Silicon induced resistance against powdery mildew of roses caused by *Podosphaera pannosa*


*Faculty of Life Sciences, Department of Plant Biology and Biotechnology, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen; and Faculty of Agricultural Sciences, Department of Horticulture, Research Centre Aarslev, Aarhus University, Kristinebjergvej 10, DK-5792 Aarslev, Denmark

Powdery mildew, caused by *Podosphaera pannosa*, is a very common disease in greenhouse potted roses, resulting in poor marketing value and hence economic losses. Alternatives to chemical control are necessary, and therefore the ability of silicon (Si) applied to roots to control the disease was investigated, as well as the mechanisms behind the observed disease reductions. Four genotypes of miniature potted roses representing different genetic backgrounds and susceptibility to disease were studied. Plants were watered with a nutrient solution containing either 3.6 mM Si (100 ppm) supplied as K₂SiO₃ (Si+) or no Si (Si−) before inoculation with *P. pannosa*. Si application increased leaf Si content two- to four-fold compared to control plants. Confocal microscopy showed that Si deposition was larger in Si+ than in Si− plants and that deposition mainly occurred in the apoplast, particularly in epidermal cell walls. Si application delayed the onset of disease symptoms by 1–2 days and disease severity was reduced by up to 48.9%. The largest reduction was found in the two most resistant genotypes, which also had the highest increase in Si uptake. The Si-induced disease protection was accompanied by increased formation of papillae and fluorescent epidermal cells (FEC) as well as deposition of callose and H₂O₂, especially at the sites of penetration and in FEC, which are believed to represent the hypersensitive response. Si treatment reduced powdery mildew development by inducing host defence responses and can therefore be used as an effective eco-friendly disease control measure.

**Keywords:** induced resistance, miniature potted roses, *Podosphaera pannosa*, powdery mildew, silicon

**Introduction**

Miniature potted roses are a very important ornamental crop for the floriculture industry. The annual world production is estimated to be more than 100 million plants, and Denmark is the largest producer with about 50% of the world’s production (Pemberton *et al.*, 2003). Powdery mildew caused by *Podosphaera pannosa* [syn: *Sphaerotheca pannosa* var. *rosae*] is one of the most widespread diseases of potted roses (Leus *et al.*, 2006). The disease results in poor marketing value for the roses because of the presence of greyish white powdery fungal growth on leaf surfaces causing leaf distortion, curling and premature defoliation. So far, powdery mildew has been managed mainly by synthetic fungicides. However, environmental considerations have necessitated increasing restrictions on the use of pesticides, and therefore environmentally friendly production methods for plant disease suppression need to be developed.

Silicon is regarded as an essential element for many plant species and Si application has been demonstrated to inhibit various plant pathogens, e.g. *Magnaporthe oryzae* infecting rice (*Rodrigues et al.*, 2004), *Blumeria graminis f.sp. tritici* infecting wheat (*Bélangé et al.*, 2003; Rému-Borel *et al.*, 2005) and *Podosphaera fuliginea* infecting cucumber (*Menzies et al.*, 1991). Plants take up Si as soluble silicic acid (H₄SiO₄), which is transported via the transpiration stream and subsequently accumulates as insoluble amorphous Si compounds primarily in the apoplast (*Bélangé et al.*, 2003). There are several hypotheses concerning the role of Si in inhibiting fungal infection. Si has been thought to protect through mechanical strengthening of the plant (*Fautex et al.*, 2005). In accordance with this, the enhanced resistance of Si-treated host plants to pathogenic fungi has been suggested to result from more efficient resistance to pathogen penetration of host tissue, e.g. due to the specific deposition of Si compounds in cell walls as suggested for rice blast (*Kim et al.*, 2002). Recent work also indicates that Si may play a more active role in plant–pathogen interactions by stimulating other plant defence responses such as deposition of phenolic compounds/phytoalexins with antifungal properties and enhanced production of defence-related enzymes.
Si-induced protection of roses against powdery mildew or other diseases and the current investigation was therefore undertaken to study the ability of Si to control powdery mildew in miniature potted roses. This was combined with studies of quantitative and temporal differences in the infection processes and defence responses against *P. panmosa* with and without amendment with Si in rose genotypes varying in resistance to the pathogen.

**Materials and methods**

**Plants**

Four genotypes of miniature potted roses, 99/9496-19, 98/8285-1 (moderately resistant), 95/5166-1 and Smart (highly susceptible), representing different genetic backgrounds and different levels of susceptibility to powdery mildew, were obtained from Poulsen Roser A/S, Fredensborg, Denmark.

Roses were propagated and maintained in a greenhouse at 23°C and 60% RH under natural daylight conditions, with supplementary light (E-Papillon, SON T 600 W). Rose cuttings were placed in 10 cm pots containing the soil mix Pindstrup Substrate A1-389 for roses (Pindstrup Mosebrug A/S), watered and covered with polyacril and polythene sheets for 2 weeks to maintain high humidity until rooting. Subsequently, the covers were removed and the plants maintained for another 2 weeks in the greenhouse. The plants were pruned for uniformity and moved to a growth chamber with cycles of 16 h light (200 μEm⁻²s⁻¹), Philips Master IL-D 36 w/865)/23°C/60% RH and 8 h darkness/20°C/80% RH.

**Treatment with silicon**

All experiments comprised 32 plants of each of the tested genotypes. For each genotype, 16 plants received 3.6 mm Si (Si⁺) or no Si (Si⁻) as a control. Soluble Si was supplied in a nutrient solution containing 3.6 mm Si (Sikal™, 91% Si and 25.5% K in the form of potassium metasilicate, K₂SiO₃; Yara Industries). The nutrient content for the 3.6 mm solution was: N (16 mm), P (0.8 mm), K (7.2 mm), Mg (0.8 mm), Ca (3.6 mm), S (0.8 mm), Fe (0.042 mm), Mn (0.021 mm), Zn (0.004 mm), B (0.021 mm), Cu (0.001 mm) and Mo (0.001 mm) (Yara Industries). For the Si⁻ solution (0 mm Si), the nutrient content was essentially the same, except for the following elements: N (13 mm) and K (4.7 mm). The 3.6 mm Si⁺ solution had a higher K content due to the high K concentration in Sikal™. Since Sikal™ is alkaline, extra nitric acid was added to the Si⁺ solution to obtain a pH of 5.5–5.7 which slightly increased the N content of the solution. The pH of the Si⁻ solution was adjusted to the same value. The electrical conductivity was 1.9 S m⁻¹ for the Si⁺ solution and 1.6 S m⁻¹ for the Si⁻ solution.

After the propagation period (4 weeks), plants were moved to the growth chamber and watered for the first time with the Si⁺ and Si⁻ solutions, respectively. Plants were subsequently watered for 10 min with the two nutrient solutions every 72 h until disease scoring, a total of 10 times. After 3 weeks in the growth chamber, the plants were inoculated with the pathogen.

**Pathogen, inoculation and disease scoring**

A single spore isolate of *Podosphaera panmosa* was maintained on the susceptible host genotype Smart in a growth chamber under cycles of 16 h light (200 μEm⁻²s⁻¹), Philips Master IL-D 36 w/865)/23°C/60% RH and 8 h darkness/20°C/80% RH. The fifth developed leaf from every branch of 7-week-old plants was labelled and inoculated by shaking rose plants infected by *P. panmosa* above the test plants in a closed chamber to reach a defined density of 1–2 conidia mm⁻².

Plants were examined daily for onset of the first microscopic symptoms of powdery mildew. Disease severity was determined visually in planta, using a magnifying glass, at 4, 5, 6, 7 and 8 days after inoculation (day) and finally, using a stereo microscope, at 9 dai on detached leaves. At each time point and for each treatment of each rose genotype, disease onset (appearance of the first visible symptoms) was recorded. Furthermore, percent coverage with powdery mildew on the surfaces of 32 individual leaflets was determined and the mean disease severity calculated as the average of the 32 leaflets.

**Measurement of Si content in rose leaves**

Si content was determined in uninoculated 7-week-old plants of all four rose genotypes (99/9496-19, 95/5166-1, 98/8285-1 and Smart). The fifth leaf from 10 Si⁺ and 10 Si⁻ plants was oven dried at 70°C for 24 h. Dried tissue was finely ground in a sample mill (Cyclotec 1093; Foss Analytical) to pass through a 40-mesh screen. Si content was determined by a modification of the autoclave-induced digestion procedure of Elliott & Snyder (1991). Briefly, 100 mg of dried leaf sample was placed in a 100 mL polyethylene tube and wetted with 2 mL 50% H₂O₂ and 3 mL 50% NaOH. Each tube was vortexed and covered with a loose fitting plastic cap. The tubes were placed in an autoclave at 138 kPa for 60 min at 126°C. Subsequently, tubes were removed and the total volume equilibrated to 50 mL with distilled water. The Si content was then determined calorimetrically as follows: 1 mL was taken from the digested plant tissue and mixed with 10 mL distilled water. The Si content was then determined calorimetrically as follows: 1 mL was taken from the digested plant tissue and mixed with 10 mL distilled water. Then, 9 mL acetic acid (20% v/v), 2.5 mL ammonium molybdate (Sigma-Aldrich) solution (100 g L⁻¹, pH 7.0), 1.25 mL tartaric acid (20% v/v) (VWR International) and 0.25 mL of a reducing solution were added. The reducing solution was prepared by dissolving 4 g sodium sulphite, 0.8 g 1-amino-2-naph-thol-4-sulphonic acid (Merck) and 50 g sodium bi-sulphite in 500 mL water. The tartaric acid was added 5 min after the ammonium molybdate, and 10 min later, the
absorbance was measured in a spectrophotometer (Milton Roy, Spectronic 401) at 650 nm. A standard curve relating the amount of Si to the absorbance at 650 nm was used to determine the Si content in the plant material. The standard curve was made by dilutions of a 1000 ppm Si standard (VWR International) using deionised water. Subsequently, the Si content was calculated, adjusted for weight of plant material used and expressed as ppm silicon in dry matter.

Confocal microscopy for localization of Si

Confocal microscopy was used to study the spatial Si deposition in the fifth developed leaf of genotype Smart both inoculated and uninoculated with the pathogen (7-week-old plants), and treated or untreated with Si. For each treatment combination, three leaves were examined. Prior to examination, epicuticular wax was removed from the rose leaves using the cellulose acetate stripping method (Silcox & Holloway, 1986). A 5% (w/v) solution of cellulose acetate in acetone was applied to the adaxial leaf surface with a small paint brush and allowed to dry, and the resulting film was gently peeled off to remove the surface wax. After wax removal, leaves were stained with the weakly basic amine LysoTracker Yellow HCK-123, a fluorescence dye (Invitrogen, final concentration 1 μM) (Descles et al., 2007). Prior to examination, leaves were stained for 2 h with a 200 nM working solution of the stain after which excess stain was removed. The stained samples were examined in a confocal laser scanning microscope (Leica TCS SP/MP; Leica Microsystems) equipped with an argon laser. The wavelength for excitation was 488 nm. The ‘green’ fluorescence of the stain was detected using an emission filter of 505–545 nm. A 660–800 nm emission filter was used for the detection of the ‘red’ autofluorescence of the chloroplasts. The objective used was HC PL APO × 20/0.70 W. For each recording, one stack of 25 images (60 μm) was made. This volume included the epidermis and uppermost mesophyll cell layer. The stack started at the level of the outer periclinal epidermal cell wall after removal of epicuticular wax and image number 3 (epidermis level) and 20 (mesophyll level) were selected for comparison of treatments.

Infection biology of P. pannosa and structural responses in rose primed with Si

Infection biology and defence responses were compared between Si-treated and untreated plants of the moderately resistant genotype 99/9496-19 and the highly susceptible genotype Smart. Leaves were collected for microscopy at 24 and 72 h after inoculation (hai) and cleared as described by Shetty et al. (2003). The fifth developed leaves were studied using bright field and epifluorescence microscopy (excitation maximum 330–385 nm, dichroic mirror DM 400 nm, barrier filter >420 nm) after staining with 0.1% Evans blue (Sigma-Aldrich) in lactoglycerol (lactic acid:glycerol:water, 1:1:1, v/v) for localization of fungal structures. Three leaves were studied at each time point in each genotype. The number of nongerminated conidia was recorded on each leaf, and subsequently the development of 50 randomly chosen germinated conidia was studied on each leaf (total of 150 germinated conidia per genotype and time point). For each conidium, it was recorded whether it formed appressoria, whether it caused penetration, whether it caused formation of single or multiple fluorescent epidermal cells (FEC) and papillae at penetration sites and whether elongating secondary hyphae (ESH) formed. Penetration was considered to occur when haustoria developed from appressoria or FEC were formed. FEC and papillae were considered to stop infection when no ESH developed from germings where these responses occurred.

For scanning electron microscopy of P. pannosa structures, 10 leaf pieces, 4 × 7 mm², were cut at 5 dai and prepared for electron microscopy as described by Shetty et al. (2003). Specimens were examined in a FEI Quanta 200 ESEM Scanning Microscope.

Accumulation of H₂O₂

Accumulation of H₂O₂ was studied by staining with 3,3’-diaminobenzidine (DAB, Sigma-Aldrich) (Shetty et al., 2003). The inoculated leaves of genotype Smart were cut from the plant at 14 and 62 hai and the cut end immediately placed in a DAB solution for 10 h. At 24 and 72 hai, the leaves were cleared as described by Shetty et al. (2003). The leaves were examined by bright field microscopy and stained with 0.1% Evans blue in lactoglycerol for identification of fungal structures. A reddish-brown staining at penetration sites indicated accumulation of H₂O₂.

Callose deposition

Inoculated leaves of genotype Smart were harvested from Si-treated and control plants at 72 hai and cleared as described above. Callose deposition was studied by staining with 0.005% aniline blue in 0.15 M K₂HPO₄ (pH 8.2) for 90 min as described by Shetty et al. (2009). In addition, callose was visualized in cleared leaves after staining with aniline blue in a SP5 confocal microscope (Leica Microsystems) using excitation and emission wavelengths of 405 nm and 460–500 nm, respectively.

Statistical analysis

Data from studies of Si content of rose leaves, onset of disease and disease severity represent continuous variables and were analysed by analyses of variance assuming a normal distribution. Variances were stabilized by appropriate transformations when necessary. Data for Si content and disease severity were analysed separately for each genotype as well as in a model with the two factors genotype and treatment. Likewise, data for onset of disease were analysed separately for each time point and
in a model with the three factors time point, genotype and treatment. Data from studies of infection biology represent discrete variables since it was recorded whether or not a certain event took place (e.g., whether a conidium germinated or not, whether a germinated conidium formed appressoria and whether appressoria with successful penetration formed haustoria or not). Consequently, these data were analysed by logistic regression, assuming a binomial distribution (corrected for over-dispersion when present) as described by Shetty et al. (2003). For comparison of variables (percentages), odds ratios were calculated using Si− plants (controls) as a reference (odds ratio = 1:0).

Data were analysed by PC-SAS (release 9.2; SAS Institute) and hypotheses were rejected at $P < 0.05$. All experiments were performed at least twice with similar results. Only representative data are presented.

Results

Si deposition in rose leaves

Treatment of the four rose genotypes 99/9496-19, 95/5166-1, 98/8285-1 and Smart with 3.6 mM Si resulted in significantly ($P < 0.001$) higher Si content than in the control plants (Fig. 1). The Si content in untreated control plants of all the genotypes was similar (3–4 ppm). The four genotypes differed significantly ($P < 0.001$) from each other in their ability to take up Si, with the moderately resistant genotypes 99/9496-19 and 98/8285-1 taking up more compared to the highly susceptible genotypes 95/5166-1 and Smart. There was a significant statistical interaction between the factors genotype and Si amendment ($P < 0.05$). Whereas application of 3.6 mM Si increased the Si uptake in all genotypes, the level of uptake varied and were 365.3%, 358.2%, 165.8% and 198.9% for 99/9496-19, 98/8285-1, 95/5166-1 and Smart, respectively, compared to the Si− control plants.

Microscope examination of leaves after staining with the fluorescence dye LysoTracker Yellow HCK-123 revealed that in the epidermis, deposition of Si occurred in both Si+ and Si− plants (Fig. 2a–d). In uninoculated leaves, Si deposition occurred to a higher degree in Si+ compared to Si− plants, as seen by the more intense fluorescence here. Si was mainly localized in the apoplast, more specifically in the cell walls (Fig. 2a,b). In the un inoculated leaves (Fig. 2a,b), Si deposition was evenly distributed and more faint than in the inoculated leaves, where Si tended to accumulate in small aggregates in patches, which were unevenly distributed in the cells (Fig. 2c,d). The tendency was most obvious in the Si amended plants (Fig. 2d).

In the mesophyll (Fig. 2e–h), Si deposition was much lower than in the epidermis and was observed only in the upper part of a few cells, just beneath the epidermis. Deposition was much lower in Si− than in Si+ plants in both un inoculated (Fig. 2e,f) and inoculated (Fig. 2g,h) leaves, with no differences between inoculated and uninoculated leaves. In non-stained leaves, no signal for Si was observed in the epidermis or mesophyll in Si+ or Si− plants (data not shown).

Disease reduction after Si uptake

Treatment of rose with a 3.6 mM Si solution resulted in a significant ($P < 0.001$) overall reduction of powdery mildew severity in all four genotypes compared to their respective controls at 9 dai (Fig. 3). In the Si− plants, genotype Smart displayed the highest disease severity and genotype 99/9496-19 the lowest, and the same general trend was seen after Si treatment. There was a significant statistical interaction between the factors genotype and Si amendment. Whereas application of 3.6 mM Si decreased the disease severity in all genotypes, the level of reduction varied and was 48.9%, 48.5%, 38.2% and 39.8% for 99/9496-19, 98/8285-1, 95/5166-1 and Smart, respectively.

The incubation period for development of powdery mildew (period until onset of visible symptoms) was delayed in Si+ compared to Si− plants of both genotypes 99/9496-19 and Smart. Control (Si−) plants of genotype Smart showed disease symptoms already at 4 dai whereas the first symptoms in Si+ plants only occurred at 6 dai. Likewise, in 99/9496-19, symptom expression was delayed from 5 dai in Si− plants to 6 dai in Si+ plants. Furthermore, disease severity after Si treatment was significantly lower in both genotypes 99/9496-19 and Smart compared to their respective controls (0.9% and 5.6%, respectively) from 5 dai (data not shown), the largest reduction being observed for 99/9496-19.
Effect of Si uptake on infection biology and host responses

*Podosphaera pannosa* conidia germinated with a short primary germ tube, appearing approximately 6 hai and establishing the first contact with the host (Fig. 4a). Subsequently one or two, occasionally up to four, appressorial or secondary germ tubes developed (Fig. 4a) and appressoria formed within 24 hai. Direct penetration of the epidermal cell wall took place from an appressorium and resulted in formation of a haustorium in the epidermal cell (Fig. 4b,c). In the case of successful establishment of the pathogen, it developed elongating secondary hyphae (ESH) on the leaf surface to spread the fungal colony (Fig. 4b–d) and from these, new haustoria formed (Fig. 4b,c). Around 5 dai, *P. pannosa* started sporulating by producing conidial chains from the superficial mycelium (Fig. 4e,f). As a response to host penetration or penetration attempts, the rose plant responded with formation of fluorescent papillae just beneath the appressorium (Fig. 4b,c) as well as formation of fluorescent epidermal cells (FEC). The latter response was seen as brightly fluorescing whole cells by epifluorescence microscopy where the pathogen had penetrated (Fig. 4c).

Results of quantitative bright field and epifluorescence microscopy of Si- and Si+ plants of the genotypes 99/9496-19 (moderately resistant) and Smart (highly susceptible) are shown in Tables 1 and 2. In Table 1, the different infection steps are analysed, based on the number of germinated conidia. None of the infection events examined was significantly affected by Si amendment in...
of appressoria causing penetration was significantly reduced (15.5%) in genotype Smart whereas there was no significant difference in 99/9496-19. Formation of haustoria and ESH were significantly lower in Si+ than in Si− plants (18.7% and 22.9%) in 99/9496-19 and Smart, respectively. Conversely, the percentages of appressoria associated with papillae and FEC was increased in 99/9496-19 (37.5% and 43.6%, respectively) and in Smart (28.6% and 28.9%, respectively). Likewise, the proportion of papillae and FEC stopping infection were significantly higher in Si+ than in Si− plants in 99/9496-19 (28.5% and 242.0%, respectively) and Smart (260.9% and 137.3%, respectively).

**Association of infection structures of *P. pannosa* with H2O2 and callose accumulation**

Staining with DAB for localization of H2O2 revealed a close relationship with infection structures (Fig. 5). Thus, H2O2 production was often observed in the vicinity of penetration attempts and infections, often around papillae (Fig. 5a). Furthermore, H2O2 production occurred in association with FEC and these cells often displayed a granulated cytoplasm (Fig. 5b). Arrest of fungal growth was often associated with H2O2 accumulation and this was seen more often and with a higher intensity in Si-treated plants than in control plants. In addition, H2O2 accumulated as early as 24 hai (Fig. 5b), with a higher intensity after Si treatment than in the control plants.

Callose deposition in cleared leaves was difficult to study due to a faint signal and intense autofluorescence (data not shown). However, confocal microscopy after staining with aniline blue revealed deposition of callose at penetration sites, especially associated with papillae and in the cell wall region (Fig. 6). Callose deposition was also observed in the haustorial region of the pathogen (Fig. 6).

**Discussion**

Rose powdery mildew caused by *Podosphaera pannosa* is an economically significant problem all over the world, particularly in greenhouse potted roses. This disease is difficult to control in a sustainable way. Here, it is shown that application of 3.6 mM Si through watering significantly reduced powdery mildew severity up to 48.9%, depending on the host genotype.

Silicon mediated protection against plant pathogenic fungi has been demonstrated in several pathosystems, with special emphasis on crops like rice (Rodrigues et al., 2003) and wheat (Bélanger et al., 2003; Guével et al., 2007). This effect has also been demonstrated with horticultural crops, including cucumber (Schuerger & Hammer, 2003) and tomato (Dannon & Wydra, 2004). Si amendment has even been tested in rose production under varying conditions, e.g. in rockwool cultures for cut flower production (Hwang et al., 2005), but the effect on powdery mildew was not assessed. However, Si amendment does not always result in reduced disease severity.
Moyer et al. (2008) found that potassium and calcium silicate application to the growth medium of gerbera daisy was not able to reduce severity of powdery mildew caused by *Erysiphe cichoracearum/Podosphaera fusca*. It was suggested that the reason for this lack of effect could be that the disease pressure was very high and that infection took place before Si had a time to exert sufficient protection. Furthermore, the final Si concentration in the gerbera daisy plants was rather low compared to, for example, the concentrations observed in roses here.

Results here show a difference in the ability of Si to protect roses of different genetic backgrounds, the highest protection being observed in the most resistant genotypes. Interestingly, after application of 3-6 mM Si, the two genotypes with the highest Si deposition also showed the highest protection against disease, whereas such a correlation was not seen in the control plants (Si−). A similar situation has been reported in rice where Si amendment of a resistant cultivar resulted in greater Si uptake and protection against rice blast compared to a susceptible cultivar (Cai et al., 2008) and could reflect the greater potential for Si uptake and defense by some host genotypes.

Si was localized almost exclusively in the epidermal cells in rose leaves, more precisely in the cell walls and extracellular spaces. These findings are in accordance with the Si deposition pattern found in various monocot species (Fauteux et al., 2005; Ma & Yamaji, 2006). In other dicots, silicon application has resulted in deposition of Si in root cell walls (Heine et al., 2007) and has been observed as a uniform pattern in Si+ leaves with the strongest deposition on and around trichomes (Samuels et al., 1991; Ghanmi et al., 2004). Previous studies on Si localization in leaves used X-ray microanalysis and transmission electron microscopy (Ghanmi et al., 2004). The preparation of material for these studies is quite laborious and time-consuming. Therefore, this study used confocal microscopy and specific staining for Si, applying the stain LysoTracker Yellow HCK-123, with the epicuticular wax initially being removed to avoid unspecific

![Figure 4](https://example.com/figure4.png)

**Figure 4** Infection structure formation of *Podosphaera pannosa* in rose genotype Smart (a–f). (a) Germinating conidia at 24 h after inoculation (hai). (b–c) Bright field and epifluorescence microscopy, respectively, showing formation of papilla with haustorium and FEC at 72 hai. (d) Penetration and formation of papillae and elongating secondary hyphae at 48 hai. (e) Scanning electron micrograph showing elongating secondary hyphae and conidial chains at 5 dai. (f) Macroscopic symptoms of infection with numerous conidial chains at 9 dai. pgt, primary germ tube; sgt, secondary germ tube; con, conidium; pap, papilla; hau, haustorium; FEC, fluorescent epidermal cell. Arrows show conidial chains in e–f.
staining. This technique is new for higher plants, but has previously been applied for studies of Si in diatoms (Descles et al., 2007) and in mammalian systems. The method was found to be quite sensitive and rapid, and furthermore it was possible to work with living plants. This technique may be applicable when there is a need to

Table 1 Quantitative studies of the infection biology of Podosphaera pannosa and defence responses in the fifth developed leaf (of every branch) of rose plants of the moderately resistant genotype 99/9496-19 and the highly susceptible Smart either treated with 3.6 mM Si (Si+) or untreated (Si−). Values are assessed on the basis of the number of germinated conidia

<table>
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<td></td>
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<td>Si−</td>
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<tr>
<td>With ESH</td>
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<td>With FEC</td>
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aOdds ratio for comparison of treatments (control used as reference, odds ratio = 1.00).

The number of asterisks indicates the degree of significance: *Significant at 0.05 < P < 0.01, **significant at 0.01 < P < 0.001, ***significant at P < 0.001, NS: nonsignificant, P > 0.05.

Table 2 Quantitative studies of the infection biology of Podosphaera pannosa and defence responses in the fifth developed leaf (of every branch) of rose plants of the moderately resistant genotype 99/9496-19 and the highly susceptible Smart either treated with 3.6 mM Si (Si+) or untreated (Si−). Infection processes were assessed based on the number of appressoria producing infection structures

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<thead>
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<th>72 hai</th>
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<td>Si+</td>
<td>Si−</td>
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<tr>
<td>Genotype 99/9496-19</td>
<td></td>
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<tr>
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<td>28</td>
<td>09</td>
<td>3.06NS</td>
<td>921</td>
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<td>With haustoria</td>
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<td>00</td>
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<td>639</td>
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<tr>
<td>With ESH</td>
<td>00</td>
<td>00</td>
<td>1.00NS</td>
<td>639</td>
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<tr>
<td>With FEC</td>
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<td>09</td>
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<td>895</td>
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<td>With papillae</td>
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<td>93</td>
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<td>With FEC stopping infection</td>
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<td>09</td>
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<td>2.16NS</td>
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aOdds ratio for comparison of treatments (control used as reference, odds ratio = 1.00).

The number of asterisks indicates the degree of significance: *Significant at 0.05 < P < 0.01, **significant at 0.01 < P < 0.001, ***significant at P < 0.001, NS: nonsignificant, P > 0.05.
obtain fast, but reliable results on the exact location of Si deposition in plants and when there is a need to study the dynamics of Si deposition in an easy way. Hence, it was observed that inoculation induced Si deposition especially in the Si+ plants and also that the appearance of the deposits changed.

There are different hypotheses concerning the role of Si in inhibition of fungal pathogens. Initially, enhanced protection of Si-treated plants against pathogenic fungi was suggested to result from increased resistance to pathogen penetration of host tissue, e.g. due to the specific deposition of Si-containing compounds in cell walls (Kim et al., 2002). Indeed, it has been reported that Si was deposited in cell walls at the site of fungal penetration (Samuels et al., 1991; Epstein, 1994; Fauteux et al., 2005). Similarly, Rodrigues et al. (2004) suggested a mechanical role of Si in protecting rice against Magnaporthe oryzae. The hypothesis that an enhanced physical strengthening is responsible for inhibition of pathogens may be quite complicated to demonstrate. Furthermore, this hypothesis has been recently questioned as it appears that amendment of plants with Si may result in enhancement of defence responses (reviewed by Ghanmi et al., 2004; Fauteux et al., 2005). Therefore, in order to further elucidate the mechanisms by which Si reduces disease severity, it is necessary to study if defence responses in the host are enhanced, i.e. whether induced resistance (sensu Kloeper et al., 1992) is involved. Various host responses have been reported in rose against infection by pathogens, including accumulation of phenolics, lignin and callose (Conti et al., 1986; Dewitte et al., 2007), PR-proteins and papillae (Dewitte et al., 2007) and reactive oxygen species (Gachomo & Kotchoni, 2008). Conti et al. (1985) reported that infected rose cells became necrotic and this resembled the hypersensitive reaction (HR). Similarly, in this study infected rose cells often fluoresced brightly when using epifluorescence microscopy and this phenomenon was termed FEC. In other host–pathogen interactions, such as the interaction between barley and Blumeria graminis f.sp. hordei, such fluorescent cells have been interpreted as an indication of the hypersensitive response (Wei et al., 1994). It is highly likely that FEC in rose also represent cells undergoing HR, as this is quite a common and efficient response to stop infection by biotrophic pathogens (Shetty et al., 2008). A correlation was observed between elicitation of FEC and arrest of fungal growth, but a formal connection between FEC and HR remains to be shown.

Figure 5  Association of infection structures of Podosphaera pannosa with H$_2$O$_2$ within rose genotype Smart. (a) Leaves treated with 0 mM Si (Si–) at 3 days after inoculation. (b) Leaves treated with 3.6 mM Si (Si+) at 24 h after inoculation. Arrows show H$_2$O$_2$ accumulation in whole cells, some with granulated cytoplasm.

Figure 6  Confocal microscopy of leaves of rose genotype Smart inoculated with Podosphaera pannosa at 72 h after inoculation (52-day-old plants) from plants treated with 3.6 mM Si (Si+) and stained with aniline blue. Each pair of images is recorded from the same optical section using excitation and emission wavelengths of (a) 405 nm and 460–500 nm. The blue colour represents callose stained with aniline blue (arrows). (b) 600–800 nm. The red colour represents autofluorescence (arrows) from host and pathogen.
The ability of *P. pannosa* to produce appressoria on the two rose genotypes at 72 hai was differently affected by Si application. Thus, in 99/9496-19, Si+ plants had a significantly lower proportion of germinated conidia forming appressoria than Si− plants, whereas no difference was seen in genotype Smart. On the other hand, the proportion of appressoria causing penetration in genotype Smart was significantly reduced in Si+ compared to Si− plants, whereas this was not seen in 99/9496-19. A possible reason for these differences could be found in the nature of the cuticle and epicuticular wax, which is known to vary between cultivars of certain plant species and thus affect the ability of pathogens to infect (Jørgensen *et al.*, 1995). Although Conti *et al.* (1985) failed to detect any apparent differences in the wax layer of the two rose cultivars they studied, the chemical composition may have varied. Furthermore, the composition of cuticle and wax may be altered by applications of Si. Wang & Galletta (1998) showed that foliar application of potassium silicate to strawberry enhanced the leaf content of fatty acids such as oleic, linoleic and linolenic acids. These fatty acids have previously been shown to be efficient inducers of resistance against another biotrophic pathogen, *Sclerospora graminicola* infecting pearl millet (Amruthesh *et al.*, 2005). Si application in roses might also act by eliciting endogenous elicitors of defence in the host.

Si was found to effectively reduce formation of haustoria and elongating secondary hyphae by the pathogen and this was correlated to an increase in papilla formation and frequency of FEC as well as in the ability of these defence responses to reduce infection. The timing and magnitude of these responses varied between Si-treated and control plants for both genotypes, with the moderately resistant 99/9496-19 giving the highest degree of reduction in accordance with its higher level of resistance. The results indicate that the reductions in formation of haustoria and ESH correspond well to the increase in formation of FEC and papillae. However, more important is the efficiency of these responses in stopping the pathogen and here the data indicate that Si amendment resulted in a considerable reduction in fungal growth. On the other hand, fungal growth was not completely stopped, indicating that neither response completely stops fungal growth.

The induced formation of papillae and FEC in response to infection attempts by *P. pannosa* was associated with the accumulation of H$_2$O$_2$ and deposition of callose, with a more prominent occurrence in Si+ plants. H$_2$O$_2$ accumulation is a host response effective in preventing development and establishment of biotrophic pathogens (Borden & Higgins, 2002; Shetty *et al.*, 2003, 2008). Accumulation of callose was observed at the penetration sites of *P. pannosa* and around the haustorial neck of the pathogen in infected cells. A similar observation was made by Kuzuya *et al.* (2006) in melon. In addition to callose fluorescence, infected rose leaves showed considerable autofluorescence. Therefore, in order to clearly verify the presence of callose, it was necessary to use confocal microscopy which can specifically detect fluorescence from callose. On the other hand, the presence of unspecific fluorescence indicates the involvement of phenolics and flavonoids as host responses to infection (Conti *et al.*, 1986). Currently, detailed analyses of metabolites induced in rose after Si application are therefore being performed, with special emphasis on flavonoids and phenolics and their role in disease resistance. This may help in identifying key metabolic intermediates in disease resistance against rose powdery mildew and subsequently in finding effective target systems for more site-directed disease control strategies.

In the present studies, root application of Si resulted in significant reductions in powdery mildew infection and growth, but did not prevent the disease completely. Nevertheless, the protection offered might still have important practical implications in commercial rose production. Si is relatively easy to apply in greenhouse systems and the reduction in disease severity and delay in onset of symptom expression are important in themselves as this may help to improve product quality, as well as reduce the disease pressure in the crop and thereby the need for disease control. In addition, boosting of the inherent ability of rose to defend itself against *P. pannosa* will result in a reduced need for pesticides and/or possibility for the use of alternative control agents, which are not effective when used alone. Therefore, use of Si as a supplement to disease control, especially in genotypes with a relatively high inherent resistance against powdery mildew, has a strong potential in integrated pest management and might help to make production of miniature potted roses more sustainable.

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**References**


