Photosynthetic gas exchange and antioxidative system in common bean plants infected by *Colletotrichum lindemuthianum* and supplied with silicon

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ABSTRACT

This study investigated the effects of silicon (Si) on the resistance of common bean plants to anthracnose caused by *Colletotrichum lindemuthianum*. The plants were grown in a nutrient solution containing 0 (control) or 2 mM Si (+Si) and both photosynthesis and antioxidative metabolism levels were evaluated. The Si concentrations in the leaf tissues of +Si plants increased by 33% in comparison to those of control plants. Anthracnose severity was reduced by 34% in +Si plants in comparison to control plants. The net carbon assimilation rate, stomatal conductance to water vapor and transpiration rate values were significantly higher in +Si plants than in control plants. The activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) tended to be higher in +Si plants than in control plants. The hydrogen peroxide concentration was significantly lower in +Si plants than in control plants. In conclusion, the Si supply was associated with lower anthracnose severity and up-regulation of antioxidant enzymes, which in turn might be associated with better gas exchange in +Si plants. The impaired photosynthetic performance in +Si plants was associated with stomatal limitations, whereas in control plants those impairments likely reflected dysfunctions at the level of biochemical reactions involved in CO_2 fixation.
Key words: *Colletotrichum lindemuthianum*, antioxidative enzymes, foliar disease, mineral nutrition, photosynthesis.

INTRODUCTION

Among the diseases that affect the common bean (*Phaseolus vulgaris* L.), anthracnose, caused by the hemibiotrophic fungus *Colletotrichum lindemuthianum* (Sacc. and Magnus) Scrib, is one of the most destructive (Pastor-Corrales, 1985). Leaves, stems, branches, pods and seeds can be infected by *C. lindemuthianum* (Hall, 1991). In seedlings, typical symptoms of anthracnose include dark lesions on the cotyledons and hypocotyls; in the leaves of adult plants, the symptoms typically consist of dark brown or brown lesions in the petioles and in the main and secondary veins. The lesions are roundish, depressed, of different sizes and with clear centers, delimited by a slightly protruding black ring and surrounded by a reddish coffee-colored border. When the relative humidity and temperature are favorable, conidia are produced in the centers of these lesions and are dispersed by water to initiate new infection cycles of the pathogen. Full crop failure can occur when infected seeds or susceptible cultivars are used and environmental conditions are favorable for the occurrence of anthracnose epidemics (Hall, 1991).

The most viable option for anthracnose control is the use of resistant cultivars, which can be difficult to achieve because there are several physiological races of *C. lindemuthianum* (Rava et al., 1994). Spraying with systemic fungicides is another option to reduce the yield losses caused by this disease (Hall, 1991). In light of these concerns, alternative methods for anthracnose control are necessary to reduce the yield losses in beans caused by *C. lindemuthianum*. Silicon (Si), although not considered an essential nutrient for plants, stands out for its potential to decrease the intensities of important diseases in several crops, especially grasses and some dicots such as beans, cucumber and soybeans (Datnoff et al., 2007). For necrotrophic pathogens such as *Rhizoctonia solani* and *Bipolaris oryzae* on rice and *Bipolaris sorokiniana* on wheat, the supply of Si to plants reduced the severity of the diseases caused these pathogens (Rodrigues et al. 2001; Dallagnol et al. 2009; Domiciano et al., 2010). For the pathosystem rice-*Pyricularia oryzae*, the increased resistance of plants supplied with Si was associated with an increase in the density of long and short silicate cells in the leaf epidermis and a thick silica layer below the cuticle that formed a physical barrier that likely prevented or slowed fungus penetration (Kim et al., 2002). The biochemical and physiological mechanisms potentiated by Si include higher concentration of phenolics, lignin and phytoalexins, an increase in the activities of defense enzymes such as chitinases and *β*-1,3-glucanases and the rapid, strong transcription of genes

related to host resistance (Datnoff et al., 2007; Brunings et al., 2009). Polanco et al. (2012) reported that higher Si concentrations in the leaf tissues of bean plants supplied with this element were associated with a reduced area under the anthracnose progress curve in association with higher activities of chitinases, phenylalanine ammonia-lyases and lipoxygenases and an increased concentration of lignin. In the *C. lindemuthianum*-bean pathosystem, Si reduced both the area under the anthracnose incidence progress curve and the area under the anthracnose severity progress curve (Moraes et al., 2006). According to Menzies et al. (1992), phenolics accumulated more rapidly in the epidermal cells of cucumber plants supplied with Si and colonized by *Podosphaera xanthii*. Oat plants deprivated of Si increased the activity of phenylalanine ammonia lyase and, consequently, the accumulation of phenolic compounds in epidermal cells colonized by *Blumeria graminis* f.sp. *avenae* (Carver et al., 1998). Furthermore, in the context of *Pythium ultimum* infection, the activities of chitinases, peroxidases and polyphenoloxidases were much higher on the roots of cucumber plants supplied with Si than on roots of plants that were not supplied with this element (Chérif et al., 1994).

Photosynthesis is a critical process for crop growth and performance, and it can be dramatically impaired when leaves are infected by pathogens (Berger et al., 2007). The decrease in the net carbon assimilation rate of infected leaves has been attributed to decreases in mesophyll and/or stomatal conductance of CO_2, impairments in the rates of biochemical reactions of photosynthesis, changes in the number or structure of chloroplasts or changes in the secretion of phytotoxic chemicals into asymptomatic tissue (Berghaus & Reisener, 1985; Meyer et al., 2001; Pinkard & Mohammed, 2006). In the bean-*C. lindemuthianum* pathosystem, changes in the net carbon assimilation rate are exhibited during the necrotrophic phase: the inhibition of the net carbon assimilation rate takes place in the green tissue around the infection site and has been associated with stomatal closure and reduced ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity (Meyer et al., 2001).

Abiotic stresses such as drought, heavy metal toxicity and salinity and biotic stresses such as pathogen infection favor the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) and hydroxyl (OH^-) radicals (Iturbe Ormaetxe et al., 1998). Upon pathogen attack, the generated ROS cause lipid peroxidation in the plasma membrane and increase the concentration of total 2-thiobarbituric acid (TBA)-reactive substances (Resende et al., 2012). To mitigate and repair the cellular damage caused by the ROS, plants have developed protective mechanisms which involve the production of low molecular weight antioxidant molecules such as ascorbate, glutathione, α-tocopherol and some carotenoids as well as antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase (Gunes et

al., 2007). Superoxide dismutase catalyzes the dismutation of O_2^- to H_2O_2 and oxygen. Because H_2O_2 is cytotoxic, it is then detoxified by the catalase to produce oxygen and water (Zhu et al., 2004). In the ascorbate-glutathione cycle, ascorbate peroxidase reduces H_2O_2 using the ascorbate molecule as an electron donor (Noctor & Foyer, 1998). The oxidized ascorbate is then reduced by reduced glutathione, which is produced from oxidized glutathione by glutathione reductase (GR) in a reaction that involves the consumption of NADPH (Lin & Kao, 2000). The activities of antioxidant enzymes in several crops, such as barley, cucumber, spinach and wheat in the presence of different types of abiotic stress were greatly improved in the presence of Si (Liang et al., 2003; Gunes et al., 2007; Gong et al., 2008).

To the best of our knowledge, the effects of Si on enhancing bean resistance against infection by *C. lindemuthianum* at the levels of both photosynthesis and antioxidative metabolism have never been investigated. Therefore, this study aimed to determine whether common bean plants supplied with Si during the course of infection with *C. lindemuthianum* could maintain photosynthesis at desirable physiological levels with a reduced oxidative metabolism.

MATERIAL AND METHODS

Nutrient solution preparation and plant growth

The nutrient solution used in this study was prepared according to Liao et al. (2004) and consisted of 3 mM KNO_3, 2 mM $Ca(NO_3)_2.4H_2O$, 0.5 mM $MgSO_4.7H_2O$, 0.25 mM $(NH_4)_2SO_4$, 0.5 mM $NH_4H_2PO_4$, 12.5 mM H_3BO_3, 1 μM $ZnSO_4.7H_2O$, 0.25 μM $CuSO_4.5H_2O$, 0.25 μM $MnCl_2.4H_2O$, 0.25 μM $(NH_4)_6Mo_7O_{24}.4H_2O$, 25 μM $FeSO_4.7H_2O$ and 25 μM Na-EDTA. The Si, provided as monosilicic acid, was obtained by passing potassium silicate through cation-exchange resin (Amberlite IR-120B, H^+ form; Sigma-Aldrich) (Dallagnol et al., 2009). The Si concentrations used were 0 (control) and 2 mM (+Si). The addition of monosilicic acid to the nutrient solution did not alter the pH. Bean seeds from the cultivar "Pérola", which is susceptible to *C. lindemuthianum* (Polanco et al., 2012) were surface sterilized in a solution of sodium hypochlorite 10% (vol/vol) and subsequently washed with sterile water for 2 min, transferred to filter paper soaked with sterile water and germinated for five days in a growth chamber (MA-835/2106UR; Marconi) at 25ºC. Seedlings were grown in a hydroponic system containing half-ionic strength nutrient solution, as mentioned above, without the addition of Si for two days to allow for acclimation. Subsequently, the seedlings were transferred to new plastic pots (25-cm-diameter) containing 5 L of nutrient solution with or without Si under aeration until the end of the experiments. The nutrient solution was changed every four days or when the electrical conductivity reached 85% of the initial value. The pH of the nutrient solution was checked daily and maintained at 5.5 using solutions of NaOH or HCl (1 M) when necessary.

Inoculation procedure

The isolate 81-538 (race 81) of *C. lindemuthianum*, obtained from the Bean Fungus Collection Culture from the Dep. de Biologia Geral, Universidade Federal de Viçosa, was used in this study. The isolate was preserved on strips of filter paper placed into glass tubes containing silica gel at 4°C. Pieces of filter paper with fungal mycelia were transferred to Petri dishes containing potato-dextrose-agar (PDA). Inoculum production was made based on Polanco et al. (2012). The suspension was filtered through double cotton gauze and adjusted to a concentration of 1.2×10^6 conidia mL^{-1} using a Neubauer-counting chamber. Plants were allowed to grow for 21 days (V5 growth stage, 5th trifoliolate unfolded at node 6 and branching) in a hydroponic culture containing 0 or 2 mM Si and then inoculated with a conidial suspension of *C. lindemuthianum* applied as a fine mist to the adaxial and abaxial leaves of each plant until runoff using a DeVilbiss atomizer No. 15. One percent gelatin (w/vol) was added to the sterile water used to prepare the conidial suspension to aid conidial adhesion to the leaves. Immediately after inoculation, the plants were transferred to a growth chamber with temperature of 20±2°C, relative humidity of 90±5% and irradiance of 700 μmol photons m^{-2}s^{-1} provided by white fluorescent lamps (12 h light/12 h dark) for 24 h. After this period, the plants were transferred to a plastic mist growth chamber (MGC) as described by Polanco et al. (2012). Non-inoculated plants were kept in a separate MGC, but exposed to the same conditions as the inoculated plants.

Evaluation of anthracnose severity

Anthracnose severity was evaluated on the fourth trifoliate leaf of each plant at 144 hours after inoculation (hai) using the diagrammatic scale with severity values ranging from 0.1 to 24% (Godoy et al., 1997).

Determination of leaf gas exchange parameters

Leaf gas exchange parameters were determined using a portable open-flow gas exchange system (LI-6400; LI-COR) equipped with a blue/red light source (LI-6400-02B). The net CO_2 assimilation rate (*A*), stomatal conductance to water vapor (g_s), internal CO_2 concentration (C_i) and transpiration rate (*E*) were measured on the fourth trifoliate leaf from 08:00 to 11:00 hours (solar time), which is when *A* was at its maximum, under artificial PAR (1,000 μmol photons m^{-2}s^{-1}) at the leaf level and 390 μmol CO_2 mol^{-1} air. All measurements were performed at ambient temperature (27±0.9°C) at 0, 36, 48, 72 and 120 hai.

Biochemical assays

Samples from the third and fourth trifoliate leaves of each plant were collected at 24, 48, 72, 120 and 144 hai. Leaf samples collected from non-inoculated plants served as the controls (0 dai). The leaf samples were kept in liquid nitrogen during the sampling and then stored at -80°C until required.

For the determination of the activities of superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), catalase (CAT, EC 1.11.1.6) and glutathione reductase (GR, EC 1.8.1.7), 0.3 g of leaf tissue was ground into a fine powder in a mortar and pestle with liquid nitrogen. The fine powder was homogenized in an ice bath with 60 mg polyvinylpolypyrrolidone and the following additional reagents: for SOD, 1 mL 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA and 0.1% v/v Triton X-100; for CAT, 1 mL 100 mM potassium phosphate buffer (pH 7.0) and 0.1 mM EDTA; for APX, 1 mL 50 mM potassium phosphate buffer (pH 7.0) and 1 mM ascorbate; and for GR, 1 mL 100 mM Tris-HCl (pH 7.5), 50 mM EDTA, 10 mM isoascorbate, 9 mM 2-mercaptoethanol and 0.1% (v/v) Triton X-100 (Resende et al., 2012). The homogenates were centrifuged at 15,000 *g* for 15 min at 4°C and the supernatants were used as crude enzyme extracts. The SOD activity was assayed by the method of Giannopolitis & Ries (1977) by measuring its ability to inhibit the photochemical reduction of *p*-nitrotetrazolium blue (NTB) to formazan by converting O_2^- in H_2O_2. The reaction was initiated after the addition of 5 μL of the crude enzyme extract to 3 mL of a mixture containing 50 mM potassium phosphate buffer (pH 7.8), 14 mM methionine, 75 μM NTB, 0.1 μM EDTA and 2 μM riboflavin. The production of formazan blue, resulting from the photoreduction of NTB, was monitored by measuring the increase in absorbance at 560 nm (Giannopolitis & Ries, 1977). One unit of SOD was defined as the amount of enzyme necessary to inhibit NTB photoreduction by 50% and was interpreted as the total superoxide scavenging capacity. For APX, the reaction was started by adding 20 μL of the crude enzyme extract to 3 mL of a mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate and 0.1 mM H_2O_2. The APX activity was determined by the rate of ascorbate oxidation at 290 nm (Nakano & Asada, 1981). The CAT activity was estimated by the rate of H_2O_2 decomposition at 240 nm (Havir & McHale, 1989). The reaction was initiated by adding 20 μL of the crude enzyme extract to 3 mL of a mixture containing 50 mM potassium phosphate buffer (pH 7.8) and 12.5 mM H_2O_2. The GR activity was determined by the rate of NADPH oxidation at 340 nm. The reaction was initiated by adding 20 μL of the crude enzyme extract to a mixture containing 50 mM Tris-HCl (pH 7.5), 10 mM reduced glutathione, 3 mM $MgCl_2$ and 0.15 mM NADPH. The decrease in absorbance was determined at 340 nm for 1 min at 30°C. An extinction coefficient of 6.22 mM^{-1}cm^{-1} (Foyer & Halliwell, 1976) was used to calculate the GR activity. For each enzyme, extractions were performed using leaf samples from the three plants of each replication of the treatments. Each reaction was performed three times using samples from the same extraction. The soluble protein contents of the extracts were measured using the method of Bradford (1976).

For hydrogen peroxide (H_2O_2) assay, a total of 0.2 g of frozen leaf tissue was ground into a fine powder using

a pestle and mortar with liquid nitrogen. The fine powder was homogenized in an ice bath in 2 mL 50 mM potassium phosphate buffer (pH 6.5) containing 1 mM hydroxylamine. The homogenate was centrifuged at 10,000 g for 15 min at 4°C (Meyer et al., 2001). The supernatant was used to determine the H_2O_2 concentration. The reaction was started by the addition of 50 µl of the supernatant to a mixture containing 25 mM sulfuric acid, 100 µM ammonium ferrous sulphate, 250 µM xylenolorange and 100 mM sorbitol in a volume of 2 mL (Gay & Gebicki, 2000). The samples were kept in the dark for 30 min and the absorbance was recorded at 560 nm. Control samples (blank) for the color of the reagents as well as for the sample extracts were prepared concurrently with the tested samples and subtracted from the absorbance value obtained for each sample. The H_2O_2 concentration was expressed as nmol g^{-1} of fresh matter (FM) with hydrogen peroxide (Sigma-Aldrich) as a standard.

Determination of Si concentration in leaf tissue

After the end of the experiments, trifoliate leaves from the plants of each replication per treatment were collected, washed with deionized water and dried at 65°C for 72 h. Next, the leaves were ground in a Thomas-Wiley mill with a 40-mesh sieve. The concentration of Si was determined by colorimetric analysis of the alkaline digestion of 0.1 g of dry leaf tissue (Resende et al., 2012).

Experimental design and statistical analysis

Two independent experiments were carried out in a completely randomized design with two treatments (0 and 2 mM Si) and six replications to evaluate the anthracnose severity, the Si concentration in the leaf tissue and the leaf gas exchange parameters. Each replication consisted of a plastic pot containing 5 liters of nutrient solution and three bean plants. A total of 180 plants were used in each experiment (18 plants per each treatment at each evaluation time). The anthracnose severity, leaf Si concentration and gas exchange parameter data from the two experiments were combined after the determination of the homogeneity of variance by Cochran's test (Gomez & Gomez, 1994). A third experiment, repeated once, was performed to obtain leaves for the biochemical assays. The H_2O_2 concentration and enzyme activity data were submitted to analysis of variance, and the treatment means were compared by t-test ($P \leq 0.05$) using SAS software (SAS Institute). The correlations among the Si concentration in the leaf tissue, severity of anthracnose and the leaf gas exchange parameters A, g_s, E and C_i were evaluated based on data pooled from two experiments.

RESULTS

Foliar Si concentration and anthracnose severity

The Si concentration in the leaf tissues of +Si plants increased by 33% in comparison to the control plants (Table 1). The anthracnose severity was reduced by 34% in the +Si plants relative to the control plants (Table 1).

TABLE 1 - Silicon (Si) concentration and anthracnose severity (Sev) in the leaves of bean plants grown in hydroponic culture containing 0 (control) or 2 mM (+Si) silicon and inoculated with *Colletotrichum lindemuthianum*.

Treatments	Si (dag kg^{-1})	Sev (%)
Control	0.84	6.30
+Si	1.25	4.16
Student's t-test	1.22*	3.34*
CV (%)	16.58	14.72

*Significant at 5% probability.
CV, Coefficient of variation.
Data are from two pooled experiments ($n = 36$).

Leaf gas exchange parameters

Silicon supplementation was able to limit the impairment of photosynthetic gas exchange upon infection by *C. lindemuthianum* as indicated by the significantly higher values of A in +Si plants from 36 to 120 hai (Figure 1A), of g_s from 48 to 120 hai (Figure 1B) and of E from 36 to 120 hai (Figure 1C). Infection with *C. lindemuthianum* led to devastating effects on A. In control plants, leaves with 6.30% severity displayed reductions of as much as 80% in A, whereas in the +Si plants, leaf severity was 4.16% and was accompanied by decreases in A of approximately 40% as severe as those in control plants at 120 hai (Table 1, Figure 1A).

H_2O_2 concentration and enzyme activities

Overall, the enzyme activities tended to be higher in +Si plants than in the control plants during the course of infection by *C. lindemuthianum*. For +Si plants in comparison to the -Si plants, SOD activity was significantly higher from 48 to 144 hai, APX activity at 0, 36, 48, 120 and 144 hai (Figure 2B), CAT activity at 0 hai (Figure 2C) and GR activity at 36, 48, 72 and 144 hai (Figure 2D). The CAT activity was significantly higher from 36 to 144 hai for -Si plants in comparison to the +Si plants (Figure 2C). The concentration of H_2O_2 was significantly lower in +Si plants than in control plants from 36 to 144 hai (Figure 3). Similar enzyme activities and H_2O_2 concentration results were observed in a pilot experiment (data not shown).

Pearson correlation

Anthracnose severity was negatively correlated with leaf Si concentration (r = -0.80). The Si concentration was positively correlated with A, E and g_s (r = 0.89, 0.79 and 0.64, respectively). Anthracnose severity also correlated negatively with A, E and g_s (r = -0.92, -0.96 and -0.89, respectively). In addition, A was correlated positively with E and g_s (r = 0.93 and 0.87, respectively). E was positively correlated with g_s (r = 0.88). All of these correlations were significant at $P \leq 0.01$.

DISCUSSION

In support of previous findings that Si can improve the resistance of several dicots against foliar pathogens
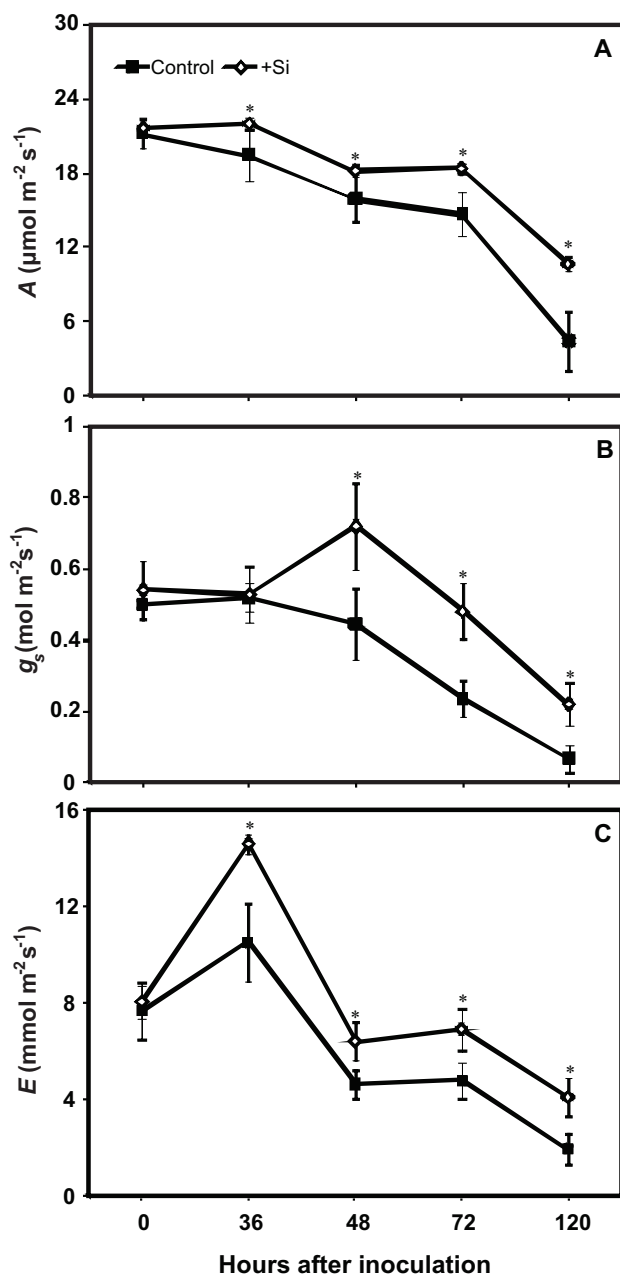
FIGURE 1 - A. Net carbon assimilation rate (*A*), **B.** Stomatal conductance to water vapor (*g*$_s$), and **C.** Transpiration rate (*E*), determined in leaves of bean plants grown in hydroponic culture containing 0 (control) or 2 mM (+Si) silicon and inoculated with *Colletotrichum lindemuthianum*. The means for the control and +Si treatments that are followed by asterisks (*) for each evaluation time are significantly different as determined by Student's t-test (*P≤0.05*). The error bars represent the standard deviation of the means of two pooled experiments (*n* = 36).

(Datnoff et al., 2007; Rodrigues et al., 2009), the present study describes the first physiological and biochemical features associated with the increase in the resistance of bean plants supplied with Si against *C. lindemuthianum* infection. The leaf Si concentration of +Si plants (values

ranging from 0.9 to up to 1.2 dag kg⁻¹) is consistent with what has been reported previously for dicots, which are able to accumulate, on average, 1 dag Si kg⁻¹ (Datnoff et al., 2007). The high Si concentration in bean leaves contributed to decreased anthracnose severity even though they were lower than those in rice, which is a known Si accumulator that exhibits Si concentrations of up to 5 dag kg⁻¹ of dry mass of shoot (Datnoff et al., 2007; Dallagnol et al., 2009).

It is well known that foliar diseases can dramatically decrease photosynthesis in several crops (Chou et al., 2000; Scholes & Rolfe, 1996; Berger et al., 2007; Dallagnol et al., 2013; Resende et al., 2012). In the present study, infection by *C. lindemuthianum* on bean leaves caused devastating effects on photosynthetic gas exchange. The effects observed were more dramatic than those previously reported by Bassanezi et al. (2001), who found that *A* was reduced by more than 50% in bean leaves with 10% of anthracnose severity and was reduced by almost 100% when the severity was above 20%. In the bean-*C. lindemuthianum* interaction, the production of lytic enzymes and non-specific toxins and their diffusion into the leaf tissues, particularly the veins, which are not yet colonized by the fungus, is likely to contribute greatly to the impairment of *A*. Nevertheless, these negative impacts were alleviated to some extent by Si supplementation. It is possible that Si acts to potentiate mechanisms of resistance that could reduce the leaf tissue damage caused by *C. lindemuthianum* and ultimately contribute to preventing any abrupt decrease in *A*.

Overall, the decreases in *A* during the course of *C. lindemuthianum* infection took place in parallel with decreases in *g*$_s$. The observed decreases in *A*, particularly at 120 hai, are caused by non-stomatal factors, possibly associated with dysfunctions at the level of the biochemical reactions involved in CO₂ fixation. These data suggest that stomatal limitations likely play a prominent role in constraining *A* upon infection by *C. lindemuthianum* in +Si plants. It is known that *C. lindemuthianum* causes stomatal closure in the green areas of bean leaves that are apparently healthy, reducing *A* of the entire leaf even under lower disease severity (Meyer et al., 2001). This may be the result of the vein necrosis that often occurs as anthracnose progresses, which abrogates the transport of water and solutes, leading to stomatal closure and, ultimately, imposing dramatic limits on CO₂ fixation, as observed in the control plants at advanced stages of fungal infection.

The lower concentration of H₂O₂ in the leaves of +Si inoculated plants support the hypothesis that Si provides a beneficial effect to plants that undergo major oxidative damage due to pathogen infection, as is the case of *C. lindemuthianum*. Indeed, Si has been shown to improve the ROS-scavenging antioxidant system in several plant species in response to both abiotic and biotic stresses (Datnoff et al., 2007; Gunes et al., 2007; Gong et al., 2008; Resende et al., 2012). Overall, these data showed that the activities of SOD, APX and GR were higher in the leaves of plants supplied with Si, which could contribute to limit
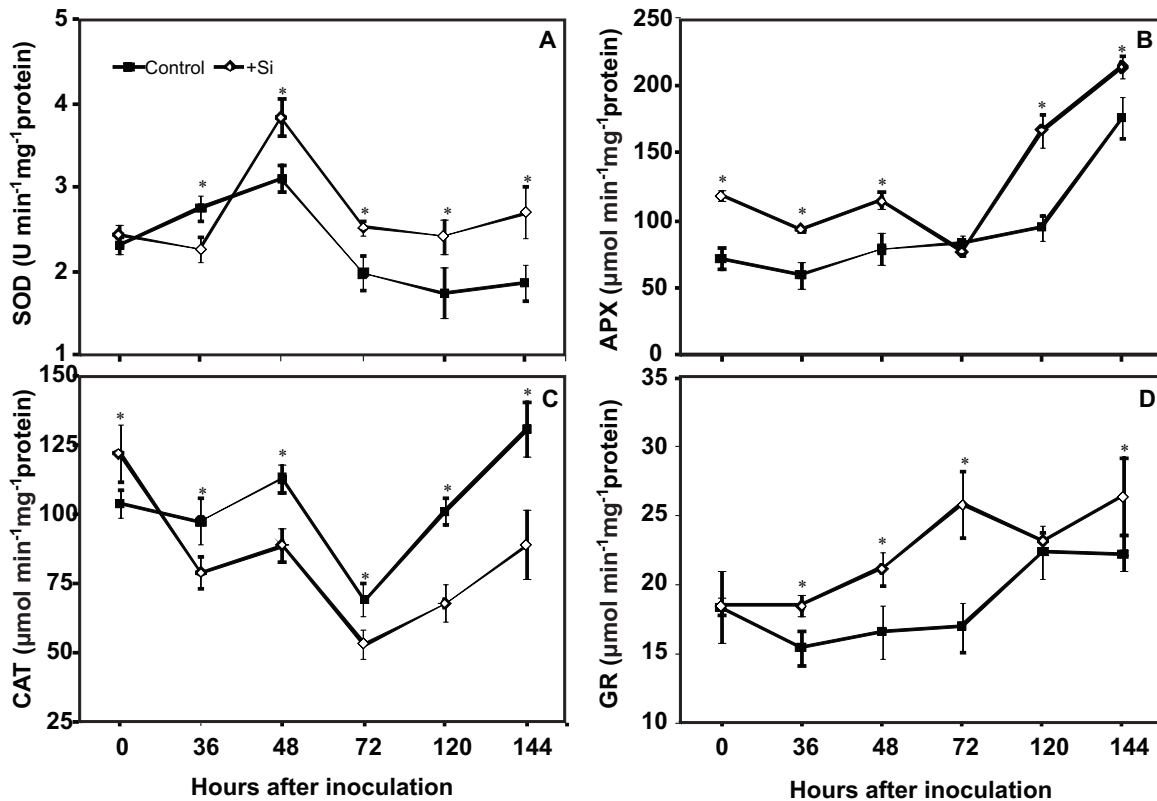
FIGURE 2 - Activities of **A.** Superoxide dismutase (SOD), **B.** Ascorbate peroxidase (APX), **C.** Catalase (CAT), and **D.** Glutathione reductase (GR), in leaves of bean plants grown in hydroponic culture containing 0 (control) or 2 mM (+Si) silicon after the inoculation with *Colletotrichum lindemuthianum*. The means for the control and +Si treatments that are followed by asterisks (*) for each evaluation time are significantly different as determined by Student's t-test (*P*≤0.05). The error bars represent the standard deviation of the mean (*n* = 18).
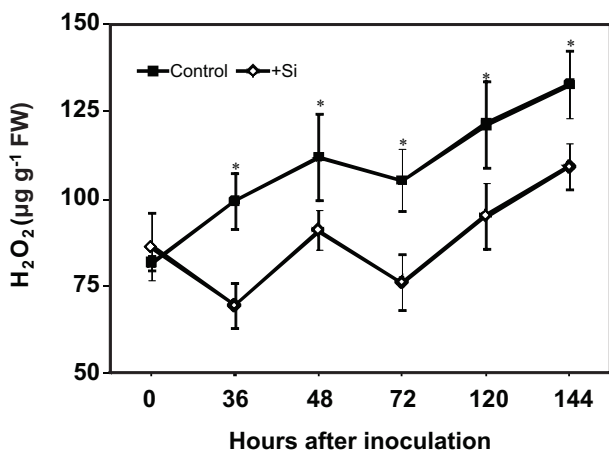
FIGURE 3 - Concentration of hydrogen peroxide (H_2O_2) in leaves of bean plants grown in hydroponic culture containing 0 (control) or 2 mM (+Si) silicon after the inoculation with *Colletotrichum lindemuthianum*. The means for the control and +Si treatments that are followed by asterisks (*) for each evaluation time are significantly different as determined by Student's t-test (*P*≤0.05). The error bars represent the standard deviation of the means of two pooled experiments (*n* = 18). FW = fresh weight.

the tissue damage caused by *C. lindemuthianum* infection. Intriguingly, CAT activity during the infection of bean leaves by *C. lindemuthianum* was down-regulated by Si supplementation and no explanation for this response is immediately evident. The affinity of CAT for H_2O_2 is lower than that of APX (Smirnoff, 1995). Consequently, the greater APX activity coupled with greater GR activity could compensate for the lower CAT activity in +Si plants. In summary, it is proposed that the high activities of SOD, APX and GR in the leaves of +Si plants may have translated into an effective removal of H_2O_2 from the infected leaf tissues. Indeed, defects in gas-exchange performance upon fungal infection can be ameliorated in the presence of Si.

In conclusion, it was demonstrated that the Si supply to the bean plants was associated with lower disease severity in parallel with the up-regulation of key enzymes in the antioxidant system, which in turn might be associated with improved gas exchange performance. It was also demonstrated that the impaired photosynthetic performance in +Si plants was largely associated with stomatal limitations, whereas in the control plants, those impairments likely reflected dysfunctions at the level of the biochemical reactions involved in CO_2 fixation. Based

on the above observations, it is tempting to speculate that Si can generate a signal that can help bean plants to cope with the deleterious effects of ROS on the cells infected with *C. lindemuthianum*. The confirmation and further identification of the exact nature of this signal, as well as the determination of the precise biological function of Si nutrition per se, remain important issues and merit future study.

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