluated: (a) Larval stage: weights at 10 and 15 days of age, leaf area consumed, mortality, and duration of stage; (b) Pupal phase: weight at 24 h after formation, viability, and duration of phase; and (c) Adult phase: longevity and sex ratio.

Leaf consumption of the caterpillars was determined using the portable leaf area meter AM300 (ADC BioScientific Ltd., England). The unconsumed leaf debris was placed in paper bags (18×42 cm), identified, and dried in a 60ºC oven. The leaf debris was then ground in a micro-mill type Willye (TECNAL, Piracicaba, Brazil), placed in plastic bags (5×23 cm), labeled, and sent to the Laboratory of Fertilizers, Universidade Federal de Uberlândia, Institute of Agricultural Sciences, Minas Gerais, Brazil (UFU/LAFER) for the determination of Si, according to the methodology of Korndorfer *et al*. (2004a).

The pupae were weighed using a precision balance (TEPRON, São Paulo, Brazil) and transferred to plastic cups with lids (5 cm tall; 7 cm wide) until the emergence of adults. The adults received food from a cotton ball soaked in honey and water, which was placed in a 50 mL plastic cup and changed every 2 days to monitor the longevity of adults.

For lignin content analysis, unconsumed leaves were cut using scissors, placed in paper bags (18×42 cm), and dried in a 60°C oven. The dried leaf samples were then crushed, placed in plastic bags (5×23 cm), identified, and sent to the Laboratory of Plant Products, Department of Food Science at UFLA for lignin analysis using the methodology described by Van Soest (1967).

Statistical Analysis

Data were subjected to ANOVA and the generated mean values were compared using the Scott–Knott test (P≤ 0.05). The counts and percentage of mortality were transformed into $\sqrt{X+0.5}$ and arcsine $\sqrt{X/100}$, respectively, before analysis. To identify significant variables, Pearson parametric linear correlation was performed. All analyses were performed using the statistical and genetic analysis software, SAEG 9.0 (Ribeiro Júnior, 2001).

RESULTS AND DISCUSSION

Table 1 shows the results of the analysis of the biological parameters of *C. lacinia saundersii*, with no significant differences (P> 0.05) among treatments for the duration of larval and pupal phases, pupal viability, longevity of the adult phase, and sex ratio. The lack of response of the sunflower caterpillar to the resistance inducers may be attributable to a complex and difficult to predict insect-plant-silicon relationship (Korndorfer *et al.*, 2004b), because the accumulation of silicon in the plant may not always affect the herbivore development (Reynolds *et al.*, 2009).

Our results are in accordance with the results of Goussain *et al.* (2002) and Santos *et al.* (2012), in which the application of different sources of Si in soil did not affect the larval and pupal stages of *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) on maize and *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) on tomato, respectively. However, many researchers have observed differences in biological parameters of pests such as *Alabama argillacea* (Huebner, 1818) (Lepidoptera: Noctuidae) on cotton (Tomquelski *et al.*, 2007) and *S. graminun* on wheat (Pereira *et al.*, 2010), by application of Si and ASM. Thus, Si, as an elicitor, can differentially affect insects belonging to specific feeding guilds (Keeping and Kvedaras, 2008).

Moreover, the application of Si and ASM alone or jointly caused significant reduction (P≤ 0.05) in the weight of the caterpillars at 10 days (Table 2); in particular, the application of Si resulted in a weight four-fold lower than that of the control plants. At 15 days, there was a 52.5% decrease in weight in caterpillars fed with Si-treated plants in comparison with the caterpillars that consumed untreated plants or plants simultaneously treated with both elicitors (Table 2). However, the treatments showed significant similarities (P> 0.05) in effects in terms of variables such as leaf area consumed, mortality of caterpillars, and pupae weight (Table 2).

Regarding the content of Si, the application of silicic acid alone or jointly with ASM favored the accumulation of this mineral (Figure 1). Furthermore, the use of ASM alone provided the highest lignin content in sunflower plants compared with the other treatments (Figure 2). Thus, the higher accumulation of Si and lignin may confer greater protection to sunflower plants against feeding herbivores, mainly defoliators such as *C. lacinia saundersii*.

As demonstrated, the resistance inducers did not affect the leaf area consumed by insects, but there was reduction in the weight of caterpillars, possibly due to changes in nutritional quality of the food in relation to the greater accumulation of Si and lignin observed in this work, which may

Table 1. Durations of the larval and pupal stages, pupal viability, longevity of adult phase, and sex ratio of *C. lacinia saundersii* subjected to different treatments.

Treatment	Duration of larval stage (Days) [a]	Duration of pupal stage (Days) [a]	Pupal viability (%) [a]	Longevity of adult phase (Days) [a]	Sex ratio [a]
Si	22.8 ± 0.74	3.6 ± 0.56	40.8 ± 13.97	9.0 ± 1.69	0.7 ± 0.21
ASM	21.4 ± 0.85	3.3 ± 0.52	36.8 ± 12.59	4.3 ± 1.33	0.6 ± 0.24
Si + ASM	21.2 ± 0.98	4.0 ± 0.64	60.2 ± 13.31	7.2 ± 1.86	0.4 ± 0.18
Control	21.7 ± 1.00	4.8 ± 0.41	72.9 ± 9.96	8.7 ± 1.47	0.8 ± 0.15
CV(%)	14.06	25.00	42.50	58.58	82.10

[a] No significant difference, *F* test (P> 0.05).

Table 2. Larval weights at 10 and 15 days, consumed leaf area, percentage of caterpillar mortality, and pupal weight of *C. lacinia saundersii* subjected to different treatments.

Treatment	Weight at 10 days (mg)[a]	Weight at 15 days (mg)[a]	Consumed leaf area (cm^2)[b]	Percentage of caterpillar mortality (%)[b]	Pupal weight (mg)[b]
Si	3.5 ± 0.20 d	21.5 ± 1.55 c	62.0 ± 3.80	79.0 ± 0.04	152.7 ± 5.06
ASM	6.7 ± 0.86 c	33.9 ± 1.97 b	59.1 ± 3.38	74.0 ± 0.04	147.5 ± 5.21
Si + ASM	10.3 ± 0.62 b	40.7 ± 3.37 a	47.1 ± 4.04	79.0 ± 0.03	124.7 ± 15.96
Control	14.7 ± 0.99 a	49.8 ± 3.89 a	50.0 ± 3.14	67.0 ± 0.04	132.0 ± 8.14
CV (%)	23.79	25.05	20.71	17.78	16.81

[a] Means followed by the same letter in the column do not differ significantly by the Scott–Knott test ($P \leq 0.05$), [b] No significant difference, *F* test ($P > 0.05$);

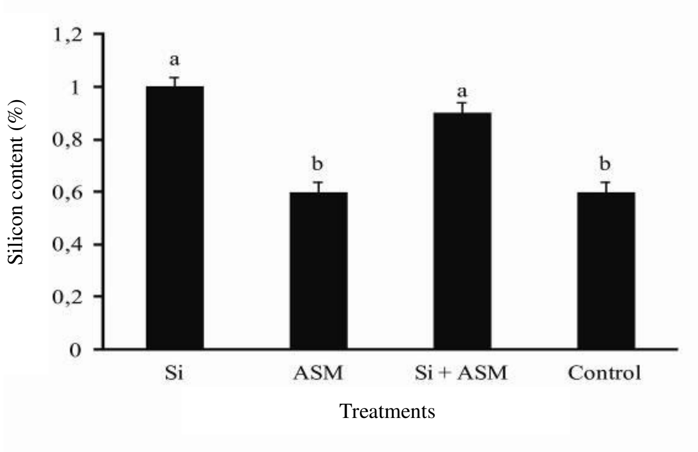

Figure 1. Si content (SiO_2 (%) ±SE) in sunflower leaf debris, subjected to different treatments, and not consumed by *C. lacinia saundersii*. Different letters in the columns indicate significant differences by Scott–Knott test ($P \leq 0.05$). Vertical bars indicate standard error (±).

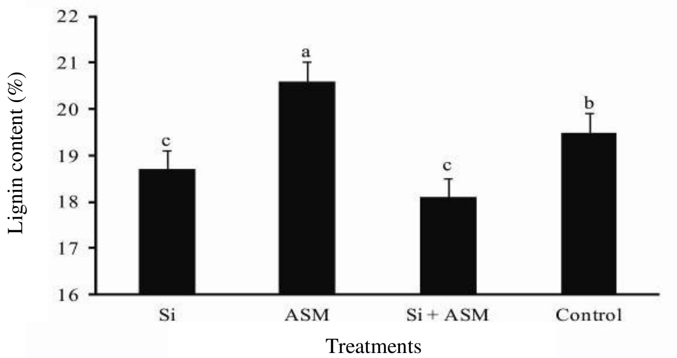

Figure 2. Lignin content (%) ±SE in sunflower leaves in pots subjected to different treatments. Different letters in columns indicate significant differences by the Scott–Knott test ($P \leq 0.05$). Vertical bars indicate standard error (±).

have altered the digestibility of the food.

In general, dicotyledons do not accumulate high amounts of Si compared with members of Poaceae. However, Kamenidou *et al.* (2008) observed higher concentrations of Si in the leaves, flowers, and stems of sunflowers, depending on the source of Si used. Similarly, Carvalho *et al.* (2009) showed that the application of potassium silicate resulted in an increase in Si content in leaves, roots, and flowers, confirming that sunflower can absorb and accumulate Si in its tissues (Sangster *et al*., 2001) and corroborating the results obtained in this work.

Similarly to what happens with the Si, lignin also acts against the attack of herbivores because the hardness of the lignified structures affects the nutritional quality of the food by reducing the digestibility of vegetal tissues (Vendramim and Guzzo, 2009) or by reducing insect biomass as reported in this work. In the soybean cultivar IAC-19, a high percentage of lignin was attributed to plants treated with ASM or Si (Moraes *et al*., 2009), however, in this work, ASM only contributed to accumulation of lignin.

Furthermore, significant linear correlations were established among the variables, with a negative correlation between the weight of 10- and 15-day-old caterpillars and lignin and Si content (Table 3), i.e. as the accumulation of Si increased in the plant, there was a reduction in weight of caterpillars and in lignin content.

The correlation between Si content and weight of caterpillars found here may be related to chemical causes because there was no difference in leaf area consumed by *C. lacinia saundersii* in spite of the accumulation of this element in the plants.

The results of this study suggest that the application of Si or ASM provide promising and viable alternatives that can be integrated into the management of *C. lacinia saundersii* in sunflower production.

CONCLUSIONS

The use of silicic acid or ASM inhibits the development of *C. lacinia saundersii*, thus conferring plant resistance by inducing accumulation of Si and lignin in plant tissues. This approach may potentially serve as an alternative technique that can be integrated into sunflower pest management.

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Table 3. Pearson parametric linear correlation between the Si content, weights of caterpillars at 10 and 15 days, and lignin content.

Parameter	Weight of caterpillars at 10 days[a]	Weight of caterpillars at 15 days[a]	Lignin content[a]
Si	-0.41	-0.45	-0.52
$P \leq 0.05$	0.0043	0.0016	0.0003

[a] Significant, *t* test ($P \leq 0.05$).

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القای مقاومت به لارو بالپولکی در آفتابگردان با استفاده از سیلیکون و Acibenzolar-S-Methyl

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چکیده

لارو بالپولکی آفتابگردان *Chlosyne lacinia saundersii* (Lepidoptera: Nymphalidae) از آفت های عمده این گیاه محسوب می شود که صدمات شدیدی به برگ ها وارد میکند و در نتیجه تولید روغن را در معرض تهدید قرار می دهد. روش کنترل شیمیایی رایج ترین روش مبارزه با این آفت برگخوار است. برای مبارزه با آفت های حشره ای کار برد مواد شیمیایی با مسمومیت کم که قادر باشند گیاه را تحریک به استفاده از سازوکارهای دفاعی خود نمایند ضروری می نماید. بنا بر این، در پژوهش حاضر، قدرت تحریک گیاه به وسیله سیلیکون (Si) و (acibenzolar-S-methyl (ASM بر علیه *C. lacinia saundersii* در آفتابگردان بررسی شد. آزمون زیستی (bioassay) با یک طرح آماری کاملا تصادفی با ۴ تیمار در ۱۰ تکرار انجام شد. تیمار ها عبارت بودند از (۱) Si، (۲) ASM، (۳) Si + ASM، و (۴) شاهد. اندازه گیری ها شامل پارامترهای زیستی لاروها و تجمع Si و لیگنین در گیاه بود. نتایج نشان داد که کار برد مواد محرک مقاومت گیاه وزن لاروهای بالپولکی را در سن ۱۰ و ۱۵ روزه کم کرد. کار برد Si به تنهایی یا Si + ASM منجر به تجمع این ماده در گیاه شد، با این وجود، فقط ASM مقدار لیگنین گیاه را افزایش داد. نیز، رابطه ای منفی بین محتوای سیلیکون و وزن لاروها و بین محتوای سیلیکون و محتوای لیگنین مشاهده شد. بنا بر این، کار برد سیلیسیک اسید یا ASM می تواند جلو رشد لارو *C. lacinia saundersii* را بگیرد و مقاومتی را در گیاه ایجاد کند که به تجمع سیلیکون و لیگنین نسبت داده می شود و از این قرار به عنوان روشی جایگزین قلمداد می شود که قابلیت دارد تا در مدیریت مبارزه با این آفت آفتابگردان به کار رود.