Eur J Plant Pathol (2011) 131:621–630
DOI 10.1007/s10658-011-9835-x

Protective role of silicon in the banana-*Cylindrocladium spathiphylli* pathosystem

Marie-Liesse Vermeire · Lucie Kablan · Marc Dorel · Bruno Delvaux · Jean-Michel Risède · Anne Legrève

Accepted: 30 June 2011 / Published online: 30 July 2011

Abstract Silicon (Si) is known to reduce the incidence of pathogens on many plants. Little information is available on the potential positive effects of Si on the susceptibility of banana (*Musa acuminata*) to pathogens. Root-rot fungi of the genus *Cylindrocladium* have been reported, along with endoparasitic nematodes, to be the causal agent of toppling disease and severe yield loss. The objective of this study was to determine the effects of Si supply on *Cylindrocladium spathiphylli* infection on banana. Plantlets inoculated by dipping the root system in a conidial suspension of the pathogen were grown on a desilicated ferralsol and amended, or not, with 2 mM of soluble Si under greenhouse conditions in Guadeloupe. The root lesion severity was evaluated using the image analysis program WinRHIZO 7, 14 and 21 days after inoculation. A reduction of about 50% of root necrosis was observed 14 days after inoculation for the Si-supplied plants compared with those not supplied with Si. The Si amendment also alleviated growth reduction caused by the pathogen. These results suggest that Si could have a positive effect on banana resistance to *C. spathiphylli* and provide an environmentally friendly alternative to pesticides for the integrated control of an important crop disease.

Keywords *Musa acuminata* · Root-rot fungus · Silicon · WinRHIZO · Integrated pest management

Marie-Liesse Vermeire and Lucie Kablan contributed equally to this work.

M.-L. Vermeire · L. Kablan · B. Delvaux · A. Legrève (✉)
Earth and Life Institute, Université catholique de Louvain,
Croix du Sud 2/3,
1348 Louvain-la-Neuve, Belgium
e-mail: anne.legreve@uclouvain.be

M. Dorel · J.-M. Risède
CIRAD, UPR Systèmes de culture à base de Bananiers,
Plantains et Ananas, Station de Neufchâteau,
Sainte-Marie,
97130 Capesterre-Belle-Eau, Guadeloupe (F.W.I.)

Introduction

Silicon (Si) is the second-most abundant element in the lithosphere and plays a very important role in global biogeochemical processes, notably the global matter cycle where it contributes to regulating atmospheric CO_2 (Sommer et al. 2006).

This element is taken up by plant roots in the form of water-soluble monosilicic acid (H_4SiO_4), and follows the water flow from the roots to the aerial transpiration sites (Raven 2001), where it precipitates as particles of biogenic opal ($SiO_2 . nH_2O$) called phytoliths (Ma and Yamaji 2006). Silicon is not considered an essential nutrient in plants (Epstein 2009; Ma et al. 2001), but under a range of biotic and abiotic stresses its importance as

an element that is particularly beneficial for plants is now beyond doubt (Datnoff et al. 2007; Reynolds et al. 2009). A Si application can make a significant difference to plant performance in field conditions. The abiotic stresses that Si fertilization helps to alleviate include drought, water logging, frost, salinity and heavy metal toxicity (Keeping and Reynolds 2009). Biotic stresses against which Si provides some protection include numerous pathogens and herbivores (Epstein 1994; Rodrigues et al. 2003; Fauteux et al. 2005; Diogo and Wydra 2007). Reduction in disease severity by an increased Si supply has been observed for many plants, dicots and monocots (Fawe et al. 2001; Bélanger et al. 2003; Datnoff et al. 2007; Martinati et al. 2008).

It is now generally recognized that Si performs its functions in two ways. First, the accumulation of solid hydrated amorphous silica in cell walls acts as a mechanical barrier that prevents penetration by pathogens (Fauteux et al. 2005). Second, soluble Si plays an active role in strengthening plant disease resistance by stimulating the expression of natural plant defence reactions (Fawe et al. 2001). However, the exact nature of the interaction of soluble Si with the plant's biochemical pathways, leading to disease resistance, remains unknown (Fauteux et al. 2005). Soluble silicon might modulate the activity of post-elicitation intracellular signaling systems, resulting in the expression of target genes and the accumulation of defence compounds such as phytoalexins (Fawe et al. 2001; Fauteux et al. 2005; Epstein 2009). Fawe et al. (2001) suggested that Si could be assimilated to a modulator similar to systemic acquired resistance (SAR)-inducing compounds. Keeping and Reynolds (2009) pointed out that Si displays a marked resemblance to plant stress hormones such as jasmonate and salicylate in its ability to act as a modulator of induced resistance, and that plants could respond more efficiently or earlier to a pathogen or herbivore attack. Silicic acid could play a positive role in local and systemic resistance, given that both processes depend on primary elicitation (Fauteux et al. 2005).

The beneficial effects of Si are usually obvious in crops that actively accumulate Si, such as rice, barley and maize (Ma et al. 2001). A plant is considered to be a Si accumulator if the Si content is higher than 1% of dry weight (Ma et al. 2001). In rice, for example, the Si content can reach 10% of the shoot dry weight (Ma and Yamaji 2006). Banana plants can have more than 2% of Si in the shoot dry weight (Lahav 1995). Although this species is also a Si-accumulator, little information is available on the impact of Si on the susceptibility of banana (*Musa acuminata*) to diseases (Henriet et al. 2006).

Bananas are perennial giant herbs and occupy the first rank of world fruit production (Swennen and Vuylsteke 2001; Lassoudière 2007). Banana production plays a major role in food security for more than 400 million people in developing countries in tropical areas and is a source of income and employment for local populations (Arias et al. 2003). Bananas for export are cultivated in perennial intensive monocultures, and are therefore hampered by many recurrent pests and diseases. In banana plantations, soilborne pathogens are major constraints limiting crop production. *Cylindrocladium* spp. (teleomorph *Calonectria*) are root-rot fungi that are reported, along with endoparasitic nematodes, to cause necrotic root and corm lesions that lead to nutrient uptake reduction, root breakage and toppling of banana plants (Risède and Simoneau 2004). *Cylindrocladium spathiphylli* has been identified as the most pathogenic species within the *Cylindrocladium* genus for the banana rhizosphere (Risède 2008). *Cylindrocladium*-resistant banana cultivars remain to be selected, and the control of these pathogenic fungi needs to be adjusted within the framework of an integrated crop protection strategy, with no reliance on the systematic use of fungicides. There is a body of circumstantial evidence that bananas grown on Vertisols, which show high Si concentration in soil solution, are less affected by pathogens, whereas lodging incidence seems to be high on largely desilicicated ferrallitic soils (Henriet et al. 2006). Silicon amendment might therefore offer a promising alternative for banana disease control in the context of integrated plant protection, especially where soils are low or limiting in plant-available Si. The objective of the present study was to determine the effects of Si uptake on the susceptibility of banana (*Musa acuminata* cv. Grande Naine) to *C. spathiphylli*. The incidence of the pathogen on banana plants amended or not with soluble Si was assessed under controlled conditions.

Materials and methods

Soil materials

About 500 dm^3 of soil of a Si-deficient typical ferralsol were collected at a depth of 0–20 cm in a plot on the eastern slopes of the volcano La Soufrière (Basse-Terre, Guadeloupe) which had been used for a long time for intensive banana cropping (Farm Feneteau, 164 m asl). The area experiences heavy rains, with a mean annual rainfall of 3,296 mm. The soil is therefore highly weathered and has a very low Si content (Henriet et al. 2008b). The main soil properties, as measured by Henriet et al. (2008b) and expressed as averages: pH H_2O value: 6.25; organic carbon content: 2.53%; exchangeable bases contents: 5.2 $cmol_c\ kg^{-1}$ Ca, 2.2 $cmol_c\ kg^{-1}$ Mg, 0.1 $cmol_c\ kg^{-1}$ Na and 1.9 $cmol_c kg^{-1}$ K; cation exchange capacity (CEC): 25 $cmol_c\ kg^{-1}$; effective cation exchange capacity (ECEC): 7.8 $cmol_c\ kg^{-1}$; particle-size composition: 5.9% sand, 14.5% silt and 81.6% clay; $CaCl_2$-extractable Si content: 21.1 mg kg^{-1}. The soil was sieved at 2 cm to homogenize the structure and then sterilized twice at 100°C for 12 h, with an interval of 48 h.

Plant material and weaning

A total of 252 vitroplants of young banana plantlets (*Musa acuminata* cv. Grande Naine, AAA group, Cavendish, dessert banana) were weaned for 10 weeks on peat in 0.3–l pots (for the first 8 weeks) and then in 0.6–l pots (for 2 weeks) (one plant per pot) under quarantine greenhouse conditions, with a mean temperature of 22.5°C, a mean relative humidity of 80.1% and a global radiance of 6.65 MJ m^{-2} (day: night about 12:12). An amount of 1.5 g of Osmocote fertiliser (11-11-18+2MgO) was added to each pot.

In the first week of weaning, the plants were sprayed with tap water. Thereafter, during 9 weeks, the soil moisture content was adjusted at field capacity with an Si solution (production method described below) for half the plants (Si+ plants) and with demineralised water for the other half (Si− plants): (1) each pot, including plant and soil, was weighed to determine the reference pot weight at field capacity; (2) three times a week, each pot was weighed and distilled water or Si solution was added to reach the reference pot weight. Daily plant transpiration was deduced. Every 3 weeks, the reference pot weight was corrected by measuring the fresh biomass of one plant per treatment.

Si solution production

Si was supplied as monosilicic acid (H_4SiO_4), 2 mM. The H_4SiO_4-solution was made by dissolving $Na_2SiO_3.5H_2O$ in demineralised water, and then leaching on an acidic cation exchanger (Amberlite® IR-120) to fix Na^+ ions (Stumm and Morgan 1996). Silicon concentration was determined using inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Inoculum production

The strain of *C. spathiphylli* used in this experiment was isolated from the root-rot of banana cv. Grande Naine collected in a contaminated plot (CIRAD at Neufchâteau, Basse-Terre, Guadeloupe). First, different fungal species were isolated from root-rots following the Root Slice Plating Method (RSPM) proposed by Risède (Risède 2008). *Cylindrocladium* colonies were then transplanted onto Petri dishes of M1 medium (1% malt extract; 2% agar) and a single conidium was isolated to obtain pure strains of *C. spathiphylli* using the protocol described by Risède and Rhino (2008). One of these strains was used in this experiment. A high quantity of conidia was produced using the inoculum production technique described by Risède and Rhino (2008): heavy conidiogenesis was induced by subculturing the strain on 80 banana leaf agar (BLA) medium dishes at 25°C for 7 days under continuous light. The conidia were harvested by shaking leaf fragments of BLA in a Triton X-100 solution, and the resulting suspension was filtered though a 32-μm sieve. The suspension was then concentrated by centrifugation and the conidial concentration was adjusted to 2×10^3 conidia ml^{-1} (d2 dose) and 0.5×10^3 conidia ml^{-1} (d0.5 dose) with a Malassez haemocytometer.

Inoculation procedure

After 9 weeks of growth in the presence or in the absence of Si (during the weaning), banana plants

were inoculated with *C. spathiphylli.*, Peat was removed as gently as possible from the root system of Si− and Si+ plants under running tap water. The root system of each plant was dipped for 20 s in 250 ml of the conidial suspension (d2 dose or d0.5 dose) (Risède and Simoneau 2004). The banana plants were left to drain for 1 min before transplantation into 2 l plastic pots filled with the sterile ferralsol. Controls (non-inoculated plants, NI) were produced using the same procedure, except that the conidial suspension was replaced by sterile water amended with Triton X 100 (250 $\mu l\ l^{-1}$). A total of 35 banana plants were planted out for each of the six treatments (amended or not with Si and three doses of inoculum: Si+ d2, Si+ d0.5, Si+NI, Si− d2, Si− d0.5 and Si-NI).

Growth conditions after inoculation

The experiment was conducted for 3 weeks after inoculation. Plants were grown according to a completely randomized design under the same greenhouse conditions as described for the weaning phase. Water content adjustment was carried out after inoculation following the same method employed for weaning, using demineralized water for the Si− plants and a Si solution for the Si+ plants.

Disease assessment

Root lesion severity was assessed 7, 14 and 21 days after inoculation on 10 randomly selected plants per date and per treatment (60 plants per date in total). After removal of peat under running tap water, the roots were placed on a transparent plastic sheet and covered with a blue plastic sheet, to prevent shadows and improve contrast, and then scanned (scanner EPSON expression 1680, Seiko EPSON Corp, Japan). For each root system, a colour picture was made with a resolution of 300 dpi, using the reflected light. The pictures were analysed with the image analysis program WinRHIZO (v. Pro; Regent Instruments, Inc., Quebec, Canada), using the method of object separation from the background and classification of pixel colors. The background was defined as a colour group consisting of two colour classes, one identified at a distance from the roots and the other in the shadow area near the roots. The colour range of the healthy parts of the roots was used to define a colour group with 10 colour classes. A third colour group with seven colour classes was defined for the necrotic parts of the roots. For each image, all the pixels were allocated to the different colour classes and groups, giving the healthy and necrotic root area. A necrosis percentage [%] was obtained from the equation:

$$\text{Necrosis percentage} = (\text{necrotic area})/(\text{healthy area} + \text{necrotic area})$$

To quantitatively assess the health of the studied root systems, an indicator called healthy roots weight (HRW) [g] was computed from the following equation:

$$\text{Healthy roots weight} = \{100 - (\text{necrosis percentage})/100\} \times (\text{root dry weight})$$

Growth assessment and mineral analysis

After weaning, 10 Si+ and 10 Si− banana plants were harvested. All the plants submitted to disease assessment were also kept for analyses (10 plants per treatment and per date, 180 plants in total). The dry weight of the root system and aerial parts of all plants was measured after drying at 60°C for 1 week. Mineral analysis was carried out for each plant, to track the Si content throughout the growth of the plants (after weaning and once a week after inoculation). For each plant, all the leaves were crushed and calcinated at 450°C for 2 days, followed by borate fusion at 1,000°C and dissolution of fusion beads in 10% HNO_3 (Chao and Sanzolone 1992). Elemental concentrations were measured by ICP-AES (ICAP 6500 of Thermo Scientific).

Once a week after inoculation, plant growth was assessed by measuring the numbers of totally and partially unfurled leaves. The length (l) and width (w) of the unfurled leaves was measured and their surface area (LA) was computed for 15 plants per treatment kept in soil until the end of the experiment from the equation $LA = 0.83 \times l \times w$ (Lassoudière 2007).

Statistical tests

Statistical analyses were performed for all data using 'Enterprise Guide' (SAS institute, Inc., Cary, NC,

USA). After checking for normality and equality of variance, data were analyzed by two-way ANOVA (factors: Si treatments and inoculum doses) and treatment mean comparisons by the Student-Newman-Keuls' test (α=0.05).

Results

Silicon content

The average leaf Si concentrations at the end of weaning were 16710±2045 mg kg^{-1} dry matter (DM) for banana plants amended with Si (Si+ plants) and 2090 ±1387 mg kg^{-1} DM for banana plants not amended with Si (Si− plants). The difference in Si content between Si− and Si+ plants was highly significant (Student-Newman-Keuls' test, $P<0.0001$). Leaf Si concentrations 7, 14 and 21 days after inoculation were also significantly higher in plants amended with Si than in those grown without Si, for each dose (Student-Newman-Keuls' test, $P<0.0001$) (Table 1).

Root-rot severity

For plants inoculated with 2×10^3 conidia ml^{-1} (d2 dose) and assessed 14 days after inoculation, two-way ANOVA revealed a highly significant effect of the Si treatment (Two-way ANOVA, $P=0.0012$) and of the inoculum dose (Two-way ANOVA, $P=0.0143$) on the necrosis percentage. The average necrosis percentage of banana roots inoculated with d2 dose was 30.21% for Si− plants and 18.61% for Si+ plants, indicating that the treatment of banana plants with a Si solution resulted in a significant reduction of *Cylindrocladium* root necrotic lesion severity compared with the Si− plants (Student-Newman-Keuls' test, $P=0.0141$) (Figs. 1 and 2). The necrosis percentages observed on plants inoculated with 0.5×10^{-3} conidia ml^{-1} (d0.5) were slightly higher than those observed on the non-inoculated plants, but the values were not significantly different for the three dates of analysis. Seven and 21 days after inoculation, the necrosis percentages observed for the six treatments were similar (data not shown). There was no significant interaction between inoculation dose and Si treatments for the three dates of analysis.

Seven days after inoculation, the average HRW was similar for the six treatments (Fig. 3) and no significant difference was observed. Fourteen days after inoculation, an important reduction of growth was observed for the Si− inoculated plants, but the reduction was smaller for Si+ plants; there was no significant difference in HRW between the Si+ d2, Si+ d0.5 and Si+NI (Student-Newman-Keuls' test, $P=0.148$) treatment, but the HRW of inoculated Si− plants was very significantly lower than the Si-NI (Student-Newman-Keuls' test, $P<0.0001$) plants. Later, a dilution of the inoculation effects was observed for the Si− plants; there was no significant difference in HRW between the treatments 21 days after inoculation.

Plant growth parameters

There was no significant effect of Si supply during weaning on the growth parameters being considered. At the end of weaning, the mean root dry weight was 0.61±0.13 g, the mean shoot dry weight was 2.36± 0.32 g and the mean leaf area was 494.09±20.13 cm^2. There was no significant effect of Si supply or inoculation on root dry weight 7 days after inoculation (Table 2). Fourteen days after inoculation, the higher severity of disease for Si− plants was accompanied by a greater reduction in plant growth for this treatment. The root dry weight of the inoculated Si− plants was statistically lower than that of the non-inoculated Si− plants (Student-Newman-Keuls' test, $P=0.0006$), but there was no significant difference in root dry weight between inoculated and control Si+ plants (Student-Newman-Keuls' test, $P=0.157$) (Table 2). A relatively linear increase of the root dry weight was observed for plants grown in the presence of Si, inoculated or not, and for the Si− non-inoculated plants. This was not the case for the plants inoculated with *C. spathiphylli* and grown in the absence of Si, where root dry weight did not increase between 7 and 14 days after inoculation (Table 2). Two-way ANOVA revealed a significant effect of inoculum dose on root dry weight (Two-way ANOVA, $P=0{,}0016$) at 14 days (Table 2). There was no significant effect of Si supply or inoculation on root dry weight at 21 days after inoculation (Table 2). A significant effect of Si supply on shoot dry matter was observed at that time (Two-way ANOVA, $P=0.0054$). The shoot dry matter

Table 1 Evolution of the average leaf Si concentrations (mg kg^{-1} dry matter) in banana leaves of plants amended (Si+) or not (Si−) with Si and inoculated with 2×10^3 (d2 dose), 0.5×10^3 (d0.5 dose) conidia of *C. spathiphylli* ml^{-1} or not inoculated (NI), 7, 14 and 21 days after inoculation. Ten plants were analyzed per treatment. For the same inoculum dose, leaf Si concentration values within a column with different letters are significantly different at $P=0.05$

Si amendment	Inoculum (conidia ml^{-1})	Leaf Si concentration (mg kg^{-1} DM) x days after inoculation		
		x=7	x=14	x=21
−	2×10^3	1602±198 a	2197±475 a	2507±501 a
−	0.5×10^3	1569±198 a	2058±385 a	2506±502 a
−	0	1483±193 a	1935±259 a	2465±411 a
+	2×10^3	16084±1768 b	17820±1356 b	13817±1535 b
+	0.5×10^3	15618±2122 b	17771±2627 b	13940±2070 b
+	0	16532±2024 b	19598±1989 b	15547±2790 b

was higher for plants amended with Si than for those grown in absence of Si (Table 3).

No significant difference in leaf surface area or evapotranspiration was observed after inoculation among the six treatments. The mean leaf area at the end of the experiment was 646.96±24.11 cm^2 and the mean total evapotranspiration for the 3 weeks following inoculation was 654.61±22.60 g of water.

None of the two-way ANOVA's made on the data revealed a significant interaction between inoculation dose and Si treatments.

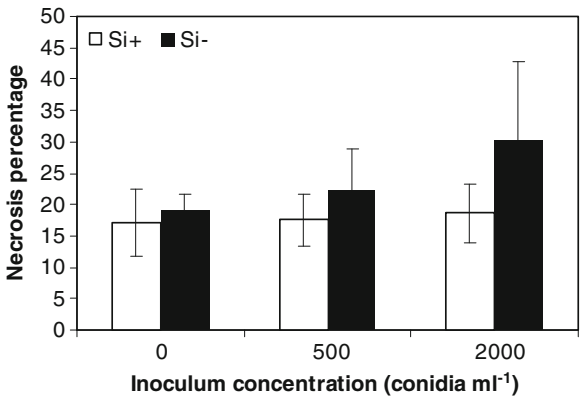

Fig. 1 Average root necrosis percentages of plants amended (Si+) or not (Si−) with Si, inoculated with 2×10^3 (d2 dose), $0.5\ 10^3$ (d0.5 dose) or 0 (NI) conidia of *C. spathiphylli* ml^{-1}, 14 days after inoculation. Each value is the mean and standard error (SE) of ten plants per treatment. The necrosis percentage observed for the Si− d2 plants is significantly higher than the necrosis percentage observed for the Si+ d2 plants (***P***=0.0141). Student-Newman-Keuls' test, $\alpha=0.05$. The necrosis percentage observed for the Si− d2 plants is significantly higher than the necrosis percentage observed for the Si− d0.5 and the Si-NI plants ($P=0.017$). Student-Newman-Keuls' test, $\alpha=0.05$

Discussion

In this study, the effect of Si uptake on the susceptibility of banana to *C. spathiphylli* was assessed under controlled conditions. Banana plants amended with 2 mM Si on a ferralsol, exhibited a reduction of about 50% in the necrosis percentage of inoculated roots 14 days after inoculation compared with plants grown in the same conditions but in the absence of Si. At that time, the HRW of the Si-amended plants (0.48 g) was also superior to the HRW of plants not amended with Si (0.38 g). These results can be correlated with the highly significant difference of leaf Si concentration between Si− and Si+ plants at the end of weaning and after transplantation in the ferralsol, and indicate that Si amendment has a positive effect on banana plants affected by *C. spathiphylli*, which is known to cause severe necrosis and yield losses.

The results of our study could partly explain the observations made in fields in Guadeloupe, indicating that bananas grown on soils with a high Si concentration in soil solution were less affected by pathogens (Henriet et al. 2006). Our results also support the results of other studies that demonstrated the efficiency of Si in controlling diseases caused by pathogens and pests on various plants, notably rice (Seebold et al. 2000), cucumber (Chérif et al. 1994; Menzies et al. 1991) and wheat (Bélanger et al. 2003). It has been shown that Si reduces the incidence of various pests and pathogens, such as herbivorous insects (see Reynolds et al. 2009 for review), parasitic plants (Epstein 1994), bacterial diseases (Diogo and Wydra 2007) and root-knot nematodes (Swain and Prasad

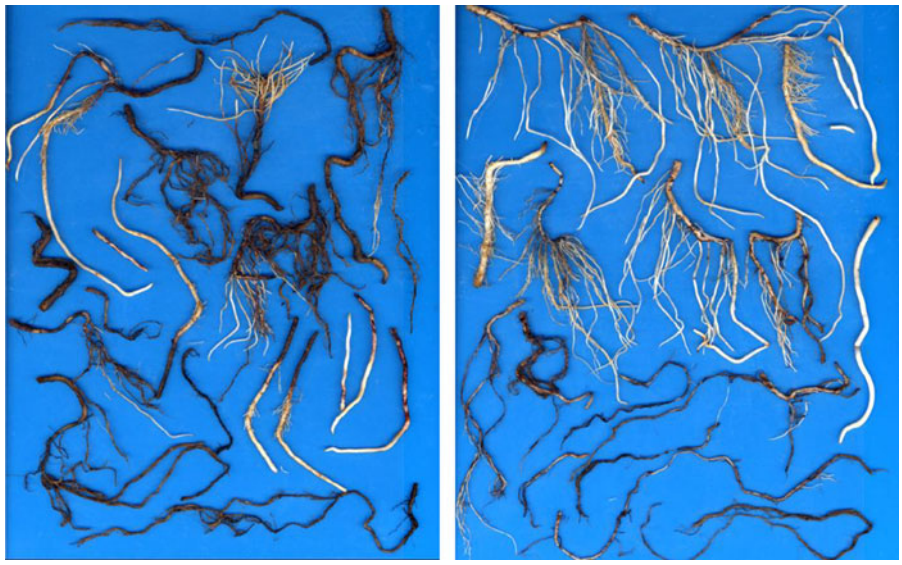

Fig. 2 Left: Si− d2 banana root system 14 days after inoculation (necrosis percentage: 45%), right: Si+ d2 banana root system 14 days after inoculation (necrosis percentage: 13%)

1988). Many studies have focused on the positive impact of Si supply on reducing the impact of pathogenic fungi (Fauteux et al. 2005). The studied fungi are mostly leaf pathogens, such as *Sphaerotheca fuliginea* (Menzies et al. 1991), *Magnaporthe grisea* (Cai et al. 2008) and *Thanatephorus cucumeris* (Rodrigues et al. 2003), but some authors have shown that a similar effect could be obtained with root pathogens, such as *Pythium ultimum* on cucumber (Chérif et al. 1992). In bananas, the positive impact of Si on *Mycosphaerella fijiensis*, a leaf pathogen, has been shown by Kablan et al. (2008). Our study demonstrated that the impact of Si can also reduce the susceptibility of banana to a root pathogen.

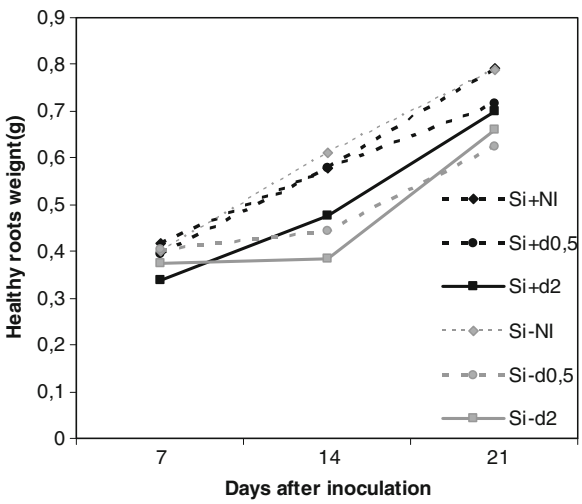

Fig. 3 Evolution of the average healthy roots weight (HRW) of plants amended (Si+), or not (Si−) with Si, inoculated with 2×10^3 (d2 dose), 0.5×10^3 (d0.5 dose) conidia of *C. spathiphylli* ml^{-1} or not inoculated (NI). Ten plants were analyzed per treatment

The mechanism by which Si reduces the incidence of *C. spathiphylli* on banana is not known. In our study, the Si concentration in leaves reached between 1.38 and 1.96% in plants amended with Si. The Si concentration in roots was not measured, but a study performed by Henriet et al. (2006) showed that Si concentration in the roots of 9-week-old banana plants amended with Si under hydroponic conditions was significantly lower than in the leaves (0.26% and 2.12%, respectively). These authors concluded that transpiration played a major role in silicon accumulation and distribution in banana. It is not known if the Si content in banana roots is in soluble or solid form, but as roots are not transpiration sites it is presumed that the Si in roots is not in solid form. This suggests that the protective effect of Si observed in the banana-*C. spathiphylli* interaction cannot be explained by the mechanical barrier theory. Chérif et al. (1992) arrived at the same conclusion with the cucumber-*P. ultimum* pathosystem, also involving a root pathogen. These authors showed that Si conferred protection against *P. ultimum* although it did not accumulate at fungal penetration sites and was almost absent from the wounded roots. Various other studies have suggested that soluble Si affects host defence mechanisms by stimulating metabolic pathways, resulting in the synthesis of defence com-

Table 2 Evolution of the average roots dry weight of plants amended (Si+) or not (Si−) with Si and inoculated with 2×10^3 (d2 dose), 0.5×10^3 (d0.5 dose) conidia of *C. spathiphylli* ml^{-1} or not inoculated (NI). Ten plants were analyzed per treatment. For a same Si treatment, values within a column with the same letter are not significantly different at $P=0.05$

Si amendment	Inoculum (conidia ml^{-1})	Roots dry weight (g) x days after inoculation		
		x=7	x=14	x=21
−	2×10^3	0.513±0.117 a	0.548±0.095 a	0.849±0.173 a
−	0.5×10^3	0.556±0.102 a	0.571±0.095 a	0.795±0.162 a
−	0	0.536±0.115 a	0.756±0.147 b	1.036±0.190 a
+	2×10^3	0.482±0.117 a	0.584±0.092 a	0.847±0.167 a
+	0.5×10^3	0.552±0.104 a	0.701±0.210 a	0.885±0.160 a
+	0	0.511±0.159 a	0.695±0.114 a	0.984±0.218 a

pounds, such as phytoalexins (Bélanger et al. 2003; Fawe et al., 1998). Silicon might play an active role, in its mobile form, in the resistance of banana to *C. spathiphylli* disease. Further physiological, cytochemical and biochemical investigation is needed to fully validate this hypothesis for bananas.

In our experiment, the reduced impact of the pathogen associated with the Si amendment can be related to the effects of Si on growth parameters after inoculation. The shoot dry weight 21 days after inoculation was significantly higher for Si+ plants than for Si− plants, and the effect of inoculation on the root dry weight 14 days after inoculation was alleviated by Si treatment. In contrast, no effect of Si supply was observed at the end of weaning on the plant growth parameters being considered (dry weight of roots and shoots, and leaf area). These results accord with earlier studies showing that Si had no effect on growth in optimal conditions (notably Henriet et al. (2006) for banana; Cornelis et al. (2010) for tree seedlings), but the beneficial effects of Si on plant growth are indirect and are evident when plants are subjected to stress conditions (Chain et al. 2009).

Table 3 Average shoot dry weights of plants amended (Si+) or not (Si−) with Si, inoculated with 2×10^3 (d2 dose), 0.5×10^3 (d0.5 dose) conidia of *C. spathiphylli* ml^{-1} or not inoculated (NI) and assessed 21 days after inoculation. Ten plants were analyzed per treatment. Values within a row with the same letter are not significantly different at $P=0.05$

Inoculum (conidia ml^{-1})	Mean shoot dry weight (g)	
	Si+	Si−
2×10^3	3.70±0.77 a	3.11±0.64 a
0.5×10^3	3.70±0.54 a	3.00±0.67 b
0	3.43±0.36 a	3.33±0.66 a

The methodology used in our study highlights three interesting points. First, the differences in Si concentration between the Si+ and Si− plants were significant, and indicated that the chosen ferralsoil was adequate for maintaining a very low Si supply to the Si− plants and that the Si amendment to the Si+ plants was well assimilated. The methodology also highlighted the importance of the inoculum concentration of *C. spathiphylli*. The observed effects of Si differed according to inoculum dose. A significant effect of the Si treatment on the necrosis percentage was observed for the d2 dose, but the d0.5 dose was not enough to create significant root necrosis and there was no significant effect of the Si treatment on the measured parameters for that dose. Finally, despite the difficulties related to the destructive methods required for studying root-rot pathogens, the disease temporal progress assessment was very important. In our case, root damage increased 7 and 14 days after inoculation, but a decrease in the effects of the inoculation occurred 21 days after inoculation. No significant difference in necrosis percentage was observed between the inoculated and control plants at the end of the experiment, for either Si− or Si+ plants. This could be attributed to a restart of growth, which diluted the effects of the inoculation. Only the conidia in contact with the roots at the time of inoculation succeeded in causing necroses, and the importance of these necroses was counter-balanced by the appearance of new healthy roots, as illustrated in Fig. 3, which shows an HRW increase 21 days after inoculation.

In conclusion, our results indicate that a Si application had a positive effect in the control of banana plant disease in a Si-deficient soil. Studies in the field are needed to confirm that Si amendment is a viable means of sustainably managing banana crops worldwide, providing an environmental friendly alternative to pesticides for controlling banana crop diseases.

Acknowledgements The authors thank A. Iserentant for ICP-AES analysis and Dr J-T Cornelis and Dr F Crutzen for critical reading of the manuscript.

References

Arias, P., Dankers, C., Liu, P., & Pilkauskas, P. (2003). *L'économie mondiale de la banane 1985–2002* (p. 102). Rome: FAO Press.

Bélanger, R. R., Benhamou, N., & Menzies, J. G. (2003). Cytological evidence of an active role of silicon in wheat resistance to powdery mildew (*Blumeria graminis f.sp. tritici*). *Phytopathology, 93*, 402–412.

Cai, K., Gao, D., Shiming, L., Resen, Z., Yang, J., & Zhu, X. (2008). Physiological and cytological mechanisms of silicon-induced resistance in rice against blast disease. *Physiologia Plantarum, 134*, 324–333.

Chain, F., Côté-Beaulieu, C., Belzile, F., Menzies, J. G., & Bélanger, R. R. (2009). A comprehensive transcriptomic analysis of the effect of silicon on wheat plants under control and pathogen stress conditions. *Molecular Plant-Microbe Interactions, 22*, 1323–1330.

Chao, T. T., & Sanzolone, R. F. (1992). Decomposition techniques. *Journal of Geochemical Exploration, 44*, 65–106.

Chérif, M., Menzies, J. G., Benhamou, N., & Bélanger, R. R. (1992). Studies of silicon distribution in wounded and *Pythium ultimum* infected cucumber plants. *Physiological and Molecular Plant Pathology, 4*, 371–385.

Chérif, M., Asselin, A., & Bélanger, R. R. (1994). Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology, 84*, 236–242.

Cornelis, J.-T., Delvaux, B., & Titeux, H. (2010). The contrasting silicon uptakes by coniferous trees: a hydroponic experiment. *Plant and Soil, 336*, 99–106.

Datnoff, L. E., Rodrigues, F. A., & Seebold, K. W. (2007). Silicon and plant disease. In L. E. Datnoff, W. H. Elmer, & D. M. Huber (Eds.), *Mineral nutrition and plant disease* (pp. 233–246). St. Paul: The American Phytopathological Society.

Diogo, R. V. C., & Wydra, K. (2007). Silicon-induced basal resistance in tomato against *Ralstonia solanacearum* is related to modification of pectic cell wall polysaccharide structure. *Physiological and Molecular Plant Pathology, 70*, 120–129.

Epstein, E. (1994). The anomaly of silicon in plant biology. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 11–17.

Epstein, E. (2009). Silicon: its manifold roles in plants. *Annals of Applied Biology, 155*, 155–160.

Fauteux, F., Rémus-Borel, W., Menzies, J. G., & Bélanger, R. R. (2005). Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiology Letters, 249*, 1–6.

Fawe, A., Abou-Zaid, M., Menzies, J. G., & Bélanger, R. R. (1998). Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. *Phytopathology, 88*, 396–401.

Fawe, A., Menzies, J. G., Chérif, M., & Bélanger, R. R. (2001). Silicon and disease resistance in dicotyledons. In L. E. Datnoff, G. H. Snyder, & G. H. Korndörfer (Eds.), *Silicon in agriculture* (pp. 159–169). The Netherlands: Elsevier Science.

Henriet, C., Draye, X., Oppitz, I., Swennen, R., & Delvaux, B. (2006). Effects, distribution and uptake of silicon in banana (*Musa* spp.) under controlled conditions. *Plant and Soil, 287*, 359–374.

Henriet, C., De Jaeger, N., Dorel, M., Opfergelt, S., & Delvaux, B. (2008). The reserve of weatherable primary silicates impacts the accumulation of biogenic silicon in volcanic ash soils. *Biogeochemistry, 90*, 209–223.

Kablan, L., Delvaux, B., & Legrève, A. (2008, October). *Impact of silicon on the susceptibility of banana plants (Musa acuminata) to black Sigatoka disease*. Paper presented at the 4th International conference of Silicon in Agriculture, Port Edward, South Africa.

Keeping, M. G., & Reynolds, O. L. (2009). Silicon in agriculture: new insights, new significance and growing application. *Annals of Applied Biology, 155*, 153–154.

Lahav, E. (1995). Banana nutrition. In S. Gowen (Ed.), *Bananas and plantains* (pp. 258–316). London: Chapman and Hall.

Lassoudière, A. (2007). *Le bananier et sa culture* (p. 383). Quae: France.

Ma, J. F., & Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends in Plant Science, 11*, 392–397.

Ma, J. F., Miyake, Y., & Takahashi, E. (2001). Silicon as a beneficial element for crop plants. In L. E. Datnoff, G. H. Snyder, & G. H. Korndörfer (Eds.), *Silicon in agriculture* (pp. 17–39). The Netherlands: Elsevier Science.

Martinati, J. C., Harakava, R., Guzzo, S. D., & Tsai, S. M. (2008). The potential use of a silicon source as a component of an ecological management of coffee plants. *Journal of Phytopathology, 156*, 458–463.

Menzies, J. G., Ehret, D. L., Glass, A. D. M., & Samuels, A. L. (1991). The influence of silicon on cytological interactions between *Sphaerotheca fuliginea* on *Cucumis sativus*. *Physiological and Molecular Plant Pathology, 39*, 403–414.

Raven, J. A. (2001). Silicon transport at the cell and tissue level. In L. E. Datnoff, G. H. Snyder, & G. H. Korndörfer (Eds.), *Silicon in agriculture* (pp. 41–56). The Netherlands: Elsevier Science.

Reynolds, O. L., Keeping, M. G., & Meyer, J. H. (2009). Silicon-augmented resistance of plants to herbivorous insects: a review. *Annals of Applied Biology, 155*, 171–186.

Risède, J. M. (2008). Isolation of *Cylindrocladium* spp. in root and soils from banana cropping systems. *Fruits, 63*, 57–61.

Risède, J. M., & Rhino, B. (2008). Long-term maintenance of *Cylindrocladium* strains and procedures for inoculum production. *Fruits, 63*, 193–197.

Risède, J. M., & Simoneau, P. (2004). Pathogenic and genetic diversity of soilborne isolates of *Cylindrocladium* from

banana cropping systems. *European Journal of Plant Pathology, 110*, 139–154.

Rodrigues, F. A., Vale, F. X. R., Korndörfer, G. H., Prabhu, A. S., Datnoff, L. E., Oliveira, A. M. A., et al. (2003). Influence of silicon on sheath blight of rice in Brazil. *Crop Protection, 22*, 23–29.

Seebold, K. W., Datnoff, L. E., Correa-Victoria, F. J., Kucharek, T. A., & Snyder, G. H. (2000). Effect of silicon rate and host resistance on blast, scald and yield of upland rice. *Plant Disease, 84*, 871–876.

Sommer, M., Kaczorek, D., Kuzyakov, Y., & Breuer, J. (2006). Silicon pools and fluxes in soils and landscapes—a review. *Journal of Plant Nutrition and Soil Science, 169*, 310–329.

Stumm, W., & Morgan, J. J. (1996). *Aquatic chemistry-chemical equilibria and rates in natural waters*. New York: Wiley.

Swain, B. N., & Prasad, J. S. (1988). Influence of silica content in the roots of rice varieties on the resistance to root rot nematode. *Indian Journal of Nematology, 18*, 360–361.

Swennen, R., & Vuylsteke, D. (2001). Bananier. In R. H. Raemaekers (Ed.), *Agriculture en Afrique Tropicale* (pp. 611–637). Bruxelles: DGCI.