LEONARDO PACKER DE QUADROS

FOLIAR APPLICATION OF A SOLUBLE SOURCE OF SILICON, ALTERNATED OR COMBINED WITH FUNGICIDE, FOR SOYBEAN RUST MANAGEMENT

Dissertation submitted to the Plant Pathology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Fabrício de Ávila Rodrigues

VIÇOSA - MINAS GERAIS
2023

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

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Q1f
2023

Quadros, Leonardo Packer de, 2023-
Foliar application of a soluble source of silicon, alternated or combined with fungicide, for soybean rust management / Leonardo Packer de Quadros. – Viçosa, MG, 2023.
1 dissertação eletrônica (43 f.): il. (algumas color.).

Orientador: Fabrício de Ávila Rodrigues.
Dissertação (mestrado) - Universidade Federal de Viçosa, Departamento de Fitopatologia, 2023.
Referências bibliográficas: f. 25-31.
DOI: https://doi.org/10.47328/ufvbbt.2023.645
Modo de acesso: World Wide Web.

1. Plantas - Efeito dos fungicidas. 2. Silício. 3. Soja - Doenças e pragas - Controle. 4. Ferrugem da soja (Doença). I. Rodrigues, Fabrício de Ávila, 1974-. II. Universidade Federal de Viçosa. Departamento de Fitopatologia. Programa de Pós-Graduação em Fitopatologia. III. Título.

CDD 22. ed. 632.952

Bibliotecário(a) responsável: Euzébio Luiz Pinto CRB-6/3317

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APPROVED: July 27, 2023.

Assent:

Leonardo Packer de Quadros
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Fabrício de Ávila Rodrigues
Adviser

ACKNOWLEDGEMENTS

To God.

To my parents.

To the Federal University of Viçosa, for the opportunity to complete the postgraduate course.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for granting the scholarship.

To the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), for granting the scholarship.

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), to granting the scholarship.

ABSTRACT

QUADROS, Leonardo Packer, M.Sc., Universidade Federal de Viçosa, July, 2023. **Foliar Application of a Soluble Source of Silicon, Alternated or Combined with Fungicide, for Soybean Rust Management** Adviser: Fabrício de Ávila Rodrigues.

Soybean rust (SR) is one of the most aggressive diseases in soybeans. Thus, alternative strategies for SR management aiming to reduce yield losses and the selection pressure exerted on the *Phakopsora pachyrhizi* population by the abusive use of fungicides deserves to be searched. This study evaluated the effectiveness of the foliar application of a soluble source of silicon [Silício Forte® (10% potassium oxide, 25% silicon, 3% magnesium oxide, and 0.01% calcium oxide), Verde Fertilizantes Ltda, Brazil; 40 g/L and syrup volume of 100 L/ha; referred to silicon source (SiS) afterward], combined or alternated with fungicide [Trifloxistrobina (375 g/L) + Ciproconazol (160 g/L); Sphere Max® - Bayer S.A., Brazil], for controlling SR under field conditions. The experiments were carried out in the 2021/22 [Experiment 1 (E1)] and 2022/23 [Experiment 2 (E2)] growing seasons and installed in a randomized block design with four replications and six treatments. The treatments conducted in E1 and E2 were as follows: (1) non-spray of SiS or fungicide (control), (2) three sprays of fungicide (F), (3) three sprays of SiS combined with fungicide (SiS+F), (4) fungicide, SiS, and fungicide, respectively, in the first, second, and third spray (F/SiS/F), (5) SiS, fungicide, and SiS, respectively, in the first, second, and third spray (SiS/F/SiS), and (6) three sprays with SiS (SiS). Plants in each plot were evaluated for severity, plant defoliation, yield assessments (yield and weight of 1000 grains), chlorophyll *a* fluorescence parameters, and concentration of photosynthetic pigments (chlorophyll *a*+*b* and carotenoids). Severity data was used to calculate the area under the rust progress curve (AURPC). The SiS inhibited the germination of *P. pachyrhizi* urediniospores from 12 to 55% as the rates increased from 20 to 80 g/L compared to the control treatment. There was no significant difference between SiS and control treatments and among F, SiS+F, and F/SiS/F treatments for AURPC, plant defoliation, weight of 1000 grains, and yield. There was a significant difference among treatments for AURPC in E1 and E2, plant defoliation in E1 (higher values for SiS and control treatment followed by treatment SiS/F/SiS, and F, SiS+F, and F/SiS/F treatments), plant defoliation in E2 (higher values for SiS/F/SiS, SiS, and control treatments followed by F, SiS+F, and F/SiS/F treatments), weight of 1000 grains and yield in E1 (higher values for F, SiS+F and F/SiS/F treatments, followed by SiS/F/SiS treatment as well as SiS and control treatments), in addition to the weight of 100 grains and yield in E2 (higher values for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments followed by SiS and control treatments). The effectiveness of SR control with SiS, alternated or combined with fungicide, ranged from 44 to 97%. The AURPC was significantly reduced by 6% for SiS treatment compared to the control treatment in E1. Plants submitted to F+SiS, F/SiS/F, and SiS/F/SiS treatments displayed less impairment on their photosynthetic apparatus (great maximum photosystem II quantum yield, effective photosystem II quantum yield, and electron transport rate values linked to lower quantum yield of non-regulated energy dissipation values) and higher concentrations of Chl *a*+*b* and carotenoids compared to plants from the control treatment. In conclusion, the present study brings a body of evidence highlighting the potential of spraying the SiS, alternated or combined with fungicide, to reduce SR severity and plant defoliation due to better physiological performance of infected plants considering that more energy could be allocated for biochemical pathways involved in host defense reactions with a positive impact on yield.

Keywords: *Glycine max*. Rust. Mineral nutrition. Photosynthesis. Integrated disease management.

RESUMO

QUADROS, Leonardo Packer, M.Sc., Universidade Federal de Viçosa, julho de 2023. **Aplicação Foliar de uma Fonte Solúvel de Silício, Alternada ou Combinada com Fungicida, no Manejo da Ferrugem da Soja**. Orientador: Fabrício de Ávila Rodrigues.

A ferrugem da soja (FS) é uma das doenças mais agressivas da cultura da soja. Assim, estratégias alternativas para o manejo da FS visando reduzir as perdas de produtividade e a pressão de seleção exercida sobre a população de *Phakopsora pachyrhizi* pelo uso abusivo de fungicidas merecem ser pesquisadas. Este estudo avaliou a eficácia da aplicação foliar de uma fonte solúvel de silício [Silício Forte® (10% óxido de potássio, 25% silício, 3% óxido de magnésio e 0,01% óxido de cálcio), Verde Fertilizantes Ltda, Brasil; 40 g/L e volume de calda de 100 L/ha; referido como fonte de silício (SiS) posteriormente], combinada ou alternada com fungicida [Trifloxistrobina (375 g/L) + Ciproconazol (160 g/L); Sphere Max® - Bayer S.A., Brasil], para controlar a FS em condições de campo. Os experimentos foram conduzidos nas safras 2021/22 [Experimento 1 (E1)] e 2022/23 [Experimento 2 (E2)] e instalados em delineamento em blocos casualizados com quatro repetições e seis tratamentos. Os tratamentos conduzidos nos E1 e E2 foram os seguintes: (1) sem aplicação de SiS ou fungicida (controle), (2) três aplicações de fungicida (F), (3) três aplicações de SiS combinado com fungicida (SiS+F), (4) fungicida, SiS e fungicida, respectivamente, na primeira, segunda e terceira pulverização (F/SiS/F), (5) SiS, fungicida e SiS, respectivamente, na primeira, segunda e terceira pulverização (SiS/F/SiS) e (6) três pulverizações com SiS (SiS). Plantas em cada parcela foram avaliadas quanto à severidade, desfolha, produção e peso de 1000 grãos, parâmetros da fluorescência da clorofila *a* e concentração de pigmentos fotossintéticos (clorofila *a*+*b* e carotenoides). Os dados de severidade foram usados para calcular a área abaixo da curva do progresso da ferrugem (AACPF). O SiS inibiu a germinação dos urediniósporos de *P. pachyrhizi* de 12 a 55% para as doses de SiS variando de 20 a 80 g/L em comparação com o tratamento controle. Não houve diferença significativa entre os tratamentos SiS e controle e entre os tratamentos F, SiS+F e F/SiS/F para a AACPF, desfolha, peso de 1.000 grãos e produção. Houve diferença significativa entre os tratamentos para a AACPF nos E1 e E2, desfolha no E1 (maiores valores para os tratamentos SiS e controle seguidos pelo tratamento SiS/F/SiS e pelos tratamentos F, SiS+F e F/SiS/F), desfolha no E2 (maiores valores para os tratamentos SiS/F/SiS, SiS e controle seguidos pelos tratamentos F, SiS+F e F/SiS/F), peso de 1000 grãos e produção no E1 (maiores valores para os tratamentos F, SiS+ F e F/SiS/F seguidos do tratamento SiS/F/SiS e também para os tratamentos SiS e controle), além do peso de 1000 grãos e produção no E2 (maiores valores para os tratamentos F, SiS+F, F/SiS/F e SiS/F/Si seguidos pelos tratamentos SiS e controle). A eficácia de controle da FS usando o SiS, alternado ou combinado com fungicida, variou de 44 a 97%. A AACPF foi significativamente reduzida em 6% para o tratamento SiS em comparação com o tratamento controle no E1. As plantas submetidas aos tratamentos F+SiS, F/SiS/F e SiS/F/SiS exibiram menor comprometimento do aparato fotossintético (maiores valores para o rendimento quântico máximo do fotossistema II, rendimento quântico efetivo do fotossistema II e taxa de transporte de elétrons e menores valores para o rendimento de dissipação de energia não regulada) e maiores concentrações de Chl *a*+*b* e carotenoides em relação às plantas do tratamento controle. Em conclusão, o presente estudo traz evidências do potencial de utilização do SiS, alternado ou combinado com fungicida, para reduzir a severidade da FS e desfolha devido ao melhor desempenho fisiológico das plantas infectadas considerando que mais energia foi alocada às vias bioquímicas envolvidos nas reações de defesa das plantas com impacto positivo na produção.

Palavras-chave: *Glycine max*. Ferrugem. Nutrição mineral. Fotossíntese. Manejo integrado de doenças.

SUMMARY

1. INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) production is of unquestionable importance for the worldwide economy for being an expensive source of protein, healthy unsaturated fats and carbohydrates for the human diet, livestock and aquaculture feed, and biofuel (Islam et al. 2022). Infection of soybean leaves by the biotrophic fungus *Phakopsora pachyrhizi* H. Sydow & P. Sydow, the causal agent of soybean rust (SR), results in profound damage to the photosynthetic apparatus and drastic decrease in the concentration of pigments (chlorophylls and carotenoids) due to intense chlorosis and necrosis with a consequent reduction on the health leaf area duration (Yang et al. 1991; Goellner et al. 2010; Hartman et al. 2015; Rios et al. 2018; Hoffmann et al. 2022). In general, yield losses (e.g., plants with fewer pods, fewer grains per pod, and grains of lower weight and reduced quality) of up to 80% are linked to the use of earlier maturation and highly productive soybean cultivars that display intense defoliation due to their great susceptibility to SR (Goellner et al. 2010; Li et al. 2010; Rios et al., 2018). Control of SR has been a challenging task to be achieved by soybean farmers nowadays. In this challenged scenario, the intensive, earlier, and sequential sprays of fungicides associated, whenever possible, with cultural practices [e.g., early-maturing cultivars, earlier sowing dates, monitoring plants for an early appearance of disease symptoms and pathogen signs, providing a 60- to 90-day period without growing soybean in the off-season (delay of disease onset), and following specific sowing periods according to the growing regions] have been adopted by the soybean farmers to reduce the negative impact of SR epidemics on soybean yield (Langenbach et al. 2016; Kashiwa et al. 2020). The fact that *P. pachyrhizi* populations in the major soybean growing regions have become less sensitive to most of the available fungicides (e.g., pre-mixtures containing at least two molecules of active ingredient belonging to different groups such as triazoles, strobilurins, and carboxamide) routinely used in soybean production regions due to the increasing presence of mutations, the cost associated with fungicides purchase and their spray, and the unavailable of resistant cultivars (Scherm et al. 2009; Klosowski et al. 2018; Kashiwa et al. 2020; Kato and Soares 2021; Müller et al. 2021; Claus et al. 2022; Machado et al. 2022) call the attention of researchers to find environmentally friendly alternatives for efficient SR control.

The literature highlights the importance of supplying silicon (Si) to plants either through foliar spray or soil amendment for the sustainable management of diseases caused by pathogens of different lifestyles (Rodrigues et al. 2015a; Debona et al. 2017, 2023; Zellner et al. 2021). Studies carried out under greenhouse and field conditions reported decrease in the intensities of different diseases [e.g., stem canker (*Diaporthe phaseolorum* f. sp. *meridionalis*), Phytophthora root rot (*Phytophthora sojae*), downy mildew (*Peronospora manshurica*), charcoal rot (*Macrophomina phaseolina*), and red crown rot (*Calonectria ilicicola*)] on soybean (Win et al. 2021; Debona et al. 2023). Particularly for the soybean-*P. pachyrhizi* interaction, Si reduced the intensity of SR on both controlled and field conditions. Under field conditions, soybean plants sprayed with potassium silicate solutions with concentrations ranging from 8 to 60 g/L showed lower SR severity (Rodrigues et al. 2009). Potassium silicate (40 g/L) was as effective as the fungicide Epoxiconazole+Pyraclostrobin in reducing SR severity and the number of pustules per cm^2 of leaf on soybean plants growing in greenhouse conditions (Rodrigues et al. 2009). Soybean plants supplied with Si displayed smaller and fewer uredinia per pustule as well as significant reductions of 27, 23, and 60%, respectively, for the number of lesions, closed uredinia, and open uredinia in comparison to non-Si supplied plants (Cruz et al. 2012). Pereira et al. (2009) also reported reduced SR severity on soybean leaves sprayed with potassium silicate. Soybean plants growing in soil amendment with Si displayed a delay in the appearance of SR symptoms of approximately 72 h and the area under disease progress curve was reduced, respectively, by 43 and 36% for plants either sprayed or growing in soil amended with Si (Lemes et al. 2011). Brunetto et al. (2022) reported a reduction in the number of uredinia per lesion and SR severity on soybean plants growing in soil with residual Si application. The resistance of plants exposed to Si and challenged by pathogens has been boosted very efficiently to cope with their infection (Debona et al. 2023). Silicon has played a passive (e.g., deposition below the cuticle to avoid or delay pathogen penetration) and or active [e.g., rapid and robust host defense reactions (defense-related genes and enzymes and production of phenolics and lignin) and a more robust antioxidative metabolism] role in the resistance of different crops against soilborne, vascular, and foliar diseases (Rodrigues et al. 2015b; Debona et al. 2017, 2023).

Considering that spraying products containing soluble Si has become a promising alternative for managing a plethora of foliar diseases, it is plausible that soybean farmers would benefit from this strategy. It is of pivotal importance to develop effective strategies for SR management that will guarantee sustainable long-term soybean production in line with the need to face the demand of society for more environmentally friendly global food production and security. Within this context, the present study hypothesized that the foliar spray of soybean plants with a source of soluble silicon, alternate or combined with fungicide, could decrease the symptoms of soybean rust and allow the plants to display a better photosynthetic performance will less impact on yield. Moreover, it is plausible to speculate that using the source of soluble Si will reduce at least one fungicide spray.

2. MATERIAL AND METHODS

2.1 Laboratory experiment: effect *in vitro* of Silício Forte® on the germination of *P. pachyrhizi* urediniospores

The sensitivity of *P. pachyrhizi* urediniospores to Silício Forte® [(10% potassium oxide, 25% silicon, 3% magnesium oxide, and 0.01% calcium oxide), Verde Fertilizantes Ltda, Brazil] was evaluated *in vitro*. Urediniospores were collected from soybean leaflets exhibiting intense *P. pachyrhizi* sporulation and used to prepare a suspension in the concentration of 10^5 urediniospores/ml. Briefly, 1 mL of the suspension was mixed with different volumes of water and Silício Forte® solution (original concentration of 100 g/L) to obtain the final concentrations of 20, 40, 60, and 80 g of Silício Forte®/L. The solutions of 20, 40, 60, and 80 g of Silício Forte®/L showed pH of 7.1 ± 0.05 and electric conductivity of 27.5, 33.7, 38.4, and 43.4 µS/cm, respectively. The control treatments corresponded to urediniospores suspension without Silício Forte® or mixed with fungicide [Trifloxistrobina (375 g/L) + Ciproconazol (160 g/L), Sphere Max®, Bayer S.A., Brazil; 2 ml/L]. A total of 60 µl of urediniospores suspension containing the different Silício Forte® concentrations was placed in glass slides. The glass slides were transferred to a growth chamber (25°C and photoperiod of 12 h of light and 12 h of dark) for 6 h. A total of 40 µl of lactofuchsine was added to each glass slide to stop urediniospores germination. One hundred urediniospores were randomly examined from each glass slide under a light microscope (Carl Zeiss AxioImager A1) at 40 × magnification. The experiment was arranged in a completely randomized design with six treatments (two controls and four Silício Forte® rates) and four replications. Each replication corresponded to one glass slide. The urediniospore with a germ tube larger than its diameter was considered germinated. The percentage of inhibition of urediniospores germination {[(percentage of urediniospores germination in the control treatment - percentage of urediniospores germinated in each treatment)*100]/[percentage of urediniospores germination in the control treatment]} was calculated for each replication. Effective concentration (EC) of Silício Forte® capable of reducing urediniospores germination by 50% (EC_{50}) was calculated using regression analysis.

2.2 Field experiment

2.2.1 Plots establishment

Two field experiments were conducted in the experimental area of the Plant Pathology Department at the Federal University of Viçosa, Viçosa City, Minas Gerais State, Brazil, located in the southeastern region of the state of Minas Gerais (20º44’44’’S, 42º50’59’’W, and 661 m above sea level). The first experiment was conducted from November 2021 to February 2022 and the second experiment from November 2022 to February 2023. Experiments were arranged in a randomized complete block design with six treatments in four replicate blocks. Each plot (experimental unit) consisted of four 5-m-long rows spaced 0.50 m apart corresponding to a total plot area of 10 m^2. The distance between adjacent plots was 1 m. The soybean cultivars DS5916IPRO and B5595CE (indeterminate growth type, maturity group 6.1, and both susceptible to soybean rust) (https://www.brevant.com.br) were used in this study. Fifteen seeds per meter of each cultivar (cultivars DS5916IPRO and B5595CE on Experiments 1 and 2, respectively) were sown to achieve a population density of 300,000 plants per hectare. Seedlings emerged on November 02, 2021 (Experiment 1) and November 15, 2022 (Experiment 2). Plots were maintained using conventional commercial soybean cultural practices (e.g., topdressing with fertilizer, insecticide sprays, weeding, and overhead irrigation when necessary) for Experiments 1 and 2. Plants were harvested at 111 and 94 days after seedlings emergence (dase) from Experiments 1 and 2, respectively. The chemical analysis of the soil (0.00-0.20 cm) in the experimental area (classified as Red Latosol) was as follows: 425 g/kg of clay (clay texture); pH in water = 5.8; P ($Mehlich^{-1}$) = 13.3 mg/dm^3; K ($Mehlich^{-1}$) = 143 mg/dm^3; remaining P = 31 mg/L; Al^{3+}, $H^{+}+Al^{3+}$, Ca^{2+}, and Mg^{2+} = 0.0, 2.31, 3.52, and 0.75 $cmol_c/dm^3$, respectively; Zn, Fe, Mn, Cu, B, and Si = 2.5, 75, 40.8, 2.2, 0.2, and 12 mg/dm^3, respectively; base saturation, effective cation exchange capacity, and cation exchange capacities at pH 7.0 = 4.64, 4.64, and 6.95 $cmol_c/dm^3$, respectively; base saturation index and aluminum saturation = 66.8 and 0.0%, respectively; and organic matter = 2.8 dag/kg. The region of Viçosa city has a Monsoon-influenced humid subtropical climate (temperate, dry winter, and hot summer) and is considered Cwa according to the Köppen classification. Weather data [precipitation, temperature (minimum, maximum, and mean), and relative humidity (mean)] were obtained from the VICOSA A510 station (Viçosa, Minas Gerais State, Brazil) (https://tempo.inmet.gov.br/TabelaEstacoes/A510).

2.2.2 Application of Silício Forte® and fungicide

Plants in the four rows of each plot were sprayed with 0.1 L of Silício Forte® [40 g/L and syrup volume of 100 L/ha; referred to silicon source (SiS) after that] and fungicide [Trifloxistrobina (375 g/L) + Ciproconazol (160 g/L), Sphere Max®, Bayer S.A., Brazil; 2 mL/L and syrup volume of 100 L/ha] solutions. The treatments used in Experiments 1 and 2 were as follows: (1) non-spray of SiS or fungicide (control), (2) three sprays of fungicide (F), (3) three sprays of SiS combined with fungicide (SiS+F), (4) fungicide, SiS, and fungicide, respectively, in the first, second, and third spray (F/SiS/F), (5) SiS, fungicide, and SiS, respectively, in the first, second, and third spray (SiS/F/SiS), and (6) three sprays with SiS (SiS). Plants were sprayed at 42, 56, and 72 dase for Experiment 1 and at 44, 57, and 72 dase for Experiment 2. The interval among sprays was approximately 15 days. The sprays corresponded to V6-V7, R1, and R4 growth stages for each experiment (Fehr and Caviness 1977). Plants in each plot were sprayed with different solutions using a backpack sprayer (Jacto model HD-20, São Paulo City, Brazil). Sprays were performed during the early morning hours to guarantee humidity above 65% and temperature below 28°C.

2.2.3 Disease, plant defoliation, and yield assessments

To avoid cross-contamination among sprayed plots, only the two central rows of each plot, omitting 0.5 m at the extremities of each row, were used to assess SR severity and plant defoliation as well as to determine yield and weight of 1000 grains. The SR severity (chlorosis and necrosis as well as fungal sporulation) was assessed on the leaves from both sides of plants in the two central rows, from basis to top, at 41, 57, 71, 84, and 99 dase for Experiment 1 and at 36, 43, 50, 57, 64, 71, 78, and 85 dase for Experiment 2 using the Horsfall-Barratt scale (Herbet 1982). The area under rust progress curve (AURPC) from the replications of each treatment was calculated using the trapezoidal integration of disease progress curves (Shaner and Finney 1977). Plant defoliation was also assessed on the same plants used to evaluate SR at 99 and 85 dase for Experiments 1 and 2, respectively, using a scale ranging from 1 to 5 as follows: 1 = defoliation up to 12.5%, 2 = defoliation from 12.5 to 25%, 3 = defoliation from 25 to 50%, 4 = defoliation from 50 to 75% and 5 = more than 75% of defoliation. A total of 30 plants at the R8 growth stage (full maturity) were obtained in the two central rows of each plot (area of 3 m^2) to determine yield (kg/ha). The grains obtained from these plants in each plot were weighed at 12% moisture content to determine the weight of 1000 grains. The moisture content of a representative subsample of grains from the sample of each plot was measured with a moisture tester (Gehaka Agri G600).

2.2.4 Imaging and quantification of Chl *a* fluorescence parameters

A total of 25 leaflets were randomly sampled on plants used to evaluate SR severity in the two central rows of each plot per treatment at 85 dase for Experiment 2. The images and parameters of Chl *a* fluorescence parameters on the leaflets sampled were obtained using the Imaging-PAM fluorometer and the Imaging Win software MAXI version (Heinz Walz GmbH, Effeltrich Germany) following the procedures described by Picanço et al. (2021).

2.2.5 Determining the concentration of photosynthetic pigments

A total of 10 leaflets were randomly sampled on plants used to evaluate SR severity in the two central rows of each plot per treatment at 99 and 85 dase for Experiments 1 and 2, respectively. Five discs (0.8 cm^2 each) obtained from each leaflet were used to determine the concentrations of Chl *a*, Chl *b*, and carotenoids according to Picanço et al. (2021).

2.2.6 Data analysis

Data from all variables and parameters obtained from Experiments 1 and 2 were subjected to analysis of variance (ANOVA) separately. Treatment means were compared by Tukey's test ($P \leq 0.05$). Data were checked for normality of residuals and homogeneity of variance before ANOVA and analyzed using the software R (R Core Team 2023).

3. RESULTS

3.1 Analysis of variance

The significance of the treatments for all variables and parameters evaluated in Experiments 1 and 2 is shown in Table 1.

3.2 *In vitro* assay

The germination of *P. pachyrhizi* urediniospores was completely inhibited in the presence of fungicide and reduced (shorter germ tube) when exposed to the doses of 20 to 80 g/L of SiS compared to the control treatment (Fig. 1a-b and c-f). The EC_{50} value was 66.77 g SiS/L (Fig. 2).

3.3 Weather conditions and overall SR development

For the period between seedlings emergence and harvest in Experiment 1, temperatures ranged from 13.6 to 32°C (average of 21.5°C), daily mean relative humidity from 71.7 to 95.7% (average of 83.4%), and total rainfall of 1359.4 mm (Fig. 3a). For the corresponding period in Experiment 2, temperatures ranged from 13 to 33°C (average of 21.8°C), daily mean relative humidity from 73.3 to 94.6% (average of 83.7%), and total rainfall of 914 mm (Fig. 3b).

In both experiments, SR severity increased over time, reaching the highest values for the six treatments at 99 and 85 dase for Experiment 1 and Experiment 2, respectively (Fig. 4a-b). Mean values for SR severity were higher for plants from control (6 to 79% from 71 to 99 dase) and SiS (5 to 74% from 71 to 99 dase) treatments compared to F, SiS+F, F/SiS/F (2 to 3% from 71 to 99 dase for these three treatments), and SiS/F/SiS (2 to 13% from 71 to 99 dase) treatments in Experiment 1 (Fig. 4a). For Experiment 2, mean values for SR severity were higher for plants from control (8 to 57% from 57 to 85 dase) and SiS (19 to 60% from 57 to 85 dase) treatments compared to other treatments and for plants from control (31 to 57% from 64 to 85 dase) and SiS/F/SiS (23 to 32% from 64 to 85 dase) treatments compared to F, SiS+F, and F/SiS/F treatments (3 to12% from 64 to 85 dase) (Fig. 4b).

The AURPC was significantly reduced from 83 to 93% for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments compared to control treatment in Experiment 1 and by 91, 74, 74, and 26% for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments, respectively, compared to SiS treatment in Experiment 2 (Fig. 4c-d). Significant decreases for AURPC ranging from 59 to 60% were obtained for F, SiS+F, and F/SiS/F treatments compared to SiS/F/SiS treatment in Experiment 1 and from 66 to 87% for F, SiS+F, and F/SiS/F treatments compared to SiS/F/SiS treatment in Experiment 2 (Fig. 4c-d). The AURPC was significantly reduced for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments (values ranging from 82-93%) compared to SiS treatment in Experiment 1 and by 92, 79, 78, and 38% for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments compared to SiS treatment in Experiment 2 (Fig. 4c-d).

Plant defoliation was significantly lower by 74, 68, 57, and 36% for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments, respectively, compared to control treatment in Experiment 1 and by 55, 47, and 53% for F, SiS+F, and F/SiS/F treatments compared to control treatment in Experiment 2 (Fig. 4e-f). Significant decreases in plant defoliation were obtained for F, SiS+F, and F/SiS/F treatments (values ranging from 33 to 59%) compared to SiS/F/SiS treatment in Experiment 1 and for F, SiS+F, and F/SiS/F treatments (values ranging from 42 to 52%) compared to SiS/F/SiS treatment in Experiment 2 (Fig. 4e-f). Plant defoliation was significantly lower for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments (values ranging from 38 to 74%) compared to SiS treatment in Experiment 1 and for F, SiS+F, and F/SiS/F treatments (values ranging from 46 to 55%) compared to SiS treatment in Experiment 2 (Fig. 4e-f).

The SR symptoms (chlorosis and many necrotic lesions containing several uredinia) were less developed on leaflets obtained from plants of F, SiS+F, and F/SiS/F treatments compared to leaflets from plants of the control treatment (Fig. 5a-d). On leaflets from plants of SiS/F/SiS and SiS treatments, SR symptoms were reduced than on leaflets from plants of the control treatment (Fig. 5a, e, and f).

3.4 Yield and weight of 1000 grains

Yield was significantly higher by 69, 60, and 57% for F, SiS+F, and F/SiS/F treatments, respectively, for Experiment 1 and by 81, 104, 104, and 74% for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments, respectively, for Experiment 2 in comparison to the control treatment (Fig. 6a-b). There were significant increases in yield of 73, 62, and

60% for F, SiS+F, and F/SiS/F treatments, respectively, for Experiment 1 and of 93, 117, 118, and 86% for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments, respectively, for Experiment 2 in comparison to SiS treatment (Fig. 6a-b). For Experiment 1, the weight of 1000 grains was significantly higher for treatments F, SiS+F, F/SiS/F, and SiS/F/SiS (values ranging from 25 to 47%) compared to control treatment, for treatments F, SiS+F, and F/SiS/F (values ranging from 14 to 17%) compared to SiS/F/SiS treatment, and for treatments F, SiS+F, F/SiS/F, and SiS/F/SiS (values ranging from 25 to 47%) compared to SiS treatment (Fig. 6c). For Experiment 2, the weight of 1000 grains were significantly higher for treatments F, SiS+F, F/SiS/F, and SiS/F/SiS (values ranging from 25 to 32%) compared to control treatment and for treatments, F, SiS+F, F/SiS/F, and SiS/F/SiS (values ranging from 28 to 35%) compared to SiS treatment (Fig. 6d).

3.5 Imaging and quantification of Chl *a* fluorescence parameters

Based on the darker regions on the images of Chl *a* parameters, remarkable damage to the photosynthetic apparatus occurred for leaflets from plants of control and SiS treatments compared to leaflets from plants of other treatments as well as for leaflets from plants of SiS/F/SiS and F/SiS/F treatments compared to leaflets from plants of F and SiS+F treatments (Fig. 7).

The F_v/F_m was significantly higher (values ranging from 8 to 10%) for leaflets from plants of SiS+F, F/SiS/F, and SiS/F/SiS treatments compared to leaflets from plants of control treatment and by 8% for leaflets from plants of SiS+F treatment compared to SiS treatment (Fig. 8a). For leaflets from plants of treatments SiS+F, F/SiS/F, SiS/F/SiS, and SiS, Y(II) was significantly higher (values ranging from 63 to 74%) compared to control treatment (Fig. 8b). The Y(NO) was significantly lower (values ranging from 23 to 26%) and ETR was significantly higher (values ranging from 62 to 73%) for leaflets from plants of F/SiS/F, SiS/F/SiS, and SiS treatments compared to leaflets from plants of the control treatment (Fig. 8b-c).

3.6 Concentrations of photosynthetic pigments

Chl *a*+*b*: for Experiment 1, significant increases were obtained for F, SiS+F, F/SiS/F, SiS/F/SiS, and SiS treatments (values ranging from 104 to 368%) compared to the control treatment; for F, SiS+F, F/SiS/F, and SiS/F/SiS treatments (values ranging from

94 to 129%) compared to SiS treatment; and of 16 and 18% for F and SiS/F/SiS treatments, respectively, compared to SiS+F treatment (Fig. 9a-b). For Experiment 2, the F, SiS+F, and F/SiS/F treatments showed significant increases (values ranging from 121 to 174%) compared to control treatment and for F, SiS+F, and F/SiS/F treatments (values ranging from 154 to 182%) compared to SiS treatment (Fig. 9a-b).

Carotenoids: for Experiment 1, there were significant increases of 28, 41, and 31% for SiS+F, F/SiS/F, and SiS/F/Si treatments, respectively, compared to the control treatment (Fig. 9c). For Experiment 2, significant increases of 44 and 67% for F and SiS+F treatments, respectively, compared to control treatment and of 45, 67, and 44% for treatments F, SiS+F, and F/SiS/F, respectively, compared to SiS treatment were obtained (Fig. 9d).

4. DISCUSSION

The abusive use of fungicides in soybean fields to ensure that SR will be efficiently controlled has been linked to the emergence of *P. pachyrhizi* populations resistant to them linked to a reduction in their long-term efficacy (Gupta et al. 2023). On top of that, the high price of fungicides on the market and the need for several sprays during the soybean season (e.g., four to five sprays depending on the sowing date and the cycle of cultivar) have contributed to an increase in the cost of soybean production besides resulting in environmental damage and human health concerns (Sarkar et al. 2021). In line with the need to find more eco-friendly and sustainable alternatives for managing diseases on soybean, the present study determined the efficiency of foliar application of a soluble source of silicon (SiS), alternated or combined with fungicide, to reduce SR development. Interestingly, the results provided herein clearly indicated that combining fungicide with SiS, using SiS on the second spray or SiS on the first and third sprays, greatly contributed to reducing SR development (smaller necrotic lesions surrounded by discreet chlorosis resulting in lower severity values) on soybean plants. It is worth mentioning that the experiments were conducted under environmental conditions favorable for SR development on plants from two cultivars susceptible to *P. pachyrhizi* infection. The most notable effect of using SiS in this scenario was the possibility of reducing at least two fungicide sprays which will positively contribute to lower the production cost. It is tempting to assume that the decision to use fungicide or SiS on the first spray will depend on the environmental conditions favorable for SR development, the level of resistance of the cultivar used, and, more importantly, the initial rust severity values (based on the amount of initial inoculum) on plants especially at the V4/V5 growth stage. Based on the *in vitro* test, the SiS inhibited the germination of *P. pachyrhizi* urediniospores at the same rate used to spray the soybean plants in the field experiments. Taking this information to a field scenario, it is plausible that the SiS will contribute to reducing the amount of initial inoculum to start the SR epidemic or even the epidemic rate without imposing any selection pressure against the population of *P. pachyrhizi* particularly considering if the sprays could be alternated with fungicide as illustrated by the outcome of F/SiS/F and SiS/F/SiS treatments. Interestingly, non-homogeneous greenish spots of dried solution of SiS noticed over the sprayed soybean leaves may become a physical impedance upon drying out to affect *P. pachyrhizi* penetration through the appressorium or possibly through an osmotic effect. It is important to point out that the deposition of SiS over the leaf surface of soybean plants forming a physical barrier will be only efficient for a short period considering plant growth over time and its wash-off by rain or sprinkler irrigation. It is worth considering that secondary cycles of *P. pachyrhizi* will dramatically decrease resulting in a lower epidemic rate with the SiS sprays especially if combined with fungicide.

Silicon has a special place owing to its overall capacity to reduce the intensities of a plethora of diseases in several crops (Rodrigues et al. 2015a; Debona et al. 2017, 2023). According to Debona et al. (2023), several solid and liquid sources of silicon have been used as soil conditioners, amendments, or fertilizers for agronomic and horticultural crops grown in the field or greenhouse. The foliar application of soluble sources of Si plays a prominent role in controlling several diseases caused by both soilborne and foliar pathogens in crops less prone to uptake and translocating Si to shoots (Debona et al. 2023). Notably, for the soybean-*P. pachyrhizi* interaction, several authors reported the potential of using Si to reduce rust development (Rodrigues et al. 2009, 2015a; Debona et al. 2017, 2023) considering the positive response of soybean plants to uptake Si from the soil solution or receiving foliar spray that will improve plant growth and increased yield (Mitani and Ma 2005; Nolla et al. 2006; Shwethakumari et al. 2018, 2021; Debona et al. 2023). A few studies have reported the potential of using the foliar spray of Si-containing products to manage diseases in soybean, especially the SR. Rodrigues et al. (2009) showed an increase in foliar Si concentration (values ranging from 8 to 10 g/kg) for soybean plants grown in the field and greenhouse resulting in lower rust severity and less number of lesions per cm^2 of leaf. The SR severity was reduced and the uredinia were fewer, smaller, and more compact indicating less production of urediniospores on the leaflets of soybean plants sprayed with potassium silicate (Cruz et al. 2012, 2013a,b).

An increased body of evidence suggests that photosynthesis is the primary physiological process perturbed by pathogens of different lifestyles on their hosts, placing them in a condition of uttermost susceptibility because several biochemical pathways involved, directly or indirectly, in defense reactions are seriously compromised due to changes in the pool of assimilates across organs (Rios et al. 2014, 2018; Silveira et al. 2015; Rodrigues et al. 2017, 2020; Aucique-Pérez et al. 2020; Dias et al. 2020; Sterling and Melgarejo 2021). Changes in the parameters related to Chl *a* fluorescence are great physiological indicators of the status of plants exposed to stress because most of the absorbed energy by the photosynthetic pigments to photochemical processes of photosynthesis is used with less efficiency (Aucique-Pérez et al. 2014; Tatagiba et al. 2015). Interestingly, the functionality of the photosynthetic apparatus of Si-supplied plants was less impaired during the infection process of pathogens of different lifestyles (Resende et al. 2012; Aucique-Pérez et al. 2014; Rodrigues et al. 2014; Tatagiba et al. 2016; Debona et al. 2017). Soybean plants infected by *P. pachyrhizi* displayed profound changes in the Chl *a* fluorescence parameters in agreement with previous studies carried out under controlled conditions (Rios et al. 2018; Einhardt et al. 2020; Picanço et al. 2022). Rios et al. (2018) reported the great impact of SR on impairing the photosynthetic apparatus of soybean leaves from plants growing in a greenhouse based on changes in Chl *a* fluorescence parameters such as lower F_v/F_m and Y(II) values accompanied by increases in Y(NO). Moreover, Einhardt et al. (2020) noticed lower values for F_v/F_m and ETR in soybean leaflets infected by *P. pachyrhizi* in addition to sharp increases in Y(NPQ) that indicated no heat dissipation to prevent PSII damage. In the present study, however, a close look at the Chl *a* fluorescence parameters obtained from leaflets of plants growing under field conditions and submitted to the F+SiS, F/SiS/F, and SiS/F/SiS treatments showed that the functioning of their photosynthetic apparatus was exceptionally preserved [great F_v/F_m, Y(II), and ETR values linked to lower Y(NO) values] with less photodamage. Even though exhibiting higher severity values, the leaflets of plants submitted to SiS treatment suffered less from fungal infection considering the higher values for Y(II), Y(NO), and ETR compared to leaflets from plants of the control treatment. It is tempting to speculate that the similar values for F_v/F_m, Y(II), Y(NO), and ETR between leaflets obtained from plants from F+SiS and control treatments and, to a lesser extent, for Y(NO) and ETR between F and F+SiS treatments indicated a possible cytotoxic effect of the fungicide on the photosynthetic performance of soybean plants. This finding reinforces the idea that replacing one spray of fungicide with SiS, not only aiming to lower production costs, may help avoid any possible hidden phytotoxic effect caused by the fungicide. Destruction of photosynthetic pigments in the chloroplasts is one of the major cytologic features associated with the infection of hemibiotrophic and necrotrophic pathogens causing foliar diseases in different crops, including soybean (Rodrigues et al. 2014; Rios et al. 2018; Einhardt et al. 2020; Picanço et al. 2022). As reported for many host-pathogen interactions, Si supply to plants helped to preserve the pools of photosynthetic pigments as a result of less disease symptoms (Rodrigues et al. 2014; Tatagiba et al. 2016; Debona et al. 2017; Silveira et al. 2021). Reduced SR development on soybean plants submitted to F, F+Si, F/SiS/F, and SiS/F/SiS treatments (less intense chlorosis and necrotic leaf tissues) contributed to preserving the pool of photosynthetic pigments (Chl *a*+*b* and carotenoids) and their highlighted efficiency in capturing the energy needed for photochemical reactions in the chloroplasts towards improved photosynthesis in close contrast to what was obtained for infected leaflets from plants from control and SiS treatments.

Decreases in plant photosynthesis due to infection by pathogens disturb carbohydrates metabolisms and their partitioning within the infected tissues resulting in lower grain yield (Misaghi 1982). Considering the need to increase soybean production worldwide (Nair et al. 2023) and the fact that *P. pachyrhizi* infection significantly compromises the functioning of the photosynthetic apparatus (Rios et al. 2018; Einhardt et al. 2020; Picanço et al. 2023), it is of exceeding importance to find strategies to slow the SR epidemic rate (Einhardt et al. 2020; Paula et al. 2021; Picanço et al. 2021, 2022). In this scenario, a greater part of the absorbed energy by the photosynthetic pigments in the leaves can be allocated to photochemical processes of photosynthesis towards better plant growth and concurrently obtaining the desired yield and grains quality (Christy and Porter 1982; Liu et al. 2006; Norman 2012; Basuchaudhuri 2016). In the present study, the reduced development of SR on plants submitted to F, F+SiS, F/SiS/F, and SiS/F/SiS treatments resulted in great yield and weight of 1000 grains in contrast to plants from SiS and control treatments. On top of that, less defoliation caused by SR noticed on plants from these treatments also played a prominent role in avoiding lower yield and weight of 1000 grains. In soybean, defoliation is a common feature associated with higher levels of SR severity (Moreira et al. 2015; Goellner et al. 2010; Langenbach et al. 2016; Rodrigues et al. 2009) as well as when plants face profound alterations in some physiological processes (Klubertanz et al. 1996; Haile et al. 1998; Islam 2014) resulting, therefore, in great yield losses.

In conclusion, the present study brings a body of evidence highlighting the potential of spraying SiS, alternated or combined with fungicide, to reduce SR severity and plant defoliation. In this scenario, infected plants displayed better physiological performance and more energy could be allocated for biochemical pathways involved in host defense reactions, positively impacting yield. It is tempting to assume that using the SiS, associated with available control strategies, may become a promising alternative for SR management, especially under a lower disease pressure towards a more sustainable soybean production (e.g., organic system) considering that fungicide spray can be reduced and the risk of *P. pachyrhizi* to develop resistance to the most used fungicide molecules will be lowered.

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TABLE AND FIGURES

Table 1. Analysis of variance for the effects of treatments on area under rust progress curve (AURPC), defoliation rate, yield components (yield and weight of 1000 grains), chlorophyll (Chl) *a* fluorescence parameters [maximum photosystem II quantum yield (F_v/F_m), effective photosystem II quantum yield (Y(II)), quantum yield of regulated energy dissipation (Y(NPQ)), quantum yield of non-regulated energy dissipation (Y(NO)), and electron transport rate (ETR)], and concentrations of photosynthetic pigments [chlorophyll *a*+*b* (Chl *a*+*b*) and carotenoids].

Variables/Parameters	*F* values	
	Experiment 1	Experiment 2
Asian soybean rust		
AURPC	**<0.001**	**<0.001**
Defoliation rate	**<0.001**	**<0.001**
Yield componentes		
Yield	**<0.001**	**<0.001**
Weight of 1000 grains	**<0.001**	**<0.001**
Parameters of Chl *a* fluorescence		
F_v/F_m	**<0.005**	nd
Y(II)	**<0.010**	nd
Y(NPQ)	0.200	nd
Y(NO)	**<0.010**	nd
ETR	**<0.010**	nd
Photosynthetic pigments		
Chl *a*+*b*	**<0.001**	**<0.001**
Carotenoids	**<0.001**	**<0.001**

Values in bold are significant at $P \leq 0.05$. nd = non-determined.

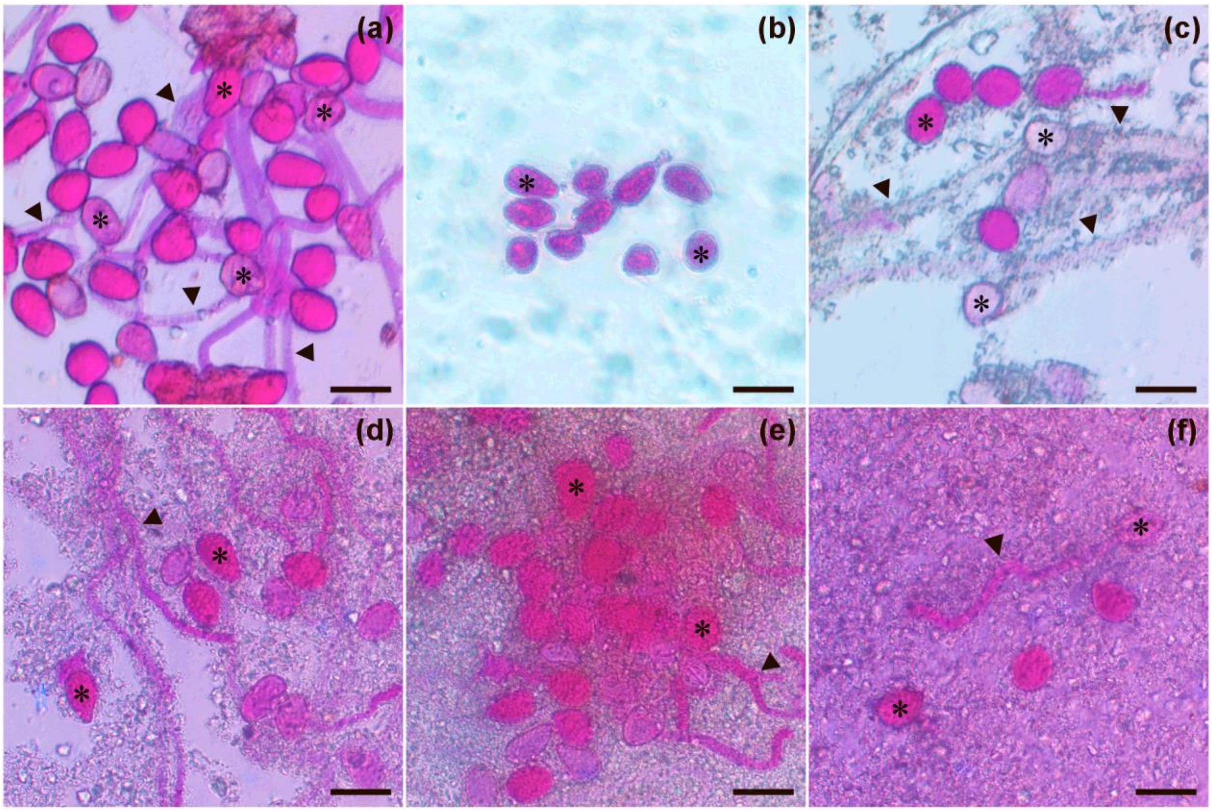

Fig. 1. Aspect of the germination of *Phakopsora pachyrhizi* urediniospores in glass slides containing different rates of silicon source (SiS) (20, 40, 60, and 80 g/L, respectively, to c, d, e, and f). Control treatments corresponded to urediniospores suspension without SiS (a) or prepared using fungicide [Trifloxistrobina (375 g/L) + Ciproconazol (160 g/L), 2 ml/L] (b). Urediniospores (asterisks) and urediniospore germ tube (arrowhead). Scale bars: 10 µm.

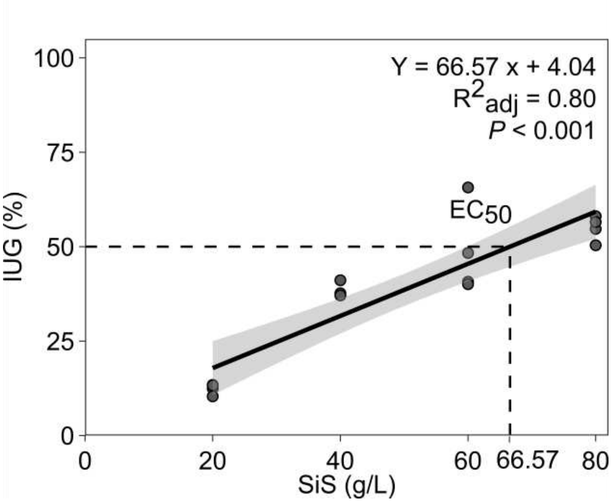

Fig. 2. Inhibition of urediniospores germination (IUG) in glass slides containing different rates of silicon source (SiS) (20, 40, 60, and 80 g/L) and effective concentration (EC_{50}) of SiS that inhibited 50% of urediniospores germination.

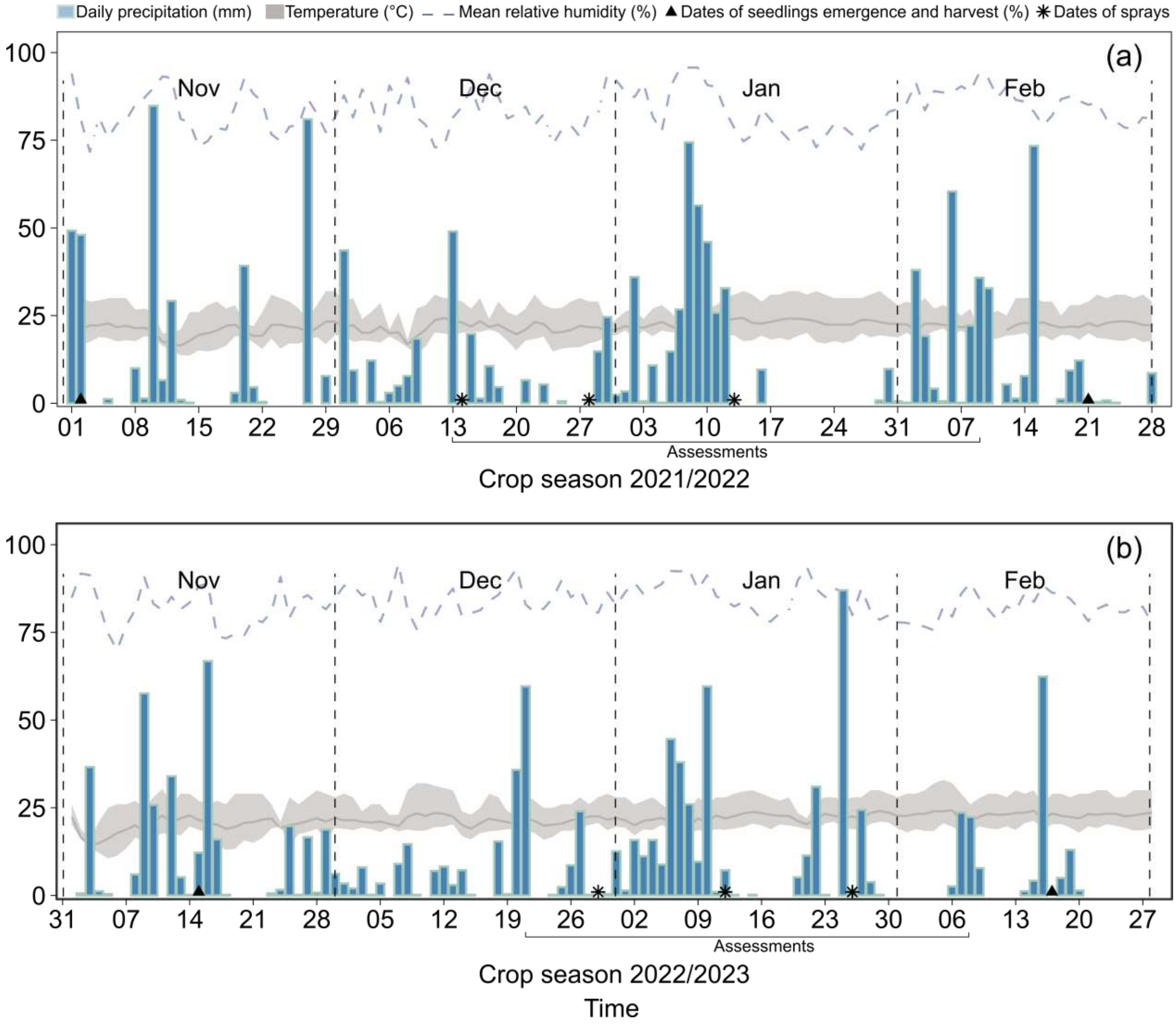

Fig. 3. Daily precipitation, temperature (mean, maximum, and minimum), and mean relative humidity obtained between November 2021 and February 2022 (crop season 2021/2022) (a) as well as between November 2022 and February 2023 (crop season 2022/2023) (b). Data were obtained from the VICOSA A510 station (Viçosa, Minas Gerais State, Brazil).

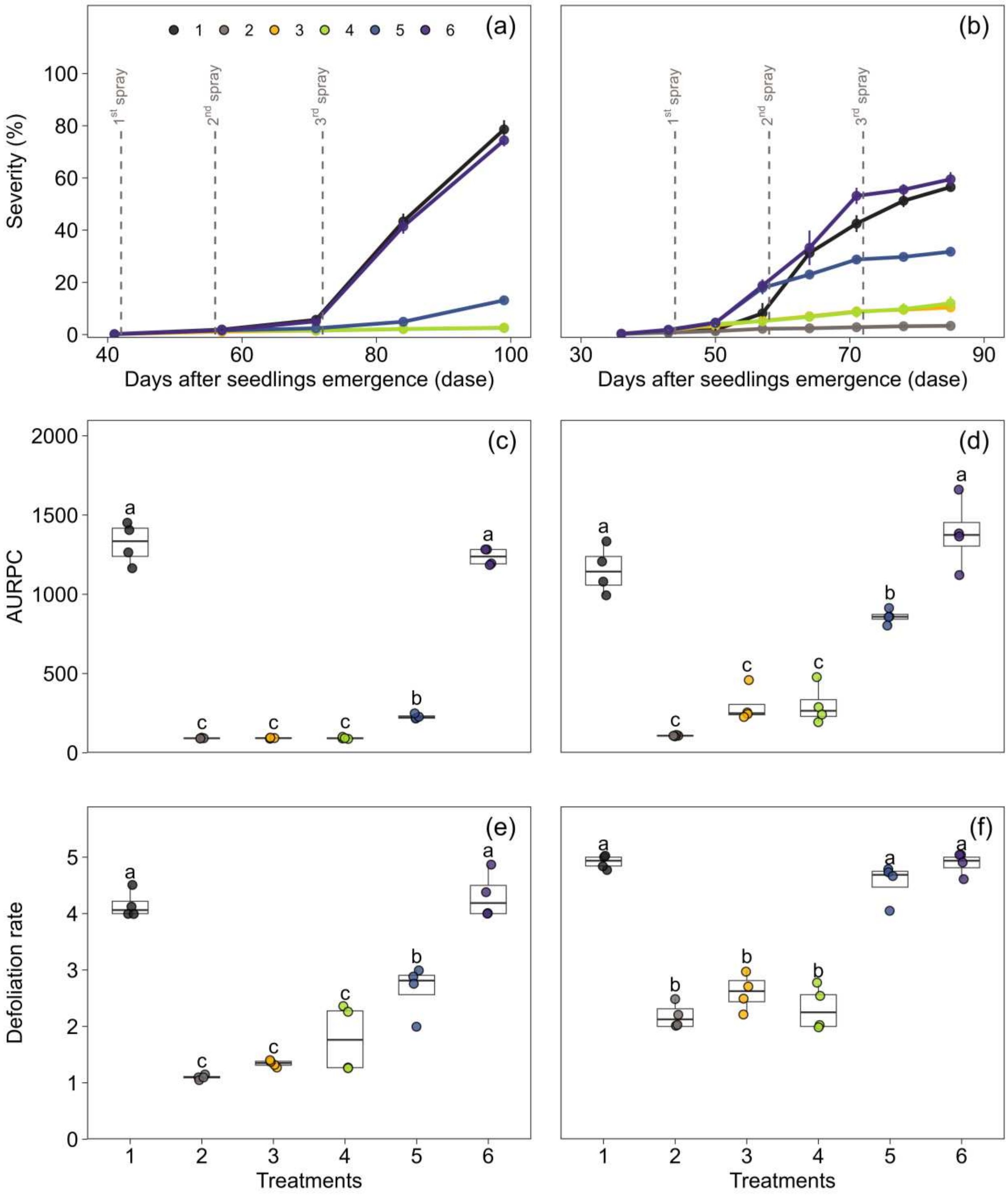

Fig. 4. Severity of soybean rust (a and b), area under rust progress curve (AURPC) (c and d), and defoliation rate (e and f) for soybean plants submitted to different treatments as follows: (1) control (non-spray with silicon source (SiS) or fungicide), (2) three sprays with fungicide, (3) three sprays with SiS combined with fungicide, (4) fungicide, SiS, and fungicide, respectively, in the first, second, and third spray, (5) SiS, fungicide, and SiS, respectively, in the first, second, and third spray, and (6) three

sprays with SiS in the crop seasons 2021/2022 (a, c, and e) and 2022/2023 (b, d, and f). Leaflets were sampled from plants of each treatment at 85 days after seedlings emergence. For AURPC (graphics c and d) and defoliation rate (graphics e and f), treatment means followed by different letters are significantly different ($P \leq 0.05$) according to Tukey's test. Bars represent the standard error of the means.

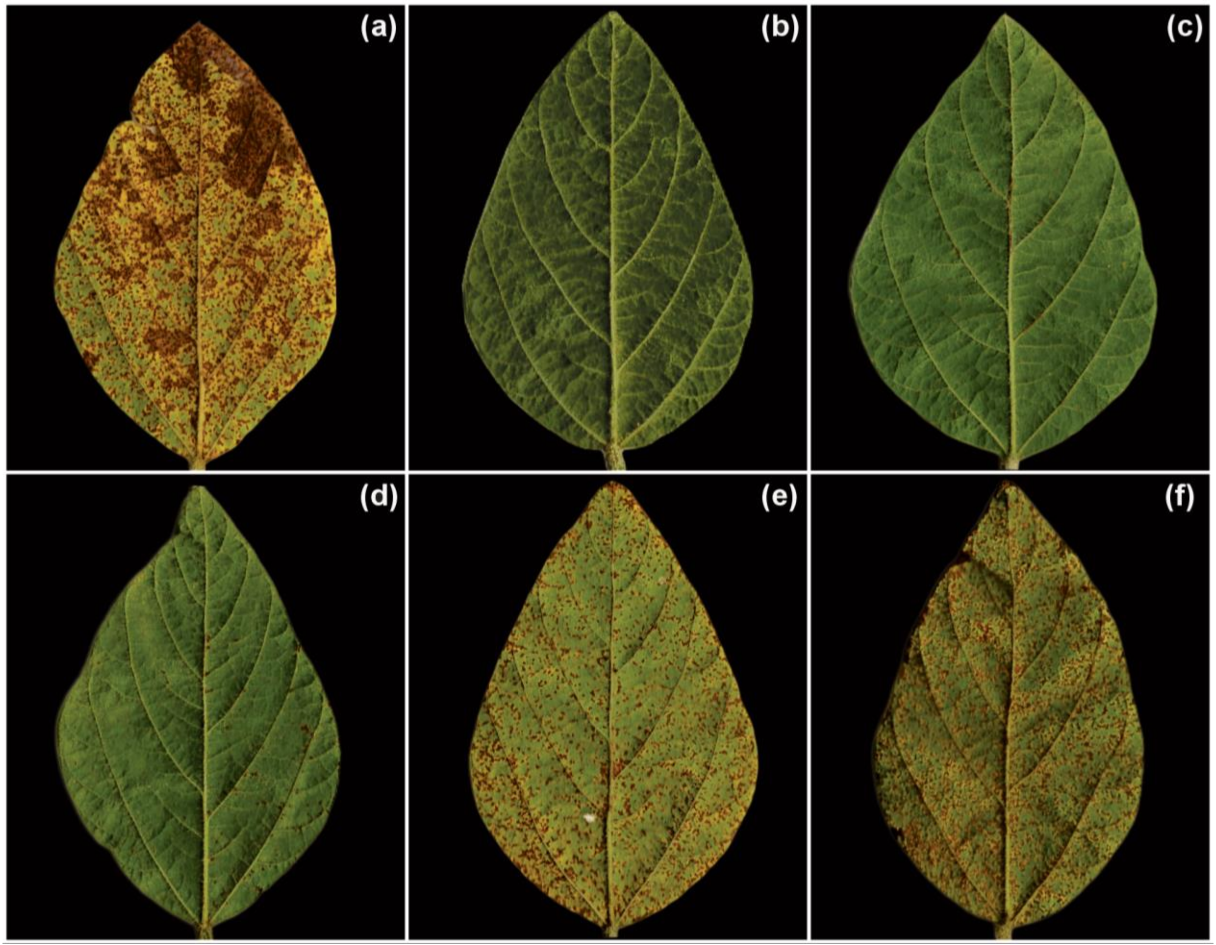

Fig. 5. Symptoms of soybean rust (chlorosis and necrosis) in the abaxial surface of leaflets from soybean plants submitted to different treatments as follows: non-spray with silicon source (SiS) or fungicide (a), three sprays with fungicide (b), three sprays with SiS combined with fungicide (c), fungicide, SiS, and fungicide, respectively, in the first, second, and third spray (d), SiS, fungicide, and SiS, respectively, in the first, second, and third spray (e), and three sprays with SiS (f). Leaflets were sampled from plants from each treatment at 85 days after seedlings emergence.

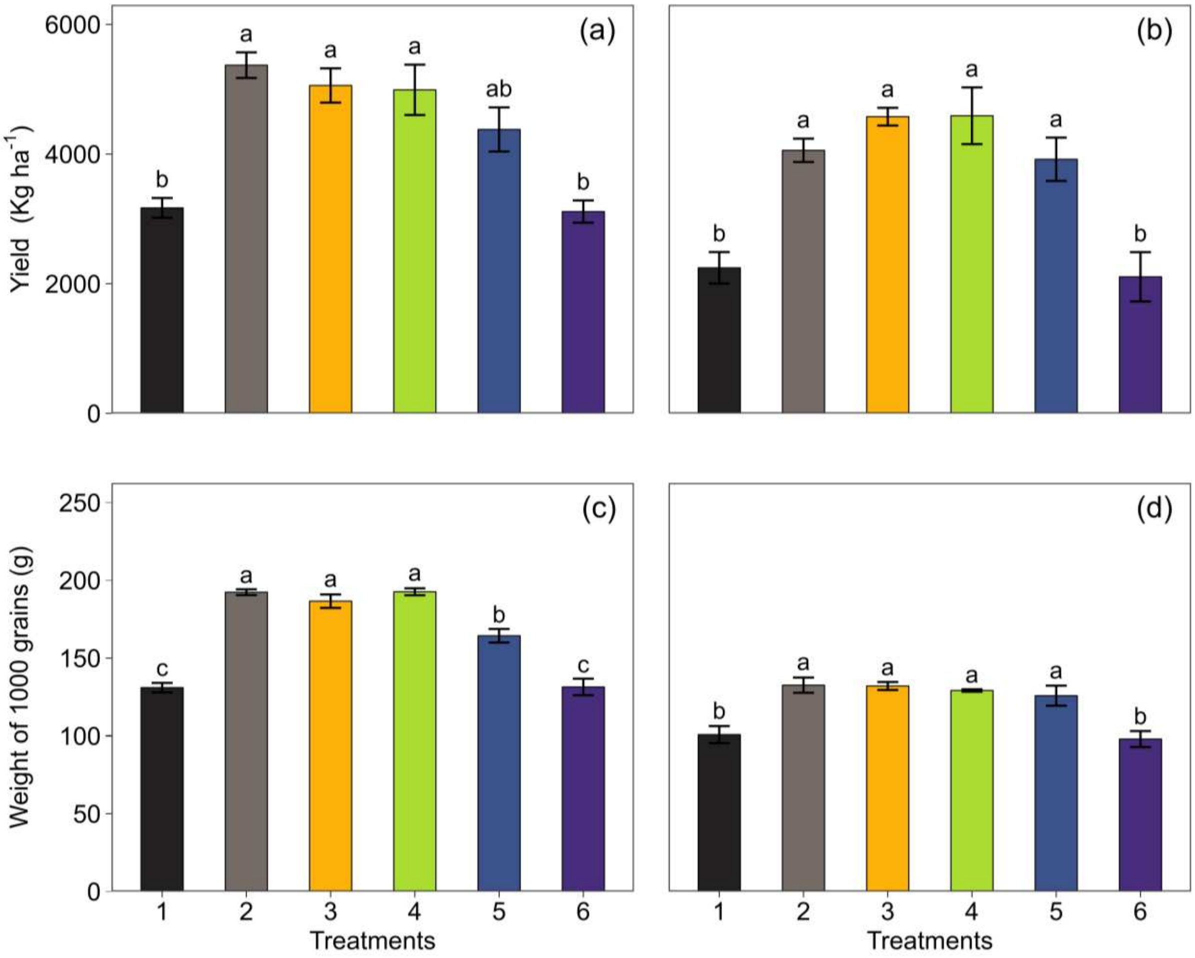

Fig. 6. Yield (a and b) and weight of 1000 grains (c and d) for soybean plants submitted to different treatments as follows: (1) control (non-spray with silicon source (SiS) or fungicide), (2) three sprays with fungicide, (3) three sprays with SiS combined with fungicide, (4) fungicide, SiS, and fungicide, respectively, in the first, second, and third spray, (5) SiS, fungicide, and SiS, respectively, in the first, second, and third spray, and (6) three sprays with SiS in the crop seasons 2021/2022 (a and c) and 2022/2023 (b and d). The yield and weight of 1000 grains were determined on plants harvested at 111 and 99 days after seedlings emergence for experiments 1 and 2, respectively. For yield and weight of 1000 grains, treatment means followed by different letters are significantly different (*P* ≤ 0.05) according to Tukey's test. Bars represent the standard error of the means.

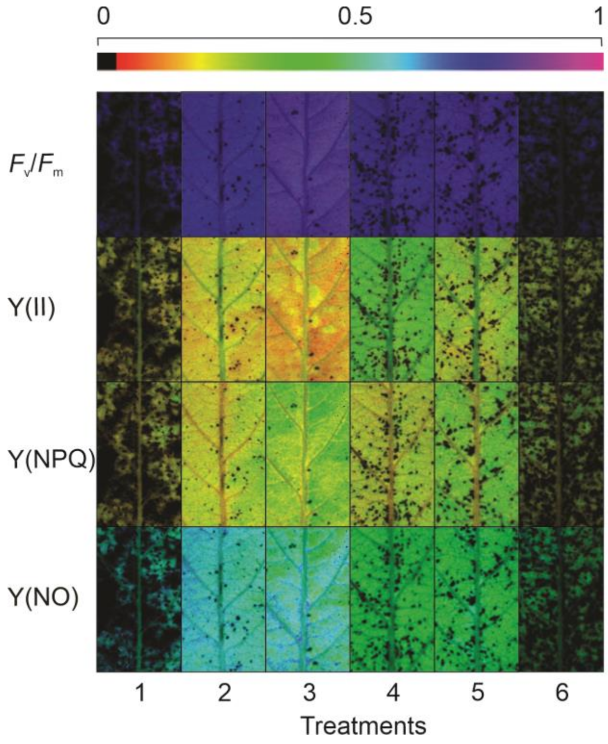

Fig. 7. Images of chlorophyll *a* fluorescence parameters maximum photosystem II quantum yield (F_v/F_m), effective photosystem II quantum yield [Y(II)], quantum yield of regulated energy dissipation [Y(NPQ)], and quantum yield of non-regulated energy dissipation [Y(NO)] determined in leaflets of soybean plants submitted to different treatments as follows: (1) control (non-spray with silicon source (SiS) or fungicide), (2) three sprays with fungicide, (3) three sprays with SiS combined with fungicide, (4) fungicide, SiS, and fungicide, respectively, in the first, second, and third spray, (5) SiS, fungicide, and SiS, respectively, in the first, second, and third spray, and (6) three sprays with SiS. Leaflets were sampled from plants from each treatment at 85 days after seedlings emergence.

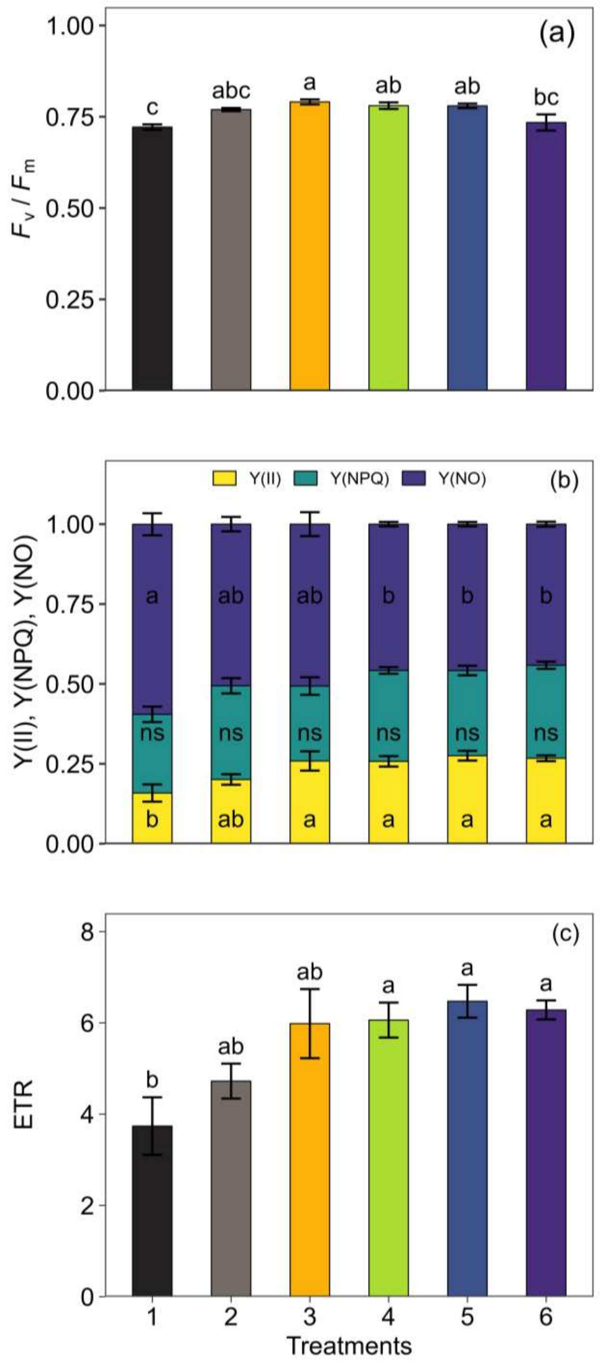

Fig. 8. Quantification of chlorophyll (Chl) *a* fluorescence parameters maximum photosystem II quantum yield (F_v/F_m) (a), effective photosystem II quantum yield [Y(II)] (b), quantum yield of regulated energy dissipation [Y(NPQ)] (b), quantum yield of non-regulated energy dissipation [Y(NO)] (b), and electron transport rate (ETR) (c) on leaflets of soybean plants submitted to different treatments as follows: (1) control (non-spray with silicon source (SiS) or fungicide), (2) three sprays with fungicide, (3) three sprays with SiS combined with fungicide, (4) fungicide, SiS, and fungicide, respectively, in the first, second, and third spray, (5) SiS, fungicide, and SiS,

respectively, in the first, second, and third spray, and (6) three sprays with SiS. Leaflets were sampled from plants of each treatment at 85 days after seedlings emergence. For each Chl *a* fluorescence parameter, treatment means followed by different letters are significantly different ($P \leq 0.05$) according to Tukey's test. Bars represent the standard error of the means. ns = non-significative.

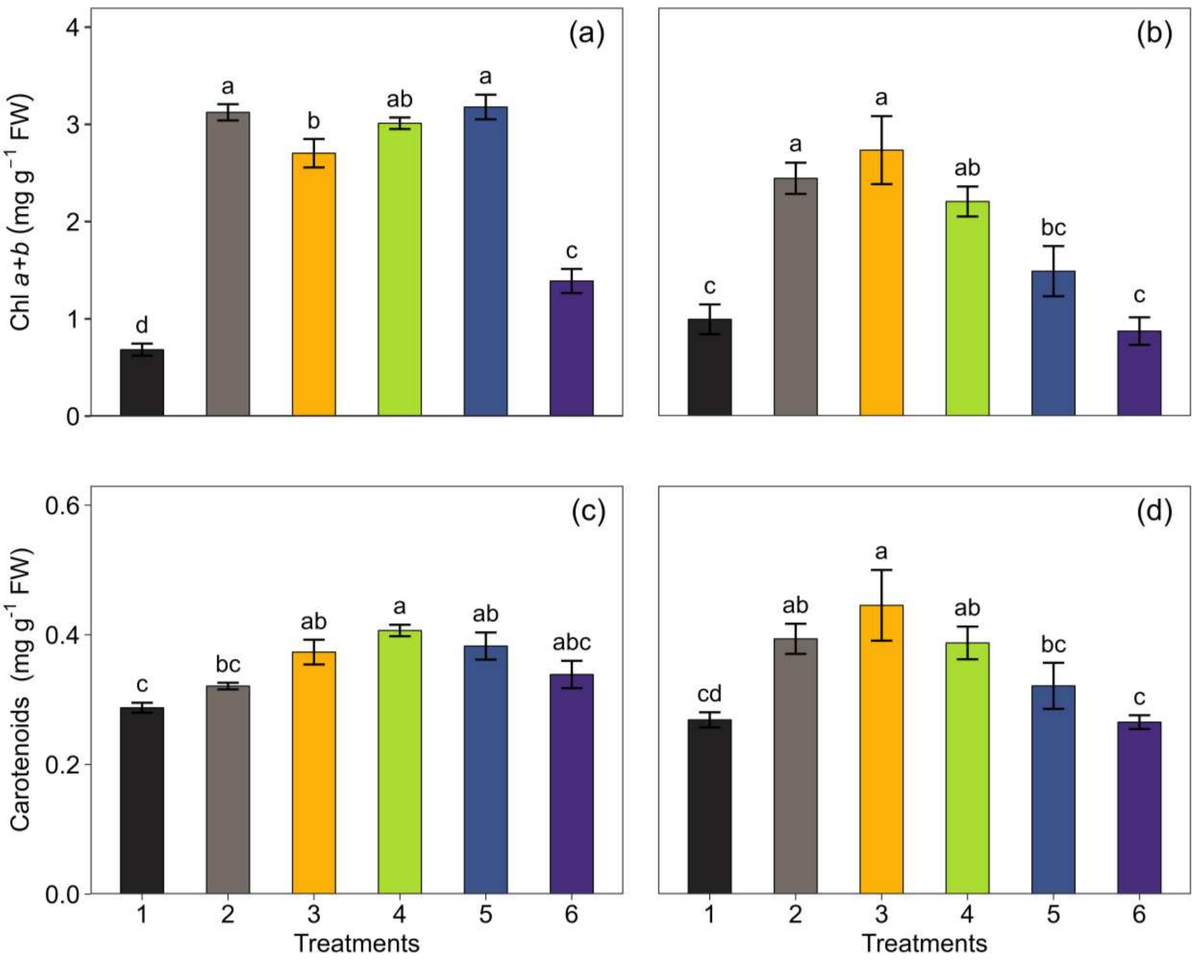

Fig. 9. Concentrations of chlorophyll *a*+*b* (Chl *a*+*b*) (a and b) and carotenoids (c and d) determined in leaflets of soybean plants submitted to different treatments as follows: (1) control (non-spray with silicon source (SiS) or fungicide), (2) three sprays with fungicide, (3) three sprays with SiS combined with fungicide, (4) fungicide, SiS, and fungicide, respectively, in the first, second, and third spray, (5) SiS, fungicide, and SiS, respectively, in the first, second, and third spray, and (6) three sprays with SiS. Leaflets were sampled from plants of each treatment at 85 days after seedlings emergence. For Chl *a*+*b* and carotenoids, treatment means followed by different letters are significantly different ($P \leq 0.05$) according to Tukey's test. Bars represent the standard error of the means. FW = fresh weight.