Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

# Effect of foliar-applied potassium silicate on coffee leaf infection by *Hemileia vastatrix*

V. Carré-Missio, F.A. Rodrigues, D.A. Schurt, R.S. Resende, N.F.A. Souza, D.C. Rezende, W.R. Moreira & L. Zambolim

Laboratory of Host–Parasite Interaction, Department of Plant Pathology, Viçosa Federal University, Viçosa, Brazil

**Keywords**
*Coffea arabica* L.; mineral nutrition; *rust*; scanning electron microscopy; silicon.

**Correspondence**
F.A. Rodrigues, Department of Plant Pathology, Viçosa Federal University, Viçosa, Minas Gerais State 36570-000, Brazil.
Email: fabricio@ufv.br

Received: 17 May 2013; revised version accepted: 5 December 2013; published online: 17 February 2014.

doi:10.1111/aab.12109

## Abstract

Coffee leaf rust, caused by *Hemileia vastatrix*, is the most devastating disease of coffee. Since limited information is available in the literature on silicon (Si) affecting plant diseases in coffee, this study was designed to investigate foliar application of potassium silicate (PS), a source of soluble (Si), on infection process of coffee leaf rust at the microscopic level. The foliar Si concentration for plants sprayed with water and PS has no significant difference (0.24 and 0.30 dag $kg^{-1}$, respectively). X-ray microanalysis indicated that the deposition of Si on the leaves of the plants that were sprayed with PS was greater in comparison to the leaf samples from the plants sprayed with water. Rust severity on leaves of plants sprayed with water or sprayed with PS reached 44% and 32%, respectively, at 36 days after inoculation (dai). Plates of polymerised PS were observed on the leaf surfaces of the plants sprayed with the product, in contrast to its absence on the leaf surfaces of plants sprayed with water. At 36 dai, a greater number of uredia were observed on the leaf surfaces of plants sprayed with water in comparison to the leaf surfaces of plants sprayed with PS. On fractured leaf tissues that were sprayed with PS, less fungal colonisation was observed in comparison to the leaves of plants sprayed with water. In conclusion, the results of this study suggest that the effect of foliar-applied Si on the control of the coffee leaf rust development may be attributed to the physical role of the polymerised PS, its osmotic effect against urediniospores germination, or both.

## Introduction

Coffee leaf rust, caused by the biotrophic fungus *Hemileia vastatrix* Berkeley & Broome, is the most devastating disease affecting coffee plants (*Coffea arabica* L.) in Brazil and can cause yield losses from 35 to 50% (Zambolim *et al.*, 2002). Pustules on the adaxial leaf blades dramatically reduce photosynthesis and cause intense defoliation, thus reducing yield (Zambolim *et al.*, 2002). Additionally, leaves supporting rapidly growing coffee berries become more susceptible to fungal infection than leaves that only support vegetative growth (Avelino *et al.*, 2006). High yielding coffee cultivars are more susceptible to *H. vastatrix* than lower yielding ones (Avelino *et al.*, 2006). Rust is managed mainly by foliar applications of contact (copper) and systemic (triazols and strobilurins) fungicides (Matiello *et al.*, 2002; Zambolim *et al.*, 2002). However, resistant populations of *H. vastatrix* can emerge due to the continuous use of systemic fungicides. The use of resistant cultivars is another approach used by coffee producers to control rust; however, their use is compromised due to genetic changes in the pathogen's population (Zambolim *et al.*, 2002).

Alternatives to the extensive use of fungicides to manage rust are needed. Silicon (Si) can be one alternative as its beneficial effects (whether direct or indirect) to plants that are under biotic or abiotic stresses have been reported in a wide variety of crops, such as barley, cucumbers, oat, rice, rye, sugarcane and wheat (Datnoff *et al.*, 2007). By amending the soil with calcium silicate or adding soluble Si to hydroponically grown plants, several

foliar and root diseases in both monocots and dicots have been controlled (Datnoff *et al.*, 2007). Many crops cannot benefit from the positive effects that Si could provide to them, such as alleviating biotic and abiotic types of stress, because they are not capable of translocating Si from the soil to the shoots (Datnoff *et al.*, 2007). The foliar application of sources of soluble Si, as in the case of potassium silicate (PS), can affect the development of some pathogens on the leaf surface through the formation of a physical barrier or by an osmotic effect (Bowen *et al.*, 1992; Liang *et al.*, 2005; Guével *et al.*, 2007; Rodrigues *et al.*, 2009). Foliar sprays of PS have been effective in controlling powdery mildew on muskmelon and zucchini (Menzies *et al.*, 1992). The foliar application of PS was shown to reduce the number of colonies of *Uncinula necator* on grape leaves by more than 14% (Bowen *et al.*, 1992). Dallagnol *et al.* (2012) reported that the area under powdery mildew progress curve was reduced by 65% and 73% for plants that were sprayed with PS or grown in a Si-supplemented substrate, respectively, compared with the control plants. Indeed, the root application of PS was more effective than foliar application in reducing the infection efficiency, colony expansion rate, colony area, conidial production and disease progress rate (Dallagnol *et al.*, 2012). Pozza *et al.* (2004) evaluated the effect of soil amendment with calcium silicate on the control of coffee leaf spot (*Cercospora coffeicola*) and observed a reduction of 63.2% for leaf lesions and 43% reduction of total lesions per plant compared to non-amended plants. The X-ray microanalysis and mapping of Si showed uniform distribution of this element on the abaxial leaf surface. On leaves of non-treated plants, little Si was detected. A scanning electron microscopy analysis showed a well-developed wax layer on the lower leaf surfaces that affected fungal penetration (Pozza *et al.*, 2004). The foliar application of PS was shown to have a negative effect on powdery mildew, which was likely caused by a direct deleterious action of the product against the fungus (Guével *et al.*, 2007). Asian soybean rust severity was reduced by foliar applications of PS (Rodrigues *et al.* (2009). According to Naidoo *et al.* (2009), it showed greater Si deposition on the lower epidermis of sugar leaves that received foliar applications of PS via X-ray microanalysis, and this deposition was associated with lower brown rust severity. Recently, Camargo *et al.* (2013) reported a reduction on brown rust incidence on sugarcane grown in different Si-deficient soils amended with calcium silicate.

Considering the limited information in the literature regarding the role of Si in controlling plant diseases in coffee, this study was designed to investigate the effect of foliar applications of PS on the progress of coffee leaf rust at the microscopic level.

## Material and methods

### Plant growth and inoculation procedure

The soil type used in the experiments was a Si-deficient typical Acrustox red-yellow latosol collected from the 'Triângulo Mineiro' savanna area. The soil had the following characteristics: 530 g $kg^{-1}$ of clay; pH in KCl = 4.8; P (Mehlich-1) = 0.5 mg $dm^{-3}$; K (Mehlich-1) = 13 mg $dm^{-3}$; $Al^{3+}$, $Ca^{2+}$, $Mg^{2+}$, $H^{+}+Al^{3+}$ = 0.1, 0.0, 0.0 and 3.8 $cmol_c$ $dm^{-3}$, respectively; base saturation = 2% and organic matter = 2.3 dag $kg^{-1}$. The concentration of available Si (extraction in $CaCl_2$) was 11.8 mg $dm^{-3}$. Plastic pots (20 cm diameter) (Ecovaso, Jaguariúna, Brazil) were filled with 1 kg of air-dried, sieved (5 mm) soil. Lime was added at a rate of 1.5 g $kg^{-1}$ of soil. The soil in each pot was incubated for 60 days with an approximate humidity of 65%.

Coffee seeds from cultivar 'Catuaí vermelho 44' were sowed in plastic trays (15 × 40 × 10 cm; Ecovaso) containing sand (10 mm). After 60 days, seedlings at the cotyledonary growth stage were transplanted to plastic pots. The soil in each pot was fertilised before sowing with 1.63 g of calcium phosphate per kg of soil and with 30 mL of a nutrient solution containing the following nutrients in g $L^{-1}$: 6.4 KCl, 3.5 $K_2SO_4$, 5.0 $MgSO_4{\cdot}7H_2O$, 2.0 $(NH_2)_2CO$, 0.009 $NH_4Mo_7O_{24}{\cdot}4H_2O$, 0.054 $H_3BO_3$, 0.222 $ZnSO_4{\cdot}7H_2O$, 0.058 $CuSO_4{\cdot}5H_2O$ and 0.137 $MnCl_2{\cdot}4H_2O$. The nutrient solution was applied every week after seedling emergence. Fifteen millilitres of a solution containing 0.27 g $FeSO_4{\cdot}7H_2O$ and 0.37 g of EDTA disodium $L^{-1}$ were also applied after seedling emergence and repeated weekly.

The abaxial leaf blades, where *H. vastatrix* penetration takes place, of the second pair of leaves of each plant were sprayed with a solution of PS (Fertisil®, PQ Silicas Brazil Ltda, São Paulo, Brazil) at a rate of 20 g $L^{-1}$. The pH was changed from 10 to 5.5 using HCl 1 M to improve monosilicic acid uptake by the leaves (Menzies *et al.*, 1992; Rodrigues *et al.*, 2009). The plant leaves that were sprayed with deionised water served as the control treatment. The leaves were sprayed with PS or water until runoff using a VL Airbrush atomizer (Paasche Airbrush Co., Chicago, IL, USA). Twenty-four hours after applying, the abaxial leaf blades were inoculated with a suspension of *H. vastatrix* (1 mg $mL^{-1}$) urediniospores using a VL Airbrush atomizer. After inoculation, the plants remained in a mist chamber at a temperature of 23 ± 1°C and a relative humidity of 90 ± 5% for 48 h in the absence of light. After this period, the plants were transferred to a growth chamber (Marconi Equipamentos para Laboratórios, São Paulo, Brazil) with temperatures ranging from 22 ± 0.5 (day) to 21 ± 0.5°C (night) for the duration of the experiments. A total

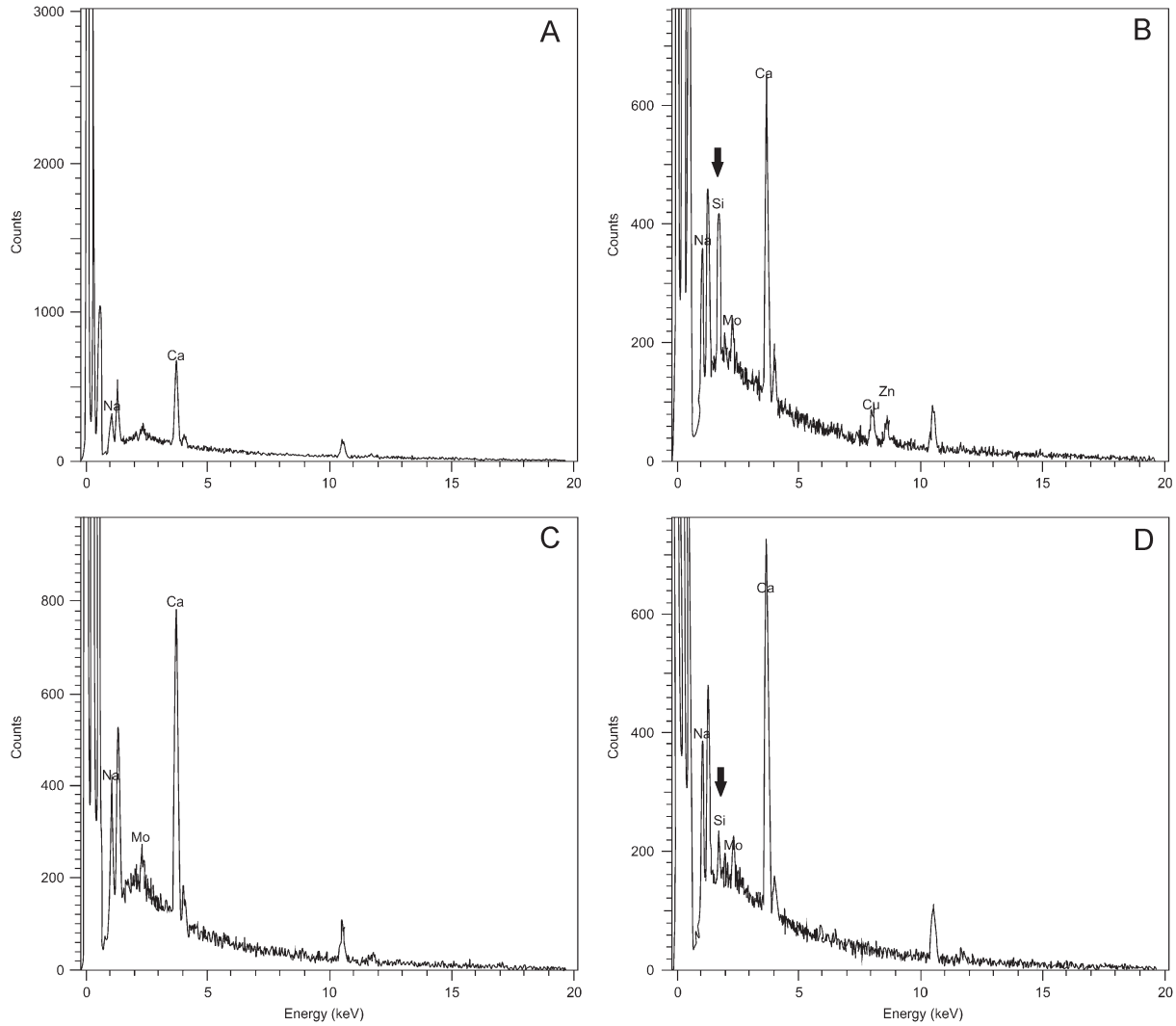


**Figure 1** X-ray microanalysis of the adaxial leaf epidermis of coffee plants that were non-sprayed (A and C) and sprayed with potassium silicate (B and D) at 10 (A and B) and 36 (C and D) days after inoculation with *Hemileia vastatrix*. Arrows (B and D) indicate peaks of silicon.

of 125 µL of inoculum was transferred to five Petri dishes containing potato-dextrose-agar media and was homogeneously distributed on each dish using a Drigalsky glass stick. Petri dishes were kept in complete darkness in a growth chamber at 25°C. After 24 h, lactophenol was added to the plates to stop urediniospore germination. Two hundred urediniospores were randomly examined from each Petri dish under a microscope (Carl Zeiss Axio Imager A1) at 400× magnification. A urediniospore was considered germinated when the germ tube was longer than its diameter. The percentage of germination was 89 ± 2.5%. The photon flux density inside the growth chamber was quantified with a Li 250A light meter (Li-Cor Environmental, Lincoln, NE, USA) and was approximately 900 µmol photons $m^{-2}$ $s^{-1}$.

### Evaluation of rust severity

Rust severity was evaluated at 10, 16, 20, 25 and 36 days after inoculation (dai) using the scale proposed by Kushalappa and Chaves (1978). This scale consists of three coffee leaves with 30%, 50% and 70% of rust severity on each leaf. Each leaf has a known area of 1%, 3%, 5%, 7% and 10% occupied by individual rust pustules.

### Plant tissue analysis for Si and potassium concentrations

After the experiments, the leaves of the plants from each replication and treatment were collected, washed in deionised water, dried for 72 h at 65°C and ground

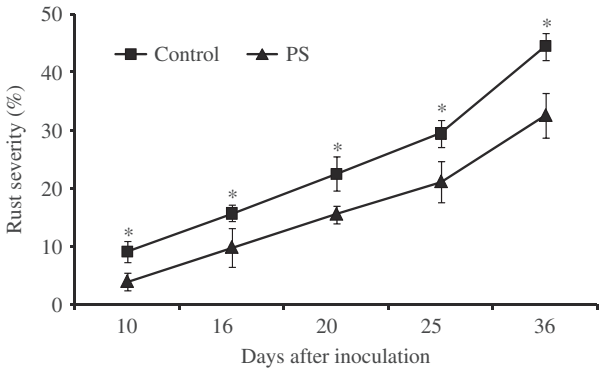


**Figure 2** Severity of coffee leaf rust on plants sprayed with water (control) or potassium silicate (PS) and inoculated with *Hemileia vastatrix*. The means for the control and PS treatments that are followed by an asterisk (*) for each evaluation time are significantly different by Student's *t*-test ($P \leq 0.05$). Bars represent the standard error of the means ($n = 20$).

in order to pass through a 40-mesh screen with a Thomas-Wiley mill. Concentration of Si in leaf tissue was determined by a colorimetric analysis of 0.1 g of dried and alkali digested tissue (Korndörfer *et al.*, 2004). Potassium concentration was determined by atomic absorption spectrophotometry.

### X-ray microanalysis of Si deposition on leaf blades

Ten to 15 leaf pieces of approximately 50 $mm^2$ in size that contained pustules were randomly collected from the leaves of each plant per treatment at 10 and 36 dai. Leaf samples were transferred to glass vials containing 10 mL of a solution composed of 3% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and stored at 4°C for 5 days. Leaf samples from all treatments were carefully washed with sodium cacodylate buffer, dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 95% and 100%) and submitted to a critical point dryer (Bal-Tec, Model CPD 030, Electron Microscopy Sciences, Hatfield, PA, USA). The abaxial leaf epidermis of the specimens was mounted onto aluminium stubs and sputter-coated with carbon in a sputter coater (Balzers Union, model FDU 010, Electron Microscopy Sciences, Hatfield, PA, USA). Specimens were examined under the scanning electron microscope (SEM) (LEO 1430VP, Carl Zeiss, Jena, Germany) with an energy dispersive X-ray analysis device (EDS, Oxford Instrument Link ISIS, Oxford, UK), operating at 10–20 kV with a working distance of 10 mm.

### Sample collection procedures for scanning electron microscopy (SEM)

A total of 25 pieces (2.5 $cm^2$ in size) of the second pair of leaves were randomly collected from each plant per replication and treatment at 6, 16 and 36 dai. The leaf pieces were carefully transferred to glass vials containing 3 mL of fixative (2.5% v/v glutaraldehyde in 0.1 mM sodium cacodylate buffer, pH 7.2). The samples were stored at 4°C for 5 days and then carefully washed with a sodium cacodylate buffer (0.1 M), dehydrated in a graded ethanol series and critical point dried in $CO_2$ (Bal-Tec, Model CPD 030, Electron Microscopy Sciences). Four specimens from each sample were mounted on aluminium stubs, sputter-coated with gold (Balzers Union, model FDU 010; Electron Microscopy Sciences) and examined and photographed using a LEO SEM (model 1430 VP) operating at 10 kV and with a working distance ranging from 10 to 30 mm. Leaf samples collected at 36 dai were carefully sliced with a scalpel in the direction of the rust pustules and examined using SEM. For each treatment, four stubs, each containing four specimens, were examined by SEM. A total of six samples were analysed.

### Experimental design and statistical analysis

The experiment was arranged in a completely randomised design with 2 treatments and 10 replications. Each experimental unit consisted of one plastic pot with one plant. The experiment was repeated once. The rust severity and foliar Si and potassium concentrations data were combined for statistical analysis after determining the homogeneity of variance and the mean squares (Gomez & Gomez, 1994). The means from the treatments at each evaluation time were compared by *t*-test ($P \leq 0.05$) using SAS (SAS Institute Inc., Cary, NC, USA).

## Results and discussion

There was no significant difference in leaf Si concentration for plants that were sprayed with water or PS (0.24 and 0.30 dag $kg^{-1}$, respectively). The foliar potassium concentration did not significantly change between plants sprayed with water or PS. The X-ray microanalysis indicated that the deposition of Si on the leaves was only observed on plants sprayed with PS at 10 and 36 dai (Fig. 1B and Fig. 1D) and not from plants sprayed with water (Fig. 1A and Fig. 1C). However, Si deposition on the leaves of the plants sprayed at 36 dai was lower than at 10 dai (Fig. 1B and Fig. 1D).

Leaf rust severity of the plants sprayed with water increased from 10 to 36 dai, when it reached 44% (Fig. 2). In contrast, rust progressed slowly and reached 32% at 36 dai on the leaves of plants sprayed with PS (Fig. 2). The highest rust severity on leaves of plants sprayed with PS from 25 to 36 dai can be linked to the reduced Si deposition at 36 dai. Plates of polymerised PS were

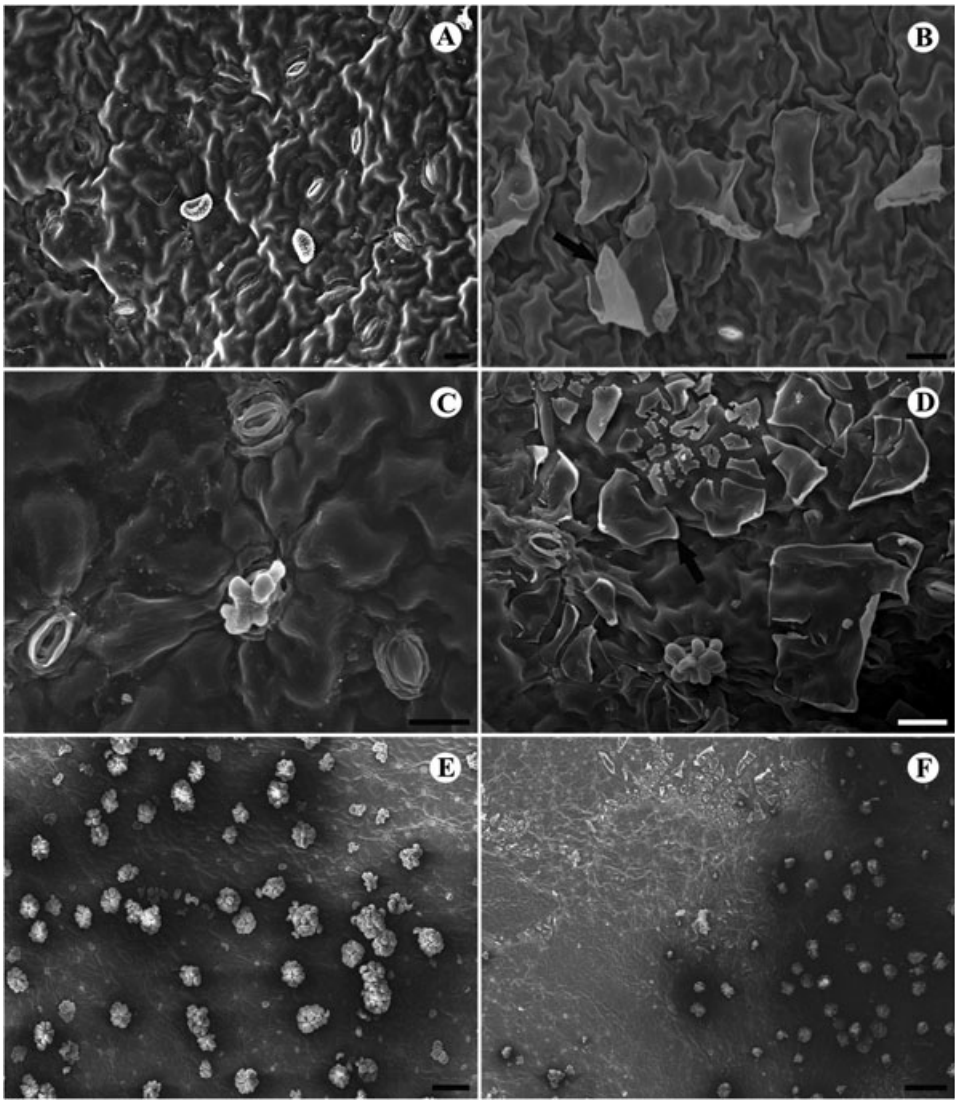


**Figure 3** Scanning electron micrographs of the adaxial leaf epidermis of coffee plants sprayed with deionised water (A, C and E) or sprayed with potassium silicate (B, D and F) 24 h before inoculation with *Hemileia vastatrix*. Leaf samples were collected at 6 (A and B), 16 (C and D) and 36 (E and F) days after water and potassium silicate spray for scanning electron microscope observations. Arrows indicate plates of polymerised potassium silicate. Bars = 20 μm (A, B, C and D), 100 μm (E) and 200 μm (F).

observed on the leaf surfaces of the plants sprayed with PS at 6, 16 and 36 dai (Fig. 3B, Fig. 3D and Fig. 3F), in contrast to its absence on the leaf surfaces of plants that were sprayed with water (Fig. 3A, Fig. 3C and Fig. 3E). At 36 dai, a greater number of uredia were observed on the leaf surfaces of the plants sprayed with water in comparison to a lesser number of uredia on the leaf surfaces of plants sprayed with PS (Fig. 3E and Fig. 3F). Less fungal colonisation was observed on leaf tissues of leaves sprayed with PS (Fig. 4B and Fig. 4D), in comparison to the leaves of plants sprayed with water (Fig. 4A and Fig. 4C).

To the best of our knowledge, the results from this study are the first to demonstrate that the spraying of PS can decrease coffee leaf rust severity at the macroscopic level. Additionally, it provides new insights regarding the positive effect of Si on the control of diseases, especially on dicots, and sheds some light on the potential of using Si-soluble products as an alternative disease management strategy. The major sources of plant-available Si are slags, PS and sodium silicate (Datnoff *et al.*, 2007). These materials are applied either to soil, soil-less media or a hydroponic system in order to increase the accumulation of Si in plant tissue and to enhance host resistance

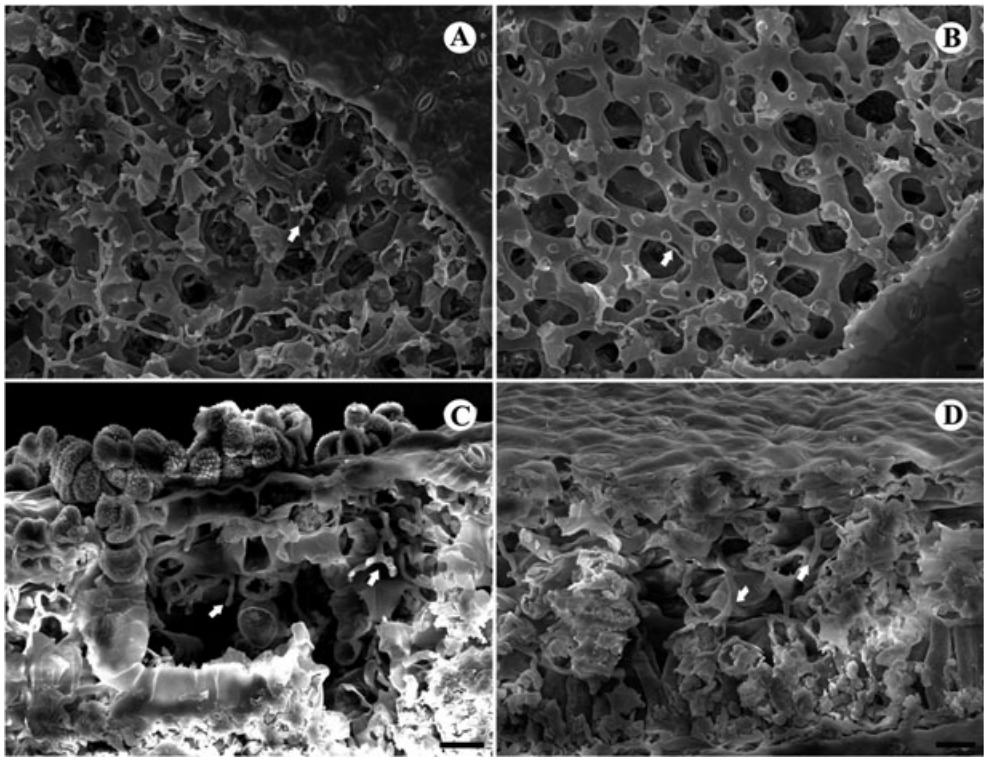


**Figure 4** Scanning electron micrographs of fractured leaves of coffee plants sprayed with deionised water (A and C) and potassium silicate (B and D) 24 h before inoculation with *Hemileia vastatrix*. Leaf samples were collected at 36 days after inoculation. Arrows indicate hyphae of *H. vastatrix*. Bars: 20 μm (A, B and D) and 30 μm (C).

against disease (Bélanger *et al.*, 2003; Rodrigues *et al.*, 2001; Datnoff *et al.*, 2007). The foliar applications of PS were shown to reduce the intensities of powdery mildews on cucumber, melon, muskmelon, zucchini and grape (Bowen *et al.*, 1992, Menzies *et al.*, 1992; Dallagnol *et al.*, 2012) and soybean rust on soybeans (Rodrigues *et al.*, 2009).

In this study, X-ray microanalysis demonstrated that Si deposition was higher on the abaxial leaf epidermis of plants sprayed with PS, even though the chemical analysis did not indicate a difference between leaf tissues of plants sprayed either water or PS. Dallagnol *et al.* (2012) also found no difference in Si concentration on leaves of melon plants sprayed either with PS or water. X-ray analyses indicated that Si accumulated only in non-rinsed leaves (Dallagnol *et al.*, 2012). Although the PS spray provided some coffee leaf rust control, this finding cannot be exclusively attributed to Si deposition on the adaxial leaf epidermis. Guével *et al.* (2007) showed that foliar spray of Si was less effective than root application for promoting powdery mildew control on wheat. The reduction of disease severity from the use of foliar Si spray was due to a direct effect of the source of Si on the pathogen rather than an effect that was mediated by the plant, as in the case of root amendments that resulted in greater disease control (Guével *et al.* 2007). The contact of *H. vastatrix* uredospores and PS may negatively affect their germination or even the germ tube growth. Additionally, this contact may physically impede the fungal ingress, as it may dry out. Liang *et al.* (2005) compared the foliar and root applications of Si on powdery mildew control in cucumber and concluded that foliar-applied Si effectively reduced infections by *Podosphaera xanthii* only through the physical barrier of Si that is deposited on the leaf surfaces. Kanto *et al.* (2007) did not observe any effect of PS spraying on the germination of conidia of *Sphaerotheca aphanis* var. *aphanis* on strawberry leaves. The authors suggested that the soluble Si did not directly act against infection by *S. aphanis* var. *aphanis*, but may change the chemical composition of the cuticle layer that can inhibit conidia germination and, consequently, reduce disease severity. Bowen *et al.* (1992) reported that thick PS deposits that coated a significant portion of the grape leaf cuticle prevented the penetration by germinating ascospores of *U. necator*. Indeed, fungal development was more extensive in the areas of the leaf surface that were not coated (Bowen *et al.*, 1992).

Coffee plants cannot uptake Si from roots and accumulate it in the shoots (Carré-Missio *et al.*, 2009) with great efficiency as compared to rice (Rodrigues *et al.*, 2001). Coffee plants also show very low leaf Si absorption efficiency, mainly due to the quick polymerisation of PS on the leaf surface before uptake can occur (Carré-Missio *et al.*, 2012). The SEM analysis revealed that the presence of thick plates of polymerised PS on the cuticle seemed to impede the formation of uredia. Menzies *et al.* (1992) reported that the polymerisation of PS on the surface of cucumber, muskmelon and zucchini squash leaves created a physical barrier that decreased penetration by *Sphaerotheca fuliginea*. Carré-Missio *et al.* (2012) observed reduced germination of *H. vastatrix* uredospores when the rates of PS were increased *in vitro*. These data are consistent with the hypothesis that there was a physical barrier provided by the Si polymerised on the leaf surface and that there was a direct effect of this product against the pathogen. This can be also explained by the lower rust severity on the leaves of plants sprayed with PS due to the reduced efficiency during the first cycle of fungal infection, reinforcing, therefore, the theory that the action of foliar application of PS is a physical–chemical barrier on the leaf surface.

In conclusion, the results of this study, in association with previous reports from other pathosystems, suggest that the effect of foliar-applied PS on the control of coffee leaf rust may be attributed to the physical role of the polymerised PS or its osmotic effect against urediniospores germination.

## Acknowledgements

Prof. F. A. R. and L. Z. thank CNPq for their fellowship. We are indebted to Prof. E.W. Kitajima (NAP/MEPA, ESALQ/USP) for his help with the X-ray microanalysis. V. C. was supported by the CNPq. The authors also would like to express their appreciation to the 'Núcleo de Microscopia e Microanálise (NMM)/CCB/UFV' for the use of the equipment. This research was supported by a grant from INEOS Silicas Brazil Ltda to Prof. F. A. R. The authors would like to thank CAPES, CNPq, FAPEMIG and FINEP for the financial support.

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