

Applications of Potassium Silicate Decrease Black Spot Infection in *Rosa hybrida* 'Meipelta' (Fuschia Meidiland™)

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Abstract. Roses in nursery and landscape settings are frequently damaged by black spot, whose causal agent is the fungus *Diplocarpon rosae* F.A. Wolf. Potassium silicate was assessed as a media-applied treatment for decreasing the severity and incidence of black spot infection. Roses were treated with 0, 50, 100, or 150 mg·L⁻¹ silicon as potassium silicate incorporated into irrigation water on either a weekly or daily schedule. Five weeks after treatments were initiated, plants were inoculated with *D. rosae*. Roses began to show visual symptoms of infection ≈4 days later. Roses that had 150 mg·L⁻¹ silicon applied on a daily schedule had significantly more silicon present in their leaves than other treatments as measured by scanning electron microscopy and energy-dispersive x-ray analysis. In addition, roses that had 100 and 150 mg·L⁻¹ silicon applied on a daily schedule had fewer black spot lesions per leaf and fewer infected leaves than any of the other treatments by the end of the experiment 7 weeks later. Although roses treated with higher levels of silicon on a daily basis fared better than roses in the other treatments, all of the roses were heavily infected with *D. rosae* by the end of the study. The results reported here indicate that using potassium silicate in irrigation water may be a useful component of a disease management system.

Silicon compounds have been shown to reduce the severity of fungal diseases in many plants (Belanger et al., 1995; Cherif et al., 1994). Silicon is actively taken up by the roots and dispersed through the plant via the transpiration stream (Samuels et al., 1991), where it inhibits fungal diseases through modifications of the epidermal layer of leaves and fruit (Samuels et al., 1991, 1993) as well as by increasing presence of low-molecular-weight metabolites (Fawe et al., 1998). In addition to the inhibition of harmful fungi, physiological effects of silicon are reported to include the promotion of growth in cucumbers and other plants (Epstein, 1994; Marschner et al., 1990; Miyake and Takahashi, 1983).

Silicon may be applied to plants in a variety of forms. For research purposes, silicon is often introduced to plants by adding soluble silicon compounds, such as sodium or potassium silicate, to a nutrient solution contacting the root zone (Belanger et al., 1995; Samuels et al., 1991). In field trials, silicon may be added as many compounds, including calcium silicate (Miyake and Takahashi, 1983), silicate slag (Ayres, 1966), or silicate rock (Hinsinger et al., 1996). The effects of adding silicon compounds to soil or growth media have been previously demonstrated and yields have

been shown to increase with these additions in certain crops, including rice, cucumbers, barley, and sugarcane (Ayres, 1966; Dyke, 1979; Epstein, 1999; Miyake and Takahashi, 1983). Supplemental applications of silicon are beneficial to crops not only because silicon is considered a necessary element for the healthy growth of many plants (Cheng, 1982; Epstein, 1999), but also because silicon additions affect soil properties such that there is an increased availability of media phosphorus (Mengel and Kirkby, 1987).

Foliar applications of silicon compounds on grapes, cucumbers, zucchini, muskmelon, and rose have revealed that this application method may also be useful for improving plant defenses (Bowen et al., 1992; Gillman and Zlesak, 2000; Menzies et al., 1992). It has been speculated that foliar applications of soluble silicon may serve to increase plant resistance to disease by absorption of silicon through the leaf (Menzies et al., 1992), or by the creation of a protective silicon barrier on the surface of the leaf (Bowen et al., 1992).

Black spot of rose, caused by the fungus *Diplocarpon rosae*, is the most troublesome disease of roses worldwide (Horst, 1983). Outdoor production situations, common in nurseries, provide free water for spore dispersal and germination. Susceptible cultivars can quickly defoliate, making them unsalable. Previous research has shown that foliar application of sodium silicate to rose cuttings decreases leaf drop and black spot infection as well as increasing rooting of cuttings (Gillman and Zlesak, 2000). The effects of soluble silicon on resistance to black spot when applied to the media in which the roses are growing,

however, has yet to be determined.

Rosa hybrida 'Meipelta' (Fuschia Meidiland™) is a popular rose for landscape use that is known to be susceptible to black spot. This study investigates the ability of potassium silicate, applied using irrigation water as a carrier, to increase host plant resistance to black spot under nursery conditions.

Materials and Methods

Plant material and experimental design. One hundred and five *Rosa hybrida* 'Meipelta' (Fuschia Meidiland) roses propagated on their own roots were planted from plugs into #1 nursery containers (2.5 L) on 22 June 2001. All roses were grown outside on a gravel pad in St. Paul, Minn. Roses were assigned to five blocks with 21 plants per block. Spray stick emitters (Netafim, Tel Aviv, Israel) were placed, one per plant, into containers. Growing media consisted of a mix of 6 parts composted pine bark : 4 parts sphagnum peat : 1 part coarse sand. Roses were watered once per day with ≈840 mL of water, delivered through the spray sticks, and were fertilized with a topdressing of 9 g of 15N–3.9P–15K (Osmocote, The Scotts Co., Marysville, Ohio) after planting. Within each block, six treatments, including three daily and three weekly potassium silicate (Pro-TeKt, Dyna-Gro, San Pablo, Calif.) applications, were applied, as well as a control that was given only water. Plants were arranged randomly within blocks. Weekly and daily potassium silicate applications included concentrations of 50, 100, and 150 mg·L⁻¹ silicon. Daily treatments consisted of applications of potassium silicate injected into the irrigation water using a fertilizer injection system (Dosmatic, Carrollton, Texas). Applications of potassium silicate to plants in weekly treatments were made by adding an additional irrigation treatment to all plants once per week with the appropriate concentration of potassium silicate being given to the weekly treatments. Treatments commenced on 2 July and continued until the termination of the experiment on 26 Sept. 2001.

Inoculum preparation. In late June 2001, leaves infected with *D. roseae* were collected from a seedling population at the Univ. of Minnesota Landscape Arboretum, Carver County. About 2500 leaflets bearing acervuli were collected and placed in plastic bags for immediate processing. Leaves were sprayed with sterile distilled water and placed in plastic boxes and incubated for 10 h in a dark growth chamber at 4 °C to release conidia from the acervuli. The leaves were then steeped in 6 L of sterile distilled water and held at 4 °C for 12 h. The water was then poured through a 1-mm² mesh strainer into a 10-L container and stirred thoroughly. In order to calculate spore concentration, ten 100-μL samples were collected from the suspension. These samples were examined at 400× magnification using a standard hemacytometer and the suspension adjusted to 100,000 spores per mL.

Inoculation procedure. All of the plants in this study were brought into a greenhouse area from outside and inoculated with *D. roseae* on

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8 Aug. To improve adhesion of spores to leaf surfaces, 1 L of 0.05% Difco agar was added to the spore suspension outlined above, diluting the mixture to a concentration of 85,714 spores per mL, and 400-mL aliquots were poured into 500-mL plastic spray bottles. The spore suspension was sprayed onto leaf surfaces until runoff. After the spore suspension was sprayed onto each plant, a 13-gal plastic bag was placed over the plant and tied shut to keep humidity near 100%. Plants were then placed in a dark growth chamber with a constant temperature of $\approx 18^\circ\text{C}$ for 40 h. After 40 h, the bags were removed and the plants were taken out of the growth chamber and replaced outside on the gravel pad. The average number of germinating spores was determined by spraying six plates of 2% Malt extract agar with the spore suspension and placing these in the same growth chamber as the roses for 48 h. After 48 h, plates were examined at 400 \times magnification and the number of spores and germinating spores were counted in each field of view in 20 predetermined locations on each plate.

Assessment of infection. Entire plants were rated on a 1–5 scale for leaf loss weekly after inoculations. Ratings consisted of a subjective measurement assessing the amount of leaves still on the plant. A rating of 1 indicated 0% to 20% leaf loss, 2 indicated 21% to 40% leaf loss, 3 indicated 41% to 60% leaf loss, 4 indicated 61% to 80% leaf loss and 5 indicated 81% to 100% leaf loss. Leaflets were examined for black spot lesion number per leaflet on six randomly selected fully expanded terminal leaflets per plant once per week starting the second week after inoculation. Leaflets were not destructively sampled. Percentage of leaflets infected was assessed weekly starting the week after inoculation and was calculated by non-destructively sampling 20 randomly selected fully expanded axillary leaflets per plant and counting the number that were infected with black spot (at least 1 spot > 1 mm in diameter). Leaflets used to assess black spot lesion number per leaflet and percent of leaflets infected were not fully expanded at the time of *D. roseae* inoculation.

Silicon concentration analysis. Seven weeks after roses were inoculated with black spot, lightly diseased leaves were collected from experimental plants to determine silicon levels. For each block and treatment, one leaflet was chosen randomly and stored at 4 $^\circ\text{C}$. For each leaflet, one 5-mm 2 piece was excised on the right side of the midrib in the middle of the leaflet. Leaflet pieces were transferred to aluminum scanning electron microscope (SEM) stubs covered with carbon tape and carbon paint was used to secure the corners of the leaf pieces to the stub. The stubs were then transferred to a cryo-quench at about -120°C for 30 s and moved to the cryo-stage on the SEM. Samples were coated with nickel using a sputter coater and transferred to the SEM operating stage. To perform the energy-dispersive x-ray analysis of leaf samples, a Hitachi S3500N variable pressure scanning electron microscope fitted with an EDAX Phoenix x-ray microanalysis system was used. The accelerating voltage for the SEM was 10 kV. The x-ray take-off angle used was

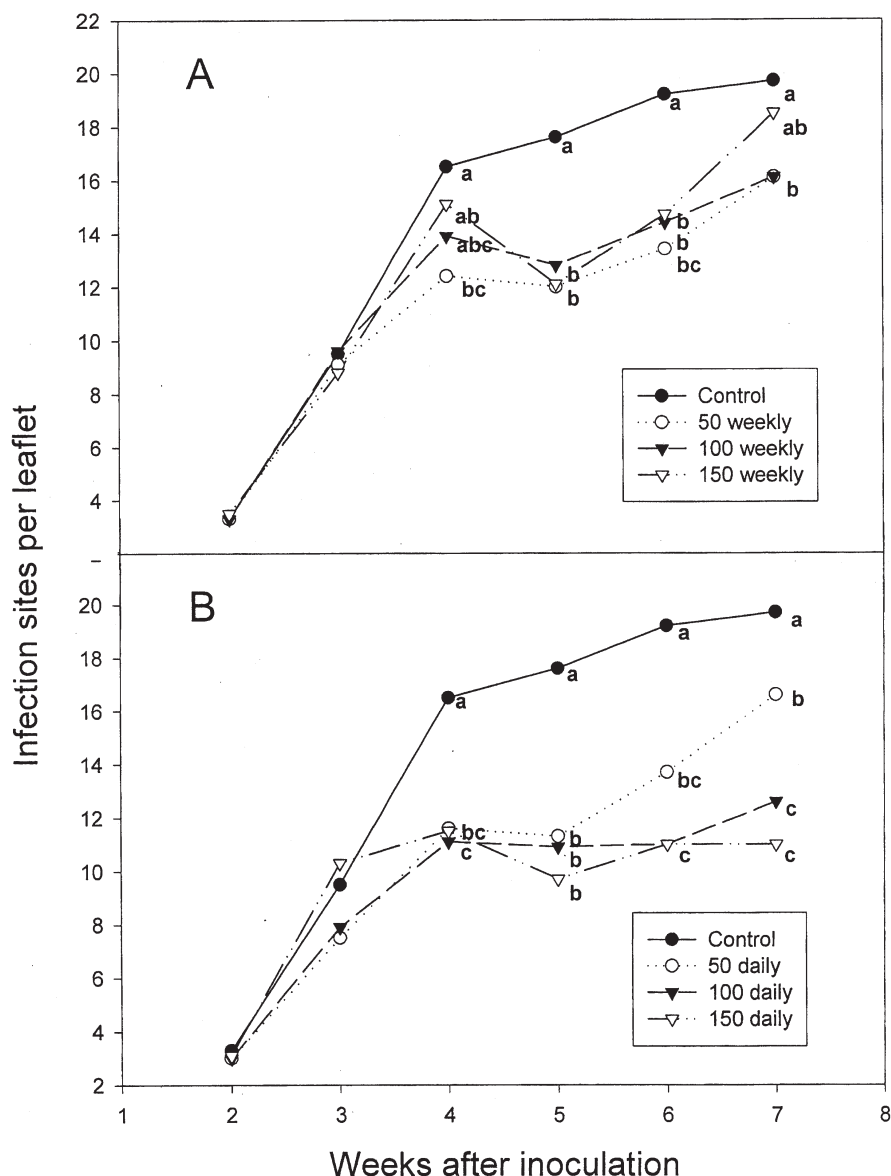


Fig. 1. Average number of black spot infection sites per leaflet on rose plants treated with 0, 50, 100, or 150 $\text{mg}\cdot\text{L}^{-1}$ of potassium silicate (A) weekly or (B) daily. Mean separations within date calculated using Duncan's multiple range test ($P \leq 0.05$).

15° , with a working distance of 16 mm. To standardize the spectra, a bare aluminum stub was used for beam current calibration at 2000 counts/s (the beam current used was ≈ 70 V). Agar blocks were used to obtain x-ray spectra for three concentrations of silicon (Si at 78,000, 7800, and 780 $\text{mg}\cdot\text{L}^{-1}$) used as standards. For all spectra collected, 8000 \times magnification was used. An area raster of $\approx 100\text{--}300$ μm^2 was used to collect each spectrum. For the agar block standards, a total of four spectra were collected for 40 s each from each agar block. For the leaf tissue, four spectra were collected from each leaf piece. Because it was determined that small pieces of dust on the leaf surface contained silicon, spectra were collected from areas free of these particles. The spectra were processed using the batch processing function in the EDAX software package. The number of silicon counts per 40 s (above background levels) were compared for all spectra. Concurrent with silicon counts,

counts of both potassium and phosphorus were taken to establish whether potassium silicate treatments affected the concentrations of these elements in leaf tissue.

Statistical analysis. Significant differences among treatment means were analyzed using Duncan's multiple range test ($P \leq 0.05$). Significant differences in percentage of infected leaflets among treatments were calculated using data prior to percentage transformation. All statistics were calculated using the general factorial command in SPSS (SPSS Inc., 1997).

Results and Discussion

Malt extract agar plates revealed a spore germination rate of 84% demonstrating that spore viability was very high. Plants showed signs of infection ≈ 4 d after inoculations were made.

Number of infections per leaflet increased across all treatments as the experiment pro-

gressed. Weekly applications of 50 mg·L⁻¹ silicon provided a lower number of infection sites per leaflet than controls from 4 weeks after inoculation until the end of the experiment. Weekly applications of 100 and 150 mg·L⁻¹ silicon showed significantly fewer infection sites per leaflet than controls from 5 weeks after inoculation until the end of the experiment, except for the weekly application of 150 mg·L⁻¹, which was not significantly different from the control on week 7 (Fig. 1A). Plants treated with daily applications of potassium silicate at all concentrations showed significantly fewer infection sites per leaflet than the control from the fourth week after inoculation until the termination of the experiment and daily treatments of 100 and 150 mg·L⁻¹ were lower than daily treatments of 50 mg·L⁻¹ at 7 weeks after inoculation (Fig. 1B). Although both the weekly and daily treatments did show significantly fewer infection sites than the control towards the end of the experiment, the daily applications of 100 and 150 mg·L⁻¹ had significantly fewer spots per leaflet than any of the weekly treatments on the sixth and seventh weeks after inoculation.

Data collected over the first 2 weeks after inoculation on leaves that were fully expanded at the time of inoculation showed that there were essentially no leaflets that were uninfected across all treatments during this time. There were no significant differences in percentage of leaflets infected between the control and the weekly treatments over the course of the experiment (Fig. 2A). Daily applications of 100 and 150 mg·L⁻¹ had significantly fewer leaflets infected than the control at 6 and 7 weeks after inoculation and the 150 mg·L⁻¹ treatment also proved to have a significantly lower percentage of leaflets infected than the control at 5 weeks after black spot inoculation (Fig. 2B).

Energy-dispersive x-ray analyses were only conducted for those plants receiving daily silicon applications due to cost and time constraints. The greater concentrations of silicon were measured in those plants treated with higher concentrations of potassium silicate indicating that silicon was taken up by the plant and deposited into the leaves (Table 1). The apparent correlation between increased silicon and decreased black spots per leaf and decreased percent of leaflets infected seems to indicate that additional silicon in the leaf is having an effect on the spread of this disease in the plant. It is likely, due to the absence of significant differences between treatments and the control early in the experiment, that the silicon applications affect secondary infections rather than the primary infection brought about by the initial inoculation. This could indicate that the plant responded to the disease only after it had begun infecting the plant, as suggested by Cherif et al. (1992). By the end of the experiment, none of the treatments resulted in lower defoliation as measured by the subjective 1–5 scale and none of the plants would be considered salable in a commercial situation.

Although other studies show that an increase in the amount of silicon applied to soil results in increased phosphorus uptake (Mengel and Kirkby, 1987), energy-dispersive x-ray analysis

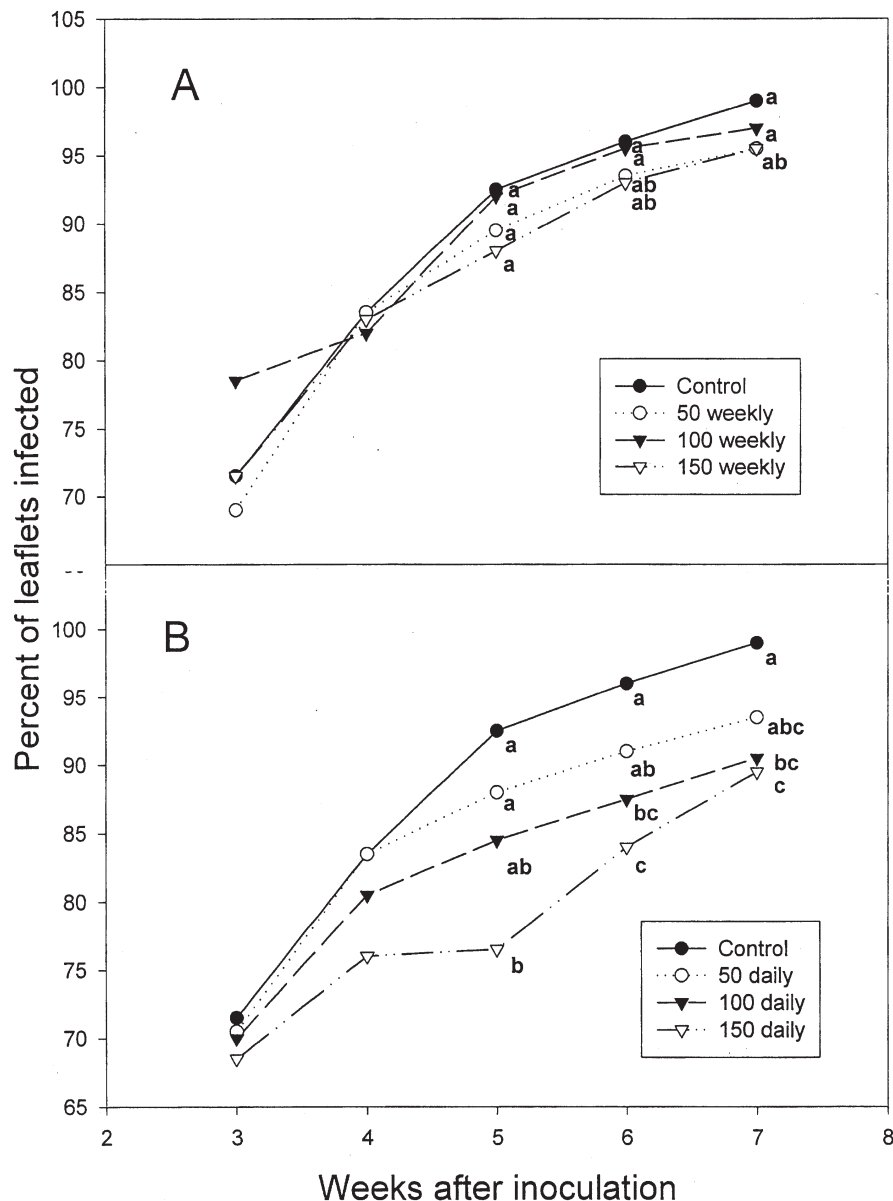


Fig. 2. Average percent of leaflets infected with black spot on rose plants treated with 0, 50, 100, or 150 mg·L⁻¹ of potassium silicate (A) weekly or (B) daily. Mean separations within date calculated using Duncan's multiple range test ($P \leq 0.05$).

Table 1. Concentrations of elements in epidermal layers of leaf tissue collected from roses treated with 0, 50, 100, or 150 mg·L⁻¹ silicon daily. Concentrations are expressed as counts per second \pm standard deviation (and as mg·kg⁻¹ plant material for Si).

Treatment (mg·L ⁻¹)	Si	K	P
0	1.470 \pm 0.659 (170.4)	2.163 \pm 1.199	0.425 \pm 0.526
50	2.475 \pm 3.356 (247.2)	1.562 \pm 0.708	0.399 \pm 0.540
100	3.965 \pm 3.495 (361.1)	2.174 \pm 0.984	0.601 \pm 0.559
150	11.135 \pm 10.641 (909.6)	2.703 \pm 1.254	0.566 \pm 0.734

of the leaves, performed at the same time that silicon was measured, showed no differences in phosphorus content between any of the treatments in this study. The additional potassium added by the treatments did not significantly affect the concentration of potassium within the leaves, as measured by energy-dispersive x-ray analysis, with the exception of the 50 mg·L⁻¹ treatment which had a slightly lower concentration of potassium than the other treatments

and the control (Table 1). It is unknown why the 50 mg·L⁻¹ treatment had a lower level of potassium than other treatments. It is possible that experimental error due to the relatively small sampling size allowed by x-ray analysis affected these readings.

Data from this study agree with previous research (Gillman and Zlesak, 2000) in showing that silicon can have a positive effect on disease control of black spot in roses. Sub-

jective measurements of black spot infection, however, indicate that this increased resistance does not necessarily result in a plant that is more salable. It is likely that silicon is not as effective when disease pressure is extremely high, as in grapes (*Vitis vinifera*) defending against powdery mildew (*Uncinula necator*) (Reynolds et. al., 1996).

Soluble silicon applications to rose do hold promise for increasing black spot resistance in roses when injected into irrigation water, but should be considered as a part of a disease management program and not as a stand-alone treatment. It seems likely that, with the silicate concentrations tested in this study, potassium silicate would have to be injected at every irrigation cycle to affect infestations of *D. rosae*.

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