Silicon Reduces Black Sigatoka Development in Banana

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Abstract

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The effect of silicon (Si) uptake on the susceptibility of *Musa acuminata* to *Mycosphaerella fijiensis* was investigated in three experiments conducted under controlled conditions. Plants were grown in the presence of Si or not, in pots adapted for a hydroponic culture system or in pots filled with compost. The banana leaves were inoculated after 4 or 6 months of plant growth by spraying conidial suspensions or by brushing mycelia fragments. The disease progress over time was assessed using quantitative and qualitative scales. At the end of each experiment, disease severity was also analyzed using the image analysis software ASSESS. The Si concentration in the leaves of plants supplied with Si reached 10 to 28 g/kg of dry matter. The first symptoms appeared 18 days after inoculation. The disease developed more rapidly and more severely on banana plants grown without Si than on plants supplied with Si. The areas under the disease progress curve (AUDPCs) calculated for plants grown with Si were significantly lower than the AUDPCs for plants not supplied with Si, regardless of inoculation method. Thus, Si supply could be a valuable tool in integrated pest management against *M. fijiensis* by reducing the disease pressure on banana.

Banana plants are perennial giant herbs and the second-most important fruit in terms of production in the world, after citrus (53). Banana production constitutes a major source of income and provides a vital source of food for more than 70 million people in Africa (44). Banana and plantain are susceptible to a wide range of serious diseases. Black sigatoka, caused by *Mycosphaerella fijiensis* M. Morelet, is currently the most important disease of banana and plantain worldwide. This airborne fungal leaf spot disease affects the photosynthetic area of the host plant, causing yield losses estimated at 33 to 69% when the disease is not controlled (8,20,39). Fungicides are used to control the spread of infection but are too expensive for small-scale farmers and are potentially environmentally damaging. The increase of fungicide-resistant *M. fijiensis* strains in some production zones has significantly affected the efficiency of these fungicides in stopping infections (49). Other strategies need to be developed to reduce losses due to this pathogen.

Silicon (Si) is the second-most abundant mineral element in the soil, after oxygen, and accounts for about 28% of the Earth's crust (6,14). It is present as silicic acid (H_4SiO_4) in soil at concentrations generally ranging from 0.1 to 0.6 mM (14,15,32). Plants absorb Si from the soil as silicic acid when the soil solution pH is below 9 (35). Si content in plant tissue ranges from 1 to 100 g/kg of dry weight (12,35).

Based on their active, passive, or exclusive Si uptake mechanisms, plant species are classified as high, intermediate, or non-accumulators, respectively (54). Three transporters responsible for the high capacity for Si uptake in roots or in shoots of rice have been identified by Ma et al (36,38) and Yamaji et al. (58).

Although Si has not been considered an essential element for higher plants, it has been shown to be beneficial for the healthy growth and development of many plants exposed to stress. The stresses that Si alleviates range from abiotic, such as salt and metal toxicities, to biotic, including pests and diseases (14,16,21, 31,32,34,52). Si reduces the severity of several economically important diseases in barley, corn, cucumber, grape, rice, strawberry, and wheat (10,43,46). It increases rice resistance to many diseases, such as blast, brown spot, leaf scald, sheath blight, and stem rot (6,9,17). It also enhances plant resistance to powdery mildew in barley, cucumber, tomato, and wheat (6,17,21). However, the mechanisms of Si-induced resistance remain unclear. Numerous studies suggest that Si performs its functions in two ways: by the polymerization of silicic acid, leading to the formation of solid amorphous, hydrated silica (24,28); and by being instrumental in the formation of organic defense compounds through the mediation of gene expression (16). Most studies on disease control by Si have focused on monocot plants such as rice, which can accumulate Si up to a level of 10% of shoot dry weight (37).

Banana plants can have more than 2% of Si in the shoot dry weight (30). Like rice, banana is an Si-accumulator monocot plant (25). There is little information, however, about the potential positive effects of Si on the resistance of banana to plant pathogens. The objective of this study was to determine whether Si could affect the susceptibility of banana (*Musa acuminata* 'Grande Naine') to *Mycosphaerella fijiensis*.

Materials and Methods

Plants materials and growth conditions. Tissue culture-derived plantlets of banana (*Musa acuminata* 'Grande Naine AAA Cavendish') supplied by Vitropic (Montpellier, France) were weaned (acclimatized) for 7 weeks in aerated nutrient solution tanks under the same conditions previously reported by Henriet et al. (25). After weaning, the plants were transplanted into pots adapted for a hydroponic culture system or pots filled with compost (pH 5 to 6.5, organic matter 25%; De Ceuster Metstoffen, Belgium), depending on the experiment. For the two hydroponic culture experiments, plantlets with an average height of 14 cm were each transferred into 2.5-liter (experiment 1) or 6-liter (experiment 2) cylindrical PVC pots. The composition of the nutrient solution in macroelements was 0.9 mM Ca $(NO_3)_2$, 0.05 mM $CaSO_4$, 0.05 mM $CaCl_2$, 0.5 mM KCl, 0.25 mM K_2SO_4, 0.05 mM $MgSO_4$, 0.1 mM NH_4Cl, 0.05 mM $(NH_4)_2SO_4$, and 0.05 mM NaH_2PO_4; and in microelements was 80 µM H_3BO_3, 80 µM FeEDTANa, 8 µM $MnCl_2$, 0.8 µM $ZnSO_4$, 0.8 µM $CuSO_4$, and 5.6 µM $(NH_4)_6Mo_7O_{24}$ (50). Si was supplied as silicic acid (H_4SiO_4). The H_4SiO_4-solution was prepared by dissolving sodium metasilicate ($Na_2SiO_3{\cdot}5H_3O$) in demineralized water, and leaching in an acidic cation exchanger (Amberlite IR-120). A concentration of 2

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or 1.7 mM Si (experiment 1 or experiment 2, respectively) was added to the nutrient solution for plants supplied with Si. The nutrient solution was supplied continuously by peristaltic pumps at a rate of 104 ml/h/pot (11). A third experiment (experiment 3) was performed with banana plants grown in pots containing 6 kg of compost and watered with a nutrient solution. Every 3 days, the plants were supplied with 1,000 ml of the same nutrient solution in the presence of Si (1.7 mM Si) or not (+Si or –Si, respectively). The plants were grown in a greenhouse with a relative humidity of 80%, 12 h of daylight, and a temperature of 28 and 25°C (day and night, respectively). The relative humidity inside the greenhouse was controlled using two humidifiers (CUOGHI type NEB-5000; BEPA SA, Jemeppe-sur-Sambre, Belgium).

Inoculum production. Two types of inoculum (conidia and mycelial fragments) were used in the experiments. Strain MUCL 47740 of *Mycosphaerella fijiensis* originated from an infected banana leaf collected in Cameroon. After 14 days of growth on potato dextrose agar (PDA) medium (39 g/liter), the fungal culture was transferred onto V8 juice agar (200 ml of V8 juice, 3 g of $CaCO_3$, and 20 g of agar; Oxoid), amended with penicillin at 60 mg/liter and streptomycin sulfate at 40 mg/liter after sterilization in order to produce conidia (adapted from Abadie et al. [1]). After 14 days, 5 ml of water was added to the petri dishes and the suspension was filtered through sterile cheesecloth (one layer, pore size of approximately 150 µm) to separate the mycelium from conidia. The conidial suspension was concentrated by centrifugation at 5,000 × *g* for 10 min. The concentration was determined using a Fuchs-Rosenthal counting chamber. Gelatin (1%) (Merck, Belgium) was added to the final conidial and mycelial fragments to facilitate inoculum adhesion to the leaf blades (41,42).

Inoculation procedure. *Experiment 1 (hydroponic).* Eight 4-month-old plants grown in 2.5-liter pots were inoculated by spraying 0.5 ml of suspension containing 10^4 conidia on two delimited areas (9 cm in diameter) or by brushing 0.3 mg of mycelial fragments on two other delimited areas on five fully unfolded leaves of banana plants. Four plants were supplied with Si (+Si), four other plants were not supplied with Si (–Si), and a further four plants (two +Si and two –Si) were not inoculated.

Experiment 2 (hydroponic). Eight 6-month-old banana plants grown in 6-liter pots were inoculated by spraying 0.5 ml of suspension containing 2×10^3 conidia or by brushing 0.3 mg of mycelial fragments on three delimited areas (9 cm in diameter) on the last three fully unfolded leaves. Four plants were supplied with Si (+Si plants), four plants were not supplied with Si (–Si plants), and two plants (one +Si and one –Si) were not inoculated.

Experiment 3 (compost). Six 6-month-old plants were inoculated by spraying 0.5 ml of suspension containing 10^4 conidia on six delimited areas on three leaves. Three of these plants were supplied with Si (+Si plants), three other plants were not supplied with Si (–Si plants), and a further two plants were not inoculated (one +Si and one –Si).

The relative humidity was maintained at 100% during the first 3 days after inoculation. To increase the humidity, plants were placed in a plastic greenhouse inside the larger greenhouse for experiment 3.

Assessment of disease progress and image analysis. The percentage of diseased leaf area was assessed every 7 days after inoculation for 34 (experiment 1) to 67 or 69 days (experiment 2 or experiment 3) using the quantitative scale described by Torres et al. (55) (scale 0 to 5, where 0 = no symptoms and 5 = more than 50% of the leaf affected). Disease severity was evaluated using the qualitative scale described by Mourichon et al. (42) for each inoculated area (scale 1 to 5, where 1= very small spots with a diameter of less than 0.5 mm and 5 = black spot with a dry, gray center, leaf completely necrotic). In all, 8 to 12 areas were inoculated per leaf stage on three or four plants in experiment 1 and experiment 2, respectively, but the infection of some areas could not be analyzed due to early senescence or accidental injury. The area under the disease progress curve (AUDPC) values were calculated with the quantitative scale using the following equation:

$$\text{AUDPC} = \sum_{i=1}^{n_i-1} (Y_i + Y_{i+1}) / 2(X_{i+1} - X_i)$$

where Y_i = disease severity at the ith observation, X_i = time at the ith observation, n_i = number of times, and i = the order index for the times.

At the end of each experiment, the percentage of diseased areas was also analyzed using the image analysis software for plant disease quantification, ASSESS (American Phytopathological Society, St. Paul, MN). Each inoculated area was scanned at a resolution of 600 pixels. For each image, the inoculated area was delimited manually. Recognition of diseased areas was achieved for each inoculation area by using the hue or intensity plane in the hue saturation intensity color space and manually adjusting the threshold levels until the diseased area was correctly recognized by the software (13). The proportion of the inoculated area that developed symptoms was assessed for each image.

Determination of Si content in plants. The total Si content in banana leaves was analyzed for each experiment. The youngest fully unrolled leaf or flag leaf was marked leaf 1 (L1) as used by Lhomme and Jimenez (33), the leaf unrolled before the flag leaf was marked L2, and so on. For the banana plants grown in hydroponic conditions, the five upper leaves (L1 to L5) were sampled in experiment 1 (4 months old) and only one leaf stage (L5) was sampled in experiment 2 (6 months old). For the banana plants grown in compost (experiment 3), the Si content was measured on the three upper leaves of each banana plant (L1 to L3). Leaves were dried at 60°C for 1 week. The mineral analysis was carried out after calcination at 450°C for 1 day and fusion in Li-metaborate (Flux LM 100; SOCACHIM-XRF Scientific, Belgium) + Li-tetraborate (Flux LT 100; SOCACHIM-XRF Scientific) at 1,000°C (7), followed by dissolution using fusion beads in 2 M HNO_3 solution. Si concentration was measured using inductively coupled plasma–atomic emission spectrometry.

Data analysis. Data from all the experiments were subjected to analysis of variance using the SAS statistical program (version 3.0; SAS Institute, Cary, NC). The treated means were compared using Fisher's protected least significant difference test ($P \leq 0.05$) for multiple mean comparisons or using a t-test for two mean comparisons ($P \leq 0.05$).

Results

Effect of Si amendment on banana plants grown in hydroponic conditions. *Experiment 1.* The Si concentration in the leaves of banana plants in which the plants were inoculated after 4

Table 1. Silicon (Si) content in the leaves of 4-month-old banana plants grown under a hydroponic system, before inoculation (experiment 1)

		Si concentration					
		In plant (g/kg of dry matter)[x]					
Si treatments[y]	In nutrient (mM)[z]	L1	L2	L3	L4	L5	Mean
+Si	2	10.14	18.02	25.75	28.2	28.3	22.07
–Si	0	0.18	0.30	0.5	0.55	0.7	0.42
P > *F*		0.0079	0.0002	0.0175	<0.0001	0.0164	<0.0001

[x] L1 = the youngest fully unrolled leaf or flag leaf, L2 = leaf 2, L3 = leaf 3, L4 = leaf 4, and L5 = leaf 5; $n = 3$.
[y] +Si = 2 mM and –Si = 0 mM. *P* value from the *t* test ($P \leq 0.05$) for the comparison between the mean Si concentrations in leaves of +Si and –Si plants.
[z] Si concentration in nutrient solution.

months was 10.14 to 28.3 g/kg depending on the leaf stage for plants amended with Si and 0.18 to 0.70 g/kg for plants not supplied with Si (Table 1). In plants inoculated with *M. fijiensis*, the first symptoms appeared 19 days after inoculation (DAI) for both the +Si and –Si treatments. The percentage of symptomatic area indicative of black sigatoka increased more rapidly on the –Si plants than on the +Si ones (Fig. 1). Stage 2 of the qualitative Mourichon scale occurred at 24 DAI for –Si plants and at 27 DAI for +Si plants inoculated with conidia. For plants inoculated with mycelial fragments, stage 2 occurred 22 DAI for –Si plants and 24 DAI for +Si ones. Thirty days after inoculation, the percent disease severity reached 20.5% for –Si plants and 18.8% for +Si plants in the plants inoculated with conidia. For plants inoculated with mycelial fragments, the percent disease severity reached 30.6% for +Si plants and 36.2% for –Si ones (Fig. 1). The AUDPC values for +Si plants were significantly lower than those for –Si ones when inoculated with conidia (Table 2). The AUDPC values were lower for leaf areas inoculated with conidial suspensions than for those inoculated with mycelial fragments (Table 2). At 34 DAI, the percentages of leaf-infected areas evaluated using ASSESS were significantly higher for plants not supplied with Si than for + Si plants (Table 3). These differences were greater for plants inoculated with mycelia fragments compared with those inoculated with conidia.

Experiment 2. The Si amendment in the nutrient solution of 6-month-old banana plants grown under hydroponic conditions led to a concentration in leaves of 14.4 to 17.6 g/kg. The Si concentration in leaves of plants not amended with Si was 1.1 to 1.2 g/kg. Therefore, the Si content was 15 times higher for +Si plants than for –Si plants, whether or not they had been inoculated with *M. fijiensis* (Table 4). In plants inoculated with *M. fijiensis,* the first symptoms appeared 18 DAI for both +Si and –Si treatments (Fig. 2). The disease developed more rapidly and more severely on banana plants grown without Si than for plants supplied with Si, whether or not plants had been inoculated with conidia or mycelial fragments. There was a delay in disease progress for plants amended with Si. In the case of mycelial inoculation, stage 2 on the Mourichon scale occurred 37 DAI for –Si plants and only 48 DAI for +Si. For plants inoculated with conidia, stage 2 occurred 43 DAI for –Si and 48 DAI for +Si (Fig. 2). The AUDPC values were 1.1 to 1.6 times higher for –Si plants than for +Si plants (Table 2). The AUDPC values of –Si and +Si plants differed significantly or not, depending on the leaf growth stage. Older leaves had a lower AUDPC value than younger leaves for + Si plants (Table 2). The disease progressed more rapidly and more severely when mycelium was used as the inoculum source (Table 2). The percentage of

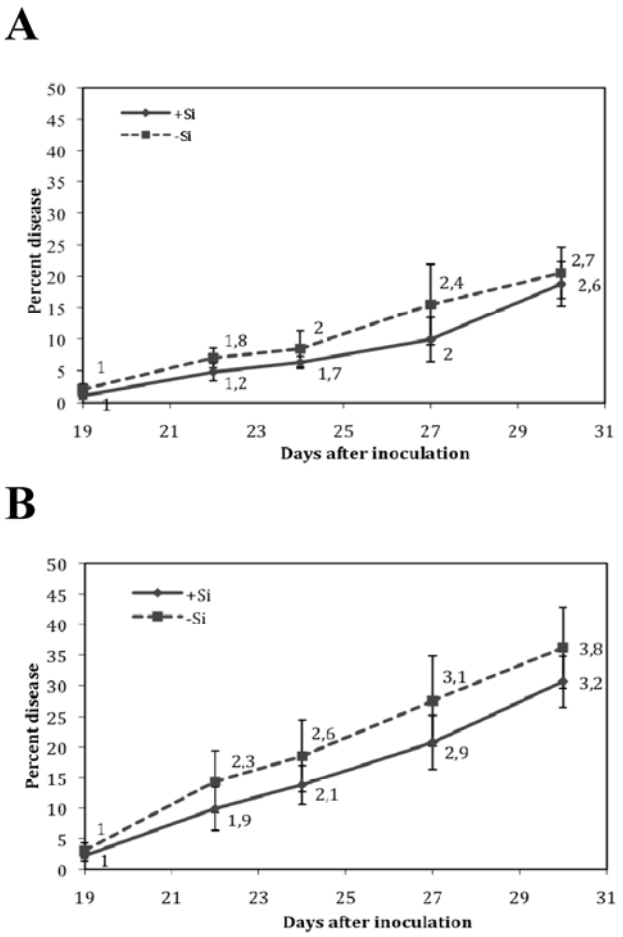

Fig. 1. Quantitative and qualitative assessment of the development of symptoms of black sigatoka on 4-month-old banana plants grown under a hydroponic culture system amended with silicon (Si) or not and inoculated with **A,** conidia or **B,** mycelia. Vertical lines show the standard deviations.

Table 2. Area under the disease progress curve (AUDPC) mean values for black sigatoka of banana grown under hydroponic culture and inoculated after 4 months (experiment 1) or 6 months (experiment 2) with mycelial fragments or conidia *of Mycosphaerella fijiensis*

	AUDPC[y]			
	Inoculated with conidia		Inoculated with mycelia	
Leaf stage[z]	–Si plants	+Si plants	–Si plants	+Si plants
Experiment 1				
L1	116 a	85 a	214 a	158 b
L2	118 a	82 b	234 a	160 b
L3	123 a	97 a	242 a	172 b
L4	106 a	82 a	222 a	158 b
L5	140 a	89 b	230 a	188 a
Mean	120 a	87 b	228 a	168 b
Experiment 2				
0 to 48 DAI				
L1	210 a	135 b	254 a	202 a
L2	151 a	134 a	286 a	175 b
L3	118 a	74 b	199 a	123 b
Mean	161 a	116 b	245 a	168 b
0 to 69 DAI				
L1	593 a	375 b	696 a	603 a
L3	390 a	314 a	600 a	448 b
Mean	506 a	347 b	650 a	522 b

[y] Si = silicon. AUDPC values followed by different letters in a same row are significantly different at $P \leq 0.05$, according to Fisher's protected least significant different test.

[z] L1 = the youngest fully unrolled leaf or flag leaf, L2 = leaf 2, L3 = leaf 3, L4 = leaf 4, L5 = leaf 5, and DAI = days after inoculation.

Table 3. Percentage of infected leaf areas calculated at the end of the experiments by image analysis for plants grown under a hydroponic system inoculated after 4 months (experiment 1) or 6 months (experiment 2) with mycelial fragments or conidia *of Mycosphaerella fijiensis*

	Infection (%)[y]			
	Conidia as inoculum		Mycelium fragments as inoculum	
Leaf stage[z]	–Si plants	+Si plants	–Si plants	–Si plants
Experiment 1				
L1	15 a	4 a	51 a	38 a
L2	20 a	9 a	66 a	29 a
L3	15 a	8 a	77 a	33 b
L4	21 a	12 a	55 a	45 a
L5	23 a	18 a	52 a	60 a
Mean	19 a	11 b	60 a	43 b
Experiment 2				
L1	54 a	43 a	69 a	61 a
L3	47 a	43 a	69 a	71 a
Mean	51 a	43 a	69 a	66 a

[y] + Si = 2 or 1.7 mM silicon (Si) and –Si = 0 mM Si. Values followed by different letters in the same row are significantly different at $P \leq 0.05$, according to Fisher's protected least significant different test.

[z] L1 = the youngest fully unrolled leaf or flag leaf, L2 = leaf 2, L3 = leaf 3, L4 = leaf 4, and L5 = leaf 5.

infected leaf calculated by image analysis was lower for +Si plants than for –Si plants; however, this difference was not significant (Table 3).

Effect of Si amendment on banana plants grown in compost. *Experiment 3.* The Si concentration in leaves of 6-month-old banana plants grown in compost amended with Si reached mean values of 17.1 to 22.6 g/kg, depending on the leaf growth stage (Table 5). These values are in the same range as the values of Si concentration obtained for plants in hydroponic solution (Table 4). The mean Si concentration of plants in pots not amended with Si varied from 1.2 to 1.8 g/kg (Table 5), which indicates that the compost did not provide plant-available Si to the plants. These values are slightly higher than those obtained for banana plants grown in hydroponic solution without Si (Table 4). Young leaves had a lower Si content than older leaves. The first symptoms of black sigatoka appeared 18 DAI on banana plants, whether or not they had been amended with Si (Fig. 3). At 67 DAI, the percent disease reached 39.5% for +Si plants and 68.9% for –Si plants (Fig. 3). Stage 2 of symptom development occurred 26 DAI for –Si plants and at 41 DAI for + Si plants (Fig. 3). The AUDPC values calculated for each leaf separately or for the whole inoculated leaves were significantly higher (from 1.7 to 2.0 times) for –Si plants than for +Si plants (Table 6). The percent disease calculated by image analysis was significantly greater for the flag leaf of plants not supplied with Si than for the flag leaf of Si-supplied plants (Table 7).

In the third experiment, black sigatoka progressed more intensively in plants grown in compost than in hydroponic solution. The percent disease on banana plants grown in compost conditions reached 70% after 67 DAI, as opposed to 30% after 69 DAI in the hydroponic system. The severity of the disease reached the last stage (stage 5) of symptom development 67 DAI, whereas only stage 3 was reached in the hydroponic system (Figs. 2 and 3).

Discussion

In hydroponic conditions or compost, the Si supply to growing banana plants led to an increase in Si concentration in the plant leaves, reaching values of 1 to 3% Si dry weight. These results confirmed findings from previous studies, which established that banana is an Si accumulator (25). In the two culture systems tested in this study, the Si content in Si-supplied plants was comparable.

In the three experiments, Si supply was not associated with an increase in the incubation time of *M. fijiensis* on banana leaves. A previous study by Rodrigues et al. (47) showed the same results for *Rhizoctonia solani* in rice. These authors did not observe any effect of Si on the length of the incubation period of sheath blight in rice, probably because *R. solani* is able to penetrate the stomata. The same explanation might be true for *M. fijiensis,* because this pathogen also penetrates via the stomata (2).

Despite the lack of any effect on incubation time, the addition of Si to the nutrient solution used to grow banana plants under controlled conditions reduced percent disease and delayed disease development of black sigatoka. These results confirmed numerous observations made on the beneficial role of Si in monocots (45).

Table 4. Silicon (Si) concentration in leaves of 6-month-old banana plants grown under a hydroponic system (experiment 2)

Silicon treatment[y]	Si in nutrient solution (mM)	*Mycosphaerella fijiensis* inoculation	Si in leaves (g/kg of dry matter)
+Si	1.7	–	14.4
+Si	1.7	+	17.6
–Si	0	–	1.1
–Si	0	+	1.2
$P > F$[z]			0.05

[y] +Si = 1.7 mM and –Si = 0 mM.
[z] P value from the t test ($P \leq 0.05$) for the comparison between the mean Si concentrations in leaves of +Si and –Si inoculated plants.

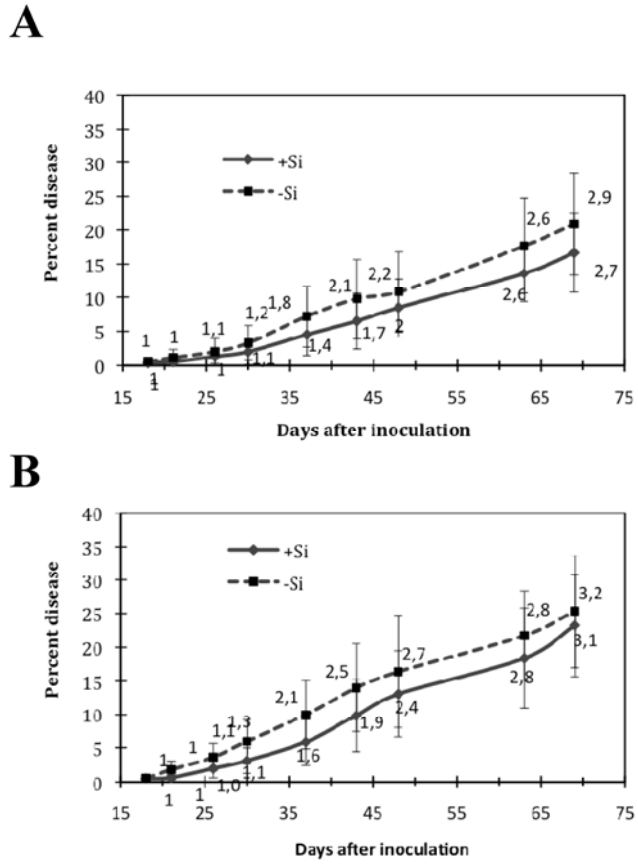

Fig. 2. Quantitative and qualitative assessment of the development of symptoms of black sigatoka on 6-month-old banana plants grown under a hydroponic culture system amended with silicon (Si) or not and inoculated with **A,** conidia or **B,** mycelia (experiment 2). Vertical lines show the standard deviations.

Table 5. Silicon (Si) concentration in leaves of 6-month-old banana plants grown in pots filled with compost (experiment 3)[v]

			Si concentration (g/kg of dry matter)[w]			
			In leaf			
Si treatment[x]	Solution (mM)[y]	Inoculation[z]	L1	L2	L3	In plant
+Si	1.7	–	17.5	nd	22.6	20.0
+Si	1.7	+	17.3	17.1	21.6	18.7
–Si	0	–	1.2	nd	1.6	1.4
–Si	0	+	1.4	1.4	1.8	1.5
$P > F$			0.006	0.009	0.02	<0.0001

[v] Mean of three plants for plants inoculated with *Mycosphaerella fijiensis*, data of one plant for plant not inoculated.
[w] L1 = the youngest fully unrolled leaf or flag leaf, L2 = leaf 2, L3 = leaf 3, and nd = no data.
[x] +Si = 1.7 mM and –Si = 0 mM. P value from the t test ($P \leq 0.05$) for the comparison between the mean Si concentrations in leaves of +Si and –Si inoculated plants.
[y] Si concentration in solution.
[z] *M. fijiensis* inoculation.

Nutrient solution concentrations of 1.7 mM Si were found to produce optimum disease reduction (40). According to Guével et al. (22), root applications of potassium silicate at a concentration of 1.7 mM gave the best results in terms of Si absorption and powdery mildew control. Si application has been shown to suppress disease in cucurbits caused by foliar and soilborne pathogens (3) and to reduce the susceptibility of rice to various pathogens (5,9). In the case of banana, it has been shown that Si amendment had a positive effect on plants infected by *Cylindrocladium spathiphylli* (57). However, the mechanistic basis for this observation is unclear. Two main hypotheses have been proposed to explain the resistance of Si-amended plants to infection by fungal pathogens (5). One hypothesis proposed that Si could accumulate in the leaves and, thereby, interfere with the pathogen's penetration as a result of a mechanical barrier (6,29). This is contradicted by other reports, suggesting that Si might mediate the natural defense mechanisms of the plant (4,18,51,52). In the present study, the older leaves of banana plants grown under hydroponic conditions were less susceptible to infection by *M. fijiensis* in 6-month old plants but not 4-month-old plants. It is known that the Si distribution in shoot organs closely follows the cumulated transpiration of these organs, which is a function of their transpiration rate and their age (28). In the case of banana, Henriet et al. (25) showed that Si concentration was higher in the older leaves compared with younger ones. The higher resistance of older leaves to *M. fijiensis* would suggest that Si acts as a physical or physiological barrier against *M. fijiensis* infection (29). This barrier would act at or after the penetration of the fungus into the leaf because *M. fijiensis* penetrates through stomata. However, this higher resistance was not apparent for 4-month-old banana plants grown in hydroponic conditions or for plants grown in compost, where the older leaves were more susceptible to *M. fijiensis* than the younger ones.

This contradiction has been observed by numerous authors (17,19) and suggests that another mechanism might be at play, possibly the involvement of Si in host defense responses, as proposed in some studies (19,21,23,40,48). Fawe et al. (18) suggested that Si is involved in the increased resistance of a host to pathogens by enhancing the activity of antifungal compounds, such as phytoalexins. Factors other than Si could also account for the difference in susceptibility to black sigatoka, depending on leaf growth stage. In the case of infection of banana by *M. fijiensis*, Jacome and Schuh (26,27) reported that older leaves were more susceptible, whereas Romero (49) observed that younger leaves were more susceptible than older ones. These contradictions are reflected in our experiments. Infection pressure, environmental conditions, and plant age might explain some of these observed differences.

The comparison of two inoculation methods showed that inoculation with mycelia fragments resulted in higher disease severity than the use of a conidial suspension. A similar result was observed by Twizeyimana et al. (56), who reported that inoculation of in vitro plantlets of banana with mycelia fragments caused significantly higher levels of black sigatoka disease and faster rates of disease progress compared with using conidial suspensions. However, they recommended conidial inoculum over mycelial fragments because of the difficulty in quantifying and standardizing concentrations of mycelia fragments. In the present study, both mycelia and conidia were used because of the difficulty in producing high concentrations of conidia.

The results presented herein suggest that Si has the potential to reduce the severity of black sigatoka development and could help in enhancing disease control, especially in combination with fungicides.

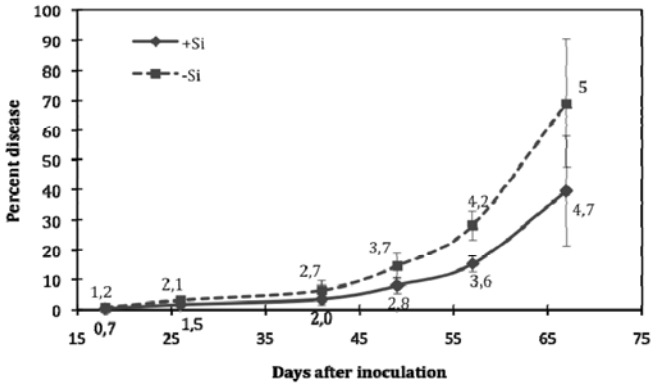

Fig. 3. Quantitative and qualitative assessment of the development of symptoms of black sigatoka on banana plants grown in compost filled pots amended or not with silicon (Si) and inoculated (experiment 3). Vertical lines show the standard deviations.

Table 6. Area under disease progress curve (AUDPC) mean values for black sigatoka of 6-month-old banana plants grown in compost-filled pots and inoculated with conidia *of Mycosphaerella fijiensis* (experiments 3)

	AUDPC for[y]	
Time scale[z]	**–Si plants**	**+Si plants**
0 to 49 DAI		
L1	191 a	99 b
L2	218 a	108 b
L3	279 a	167 b
Mean	229 a	124 b
0 to 67 DAI		
L1	793 a	431 b
L2	813 a	447 b
Mean	803 a	439 b

[y] Si = silicon. AUDPC values followed by different numbers in a same row are significantly different at $P \leq 0.05$ according to Fisher's protected least significant different test.

[z] DAI = days after inoculation. Leaf growth stage: L1 = the youngest fully unrolled leaf or flag leaf, L2 = leaf 2, and L3 = leaf 3.

Table 7. Percent disease calculated at the end of the experiment by image analysis for banana plants grown in compost-filled pots and inoculated after 6 months with conidia *of Mycosphaerella fijiensis* (experiment 3)

	Disease (%)[x]		
Leaf growth stage[y]	**–Si plants**	**+Si plants**	***P* > *F*[z]**
L1	85	46	0.0007
L2	53	43	0.49
Mean	72	45	0.43

[x] + Si = 1.7 mM and –Si = 0 mM.

[y] L1 = the youngest fully unrolled leaf or flag leaf and L2 = leaf 2.

[z] *P* value from the *t* test ($P \leq 0.05$) for the comparison between the percent disease of +Si and –Si plants.

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