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Effect of Potassium Silicate Amendments in Hydroponic Nutrient Solution on the Suppressing of *Phytophthora* Blight (*Phytophthora capsici*) in Pepper

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Amendments of a recirculating nutrient solution with potassium silicate were evaluated as a means to control *Phytophthora capsici* infections on pepper plant (*Capsicum annuum* L.). Supplying the solutions with 100 or 200 ppm of silicate significantly reduced motility, root decay, and yield losses attributed to infection of *P. capsici*. Treating inoculated plants with potassium silicate increased root dry weights and number of fruit, especially high-grade fruit. Results were slightly superior to non-inoculated controls. The two varieties, PBC 137 and PBC 602, responded similarly to the treatments. No significant differences were observed between the 100- and 200 ppm silicate treatments. Results were better when greenhouse conditions favored the spread of *P. capsici*. Silicon alone did not increase pepper yield, suggesting that it acts as a disease suppression agent rather than as a fertilizer. The phenomena by which silicon confers protection against *P. capsici* infection and disease development are not fully understood, but our results indicate that mechanisms other than a mechanical barrier to fungal penetration are involved.

Keywords **:** *Capsicum annuum* L., hydroponic culture, *Phytophthora capsici*, potassium silicate

Phytophthora spp. are responsible for crown and root rot and yield reductions of several greenhouse-grown crops (Kaufman et al., 1981). The production of pepper plant (*Capsicum annuum* L.) is particularly hampered by this problem, in which symptoms of the disease can be easily overlooked until the plants suddenly wilt (Favrin et al., 1988). To overcome this problem some producers grow peppers in hydroponic cultures because this system was reported to be disease-free. However, it was soon found that the severity of infection by *Phytophthora* was intensified by conditions in the hydroponic cultures that favor spread of the fungus (Stanghellini et al., 1988). In addition to their inherent self-dispersal mechanism (motility), passive and long-distance dispersal is facilitated by their transport in flowing surface water from nutrient solution. The latter is particularly important in greenhouse industries that employ recirculation of the irrigation water including hydroponic (Stanghellini and Rasmussen, 1994) and ebb-and flow cultural systems (Thinggaard and Anderson, 1995). In the latter industry, preventive control measures include incorporation of highly effective chemical fungicides into the irrigation water. However, the continued use and reliance on fungicides could favor the buildup of these chemicals in the nutrient solution and increase the probability of the development of pesticide-insensitive strains from the target pathogen. These potential problems, in addition to the lack of the registration of effective strategies for disease control. To solve the problem, fungicides are not registered for use in hydrophonically grown crops and *Phytophthora*-resistant cultivars are not commercially available (Zinnen, 1988).

Stanghellini et al. (1984) experimented with the control of *Phytophthora* and *Pythium* spp. in recirculating hydroponic systems by irradiating the nutrient solution with ultraviolet light. Although this approach gave good control of the disease, it has not gained wide acceptance commercially, probably because of the high cost of irradiation. In *in vitro* experiments with *Phytophthora* and *Pythium* spp., Agral, a nonionic surfactant, was shown to disrupt the integrity of the plasma membrane of fungal structures lacking a cell wall, such as zoospores and vesicles (Stanghellini and Tomlinson, 1987). However, commercial-scale experiments have yet to validate this control method on greenhouse crops. An interesting approach to disease control that has gained attention is the amendment of nutrient solutions with silicate. Several workers reported a reduction in severity of powdery mildew on cucumber and other crops with this practice (Adatia and Besford, 1986; Menzies et al., 1991). Silicon (Si) is not considered an essential nutrient for peppers (Ristaino et al., 1994; Stanghellini et al., 1996). The effect of Si on root diseases has never been studied extensively, but there was possibility that it may affect the development of *Phytophthora* infection and result in an increase in the productivity of the plants.

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Considering the current problems with *Phytophthora* diseases in hydroponic cultures and considering the few options offered to producers to overcome these problems, we examined the potential of potassium silicate for the control of *Phytophthora* root rot of pepper plants grown in hydroponic solutions.

Materials and Methods

Pathogen and host. A virulent pepper isolate of *P. capsici*, Pc-17E (race 3) was used for this experiment. A stock culture of the fungus was stored in sterile distilled water, and working cultures were reared on 10% V8 agar medium. Pepper plants, *C. annuum* PBC137 and PBC602 were employed as the moderately susceptible host.

Hydroponic cultural system. All experiments were conducted in a temperature-controlled greenhouse (24 to 32°C) containing 12 recirculating hydroponic units. Each hydroponic unit consisted of two rock wool slabs connected to a common reservoir containing 200 liters of nutrient solution (Fig. 1). Pepper seeds were sown in Grodan rockwool cubes (10 × 10 cm), and developing plants were fertilized daily for 35 days. Five 35-day-old pepper plants were then transplanted onto each rock wool slab (ten plants/hydroponic unit). Distance between plants on individual slabs was 35 cm. There were two separate experiments, and each experiment ranged in duration from 7 to 8 weeks after transplanting. Plants were grown in a base nutrient solution containing, in parts per million, 205 N, 305 K, 27 P, 151 Ca, 45 Mg, 4 Fe, 1 Mn, 0.4 Zn, 0.14 Cu, 0.3 Bo, and 0.055 Mo. The nutrient solutions were prepared with tap water (<10 ppm of Si), and final pH was adjusted to 5.8 with nitric acid. The electrical conductivity ranged from 1.8 to 2.2 mS/cm, depending on the Si concentration. Each tank originally contained 200 L of nutrient solution; the solution was replaced every 2 or 3 weeks. The temperature of the nutrient solution was investigated daily throughout the experiment, and its ranges were between 18 and 24°C. After completion of each experiment, the entire hydroponic system in the greenhouse was dismantled, surface-sterilized in sodium hypochlorite (10%), and reassembled as previously described (Stanghellini et al., 1996).

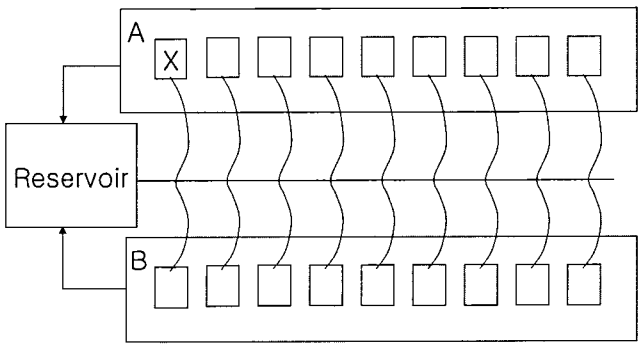

Fig. 1. Schematic of a recirculating hydroponic unit employed to evaluate the efficacy of a potassium silicate in the control of root rot of pepper caused by *Phytophthora capsici*. There were five plants on each side of the unit. Two plants (x) were inoculated with the pathogen.

Amendment of potassium silicate. Experiment 1. Nutrient solutions were supplemented with 1.7 mM (100 ppm = Si+), 3.4 mM (200 ppm = Si++), and 5.1 mM (100 ppm = Si+++) silicate in the form of potassium silicate (Kasil No. 6, 28.5% SiO_2) which was fed to the plants for the duration of the experiment. This experiment comprised five treatments: 1) no Si amendment and no inoculation with *Phytophthora* (Si–P–), 2) no Si amendment and inoculation with *Phytophthora* (Si–P+), 3) Si+P+, 4) Si++P+, and 5) Si+++P+. Each treatment was replicated twice. This experiment was maintained for 12 weeks after the seedlings were transferred to the gullies. Through out the experiment, fruit were harvested and graded according to the standard system applied in Korea (Anonymous, 1991). Root and aerial dry weights were measured after 12 weeks.

Experiment 2. Seeds of two varieties, PBC137 and PBC602, were sown. Nutrient solutions were amended with 1.7 mM (100 ppm) silicate. The four treatments were silicate amendment with (Si+P+) or without (Si+P–) *P. capsici* inoculation and no silicate amendment with (Si–P+) or without (Si–P–) *P. capsici* inoculation. Each treatment comprised two replicates. This experiment was maintained for 12 weeks after the seedlings were transferred to the gullies. The same variables as in experiment 1 were measured.

Inoculations. Inoculum was prepared from one strain of *P. capsici*, Pc-17E (race 3). The strain was grown on V8 juice agar on 9cm petri-dishes for 6 days. Inoculation was conducted as follows: a 7-mm-diameter disk cut from the advancing margin of a 6-day-old culture of the pathogen was placed in contact with the lower roots (immediately below the substrate stem) of one plant on one side (side A) of a hydroponic unit (Fig. 1). The agar disk was removed after 48 h. This method of inoculation permitted us to evaluate pathogen spread within a recirculating hydroponic system subsequent to pathogen colonization and reproduction on a single plant. Treatment included non-inoculated units in which the nutrient solution was amended with a potassium silicate. The presence of *P. capsici* was monitored by taking root and nutrient solution samples every week after the date of inoculation. Root samples were dissected from two or three plants selected at random from both replicates in each treatment, washed thoroughly in distilled water, surface-disinfested in 3% aqueous sodium hypochlorite for 5 min, rinsed for a few minutes in distilled water, blotted dry, then plated onto a selective medium (Jeffers and Martin, 1986) and incubated at 25°C. Presence of *P. capsici* was determined after 7 days of incubation. Identification was based on morphological features (Van Der Plaats-Niterink, 1981).

Scanning electron microscopy (SEM). Small portions (0.2 mm^2) of the fruit epidermis were excised with a sharp razor blade and immersed in 2.5% glutaraldehyde, buffered with 50 mM Na-cacodylate, pH 7.3 for 2 hrs. After two 15 min buffer rinses,

samples were postfixed in 1% osmium tetroxide in 50 mM Na-cacodylate, overnight at 4°C. Samples were rinsed in distilled deionized water, treated with 50 mM lanthanum nitrate for 30 min to preserve the cuticle (Sargent, 1976), dehydrated in ethanol and embedded in Spurr's resin. Ultrathin sections were cut with a razor beam with microtome (Razor blazer's CPD 550) and stained with 2% aqueous uranyl acetate for 20 min, followed by lead citrate for 5 min.

Data analysis. The effects of the treatments were analyzed by ANOVA and means were separated by DMRT ($P \leq 0.05$). The software Super ANOVA (Abacus Concepts, Berkeley, CA) was used for the statistical analysis.

Results

Plant growth. The growth of the Si treated pepper plants was prone to rapid than nontreated plants. The height increased in length from 22 to 27 cm after 10 days of Si treatment (Fig. 2). The lower leaves on the high Si plants were darker green and remained well presented to intercept light efficiently, while the leaves on the low Si plants were more prone to wilting. Basal leaves of the high Si plants were noticeably rough and the petioles particularly rough. Neither the final leaf area nor the fresh weight of the mature leaves (which included the mid vein) was affected by the addition of Si (Table 1). However, the petioles of the leaves on the low Si plants were longer and heavier. There was a small but significant increase in root fresh weight and dry weight by adding Si. The fresh weight per unit area (mg cm-2) of the interveinal laminar tissue of mature leaves, an index of leaf thickness, was greater on plants supplied with additional Si (Table 1). The dry weight per unit area (mg cm-2) of the leaves was also increased by added Si. We also found no interaction between varieties and treatment, data were pooled for further analyses. In terms of fruit produced, at the end of the experiment all fruit had been harvested, and there were no significant differences in the total number among the treatments (Table 1). For all variables

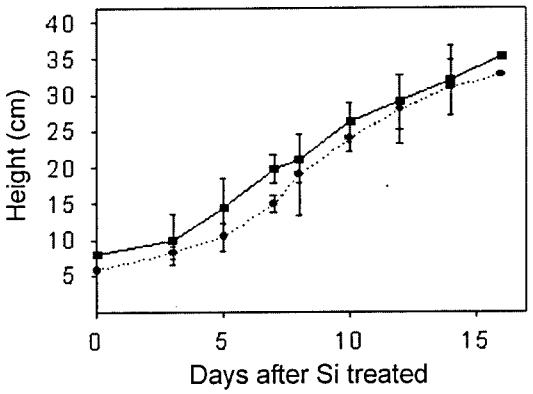

Fig. 2. Growth in length of 'PBC602' pepper over time, from the day of Si treated (day 0). Each point represents the mean length ± SE. The height of plants treated with (Si+, – ■ –) or without Si (Si–, – ● –) was not significantly different.

measured, there was no significant difference between 100- and 200 ppm treatments, and inoculated plants treated with silicate were always as productive as non-inoculated plants (Si–P–).

Mortality of pepper plant in the absence or presence of potassium silicate. All inoculated control plants started showing symptoms of wilting within 2 weeks after inoculation. The experiments were terminated 11 weeks after inoculation because most of the inoculated controls were either dead or had reached an advanced stage of wilting, root decay, and senescence and had stopped producing fruit.

In the absence of potassium silicate, all the remaining peppers (17 plants) within a recirculating hydroponic unit wilted and died within the next 15 days. Sporangia were observed microscopically on roots of the inoculated plants, and the fungus was detected in the nutrient solution by day 5 after inoculation. In contrast, no plant mortality or root infection occurred (Table 2), with the exception of the inoculated plant, in hydroponic units that were amended with the potassium silicate over the duration of the study.

Table 1. Average root fresh weight, dry weight, leaf area and petiole length of peppers, PBC137 and PBC602, grown for 12 weeks in a recirculating nutrient solution amended with different concentrations of potassium silicate and non-inoculated with *Phytophthora capsici*

Treatments[x]	Roots[y]		Twelfth leaf above cotyledons					Number of fruit
	F. wt (g)	D. wt (g)	Laminar tissue		Petiole		leaf	Total/each
			Area (cm^2)	F. wt (g)	Length (cm)	F. wt (g)	(mg cm^{-2})	
Si– P–	41.7ab[z]	19.5a	44.4a	4.9b	2.6ab	5.7a	0.6ab	7.5a
Si– P+	39.2b	17.8b	42.3a	5.4ab	2.5b	5.9a	0.5b	6.7a
Si+ P+	49.9a	19.7a	46.5a	6.3a	3.1a	5.8a	0.8a	7.7a
Si++ P+	55.5a	20.4a	46.4a	6.3a	2.9a	4.9a	0.9a	7.7a

[x] Si– = 0 ppm, Si+ = 100 ppm, and Si++ = 200 ppm of potassium silicate; P– = non-inoculated and P+ = inoculated with *P. capsici*. Each treatment represents an average of 24 plants.

[y] Sampled in early September. Each value is the mean of eight independent determinations.

[z] Means in the same column followed by a different letter are significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Table 2. Percent mortality of pepper plant after inoculation of a single plant on one side (side A) of a two-sided recirculating hydroponic unit with *Phytophthora capsici*

Treatments[z]	Side	Weeks after inoculation[y]		
		1	2	3
Si– P–	A	0.0	0.0	0.0
	B	0.0	0.0	0.0
Si– P+	A	5.6	27.7	100
	B	0.0	61.1	100
Si+ P+	A	5.6	5.6	5.6
	B	0.0	0.0	0.0

[z]Mean percent mortality from two trials

[y]Pepper plants were 75 and 69 days old at time of inoculation. there were nine plants on side A and nine plants on B of each hydroponic unit.

In the other treatments, all plants went through the production period without obvious problems with disease. Their root systems were well developed and did not show symptoms of decay. A visual comparison between inoculated plants grown with (Si+P+) and those grown without (Si–P+) potassium silicate showed that the benefits of using potassium silicate against *P. capsici* were unequivocal (Fig. 3). Interestingly, despite the healthy appearance of the Si+P+ plants, *P. capsici* was reisolated from all solutions and root samples taken from that treatment.

Depositon of silicate. In Si treated plant, the root structure was examined in more details by light and scanning electron microscopy, using sectioned material. Some cells had vaculoes filled with dark, osmophilic product, which suggests that these are glandular type and its more resistant to the pathogen. In -Si treated root, epidermis was formed a thin, smooth coating over the cells. In contrast, the +Si epidermis had distinct electron dense particles in the thick epicuticular wax covering cells (Fig. 4B, C). These particles are identical to the 'Silica bodies' which have been described in a wide range of plants (Kaufman et al., 1981).

Fig. 3. Effect of potassium silicate on control of *Phytophthora capsici* infections on pepper plants grown in a recirculating nutrient solution system. Plants on the right (Si–P+) were grown in a basic nutrient solution inoculated with *P. capsici*; plants on the left (Si+P+) were grown in a nutrient solution inoculated *P. capsici* and amended with 100 ppm of silicate.

The silica bodies had inherent electron density, and therefore can be visualized in unstained material (Fig. 4C). The silica bodies were always found embedded in the epidermis wax in the +Si treated root sells.

Discussion

The use of potassium silicate (or metasilicate) as an amendment to nutrient solution is currently gaining wide acceptance among pepper and cucumber producers (Stanghellini et al., 1996). However, while some producers have reported a yield increase as a result of its use, several have failed to report beneficial effects. Our results showed no significant differences between Si+P– and Si–P–

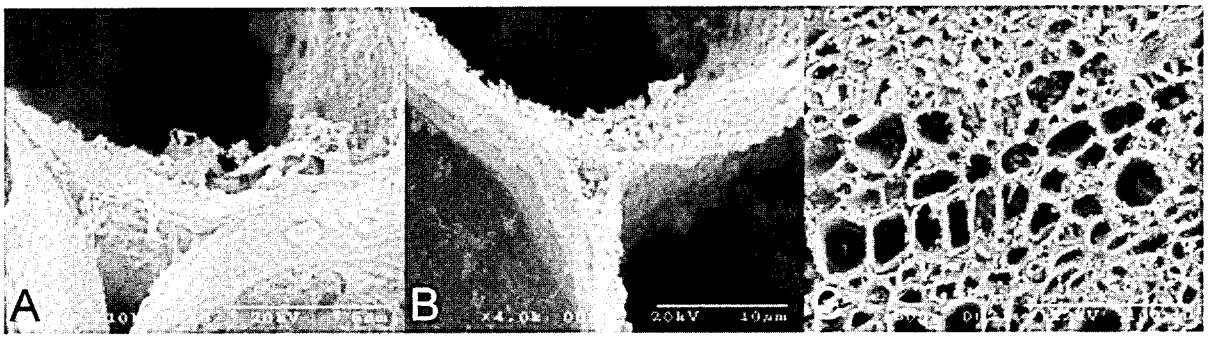

Fig. 4. Scanning electron micrographs of pepper root and basal stem epidermis sections transverse to plane of root surface. Samples were treated with lanthanum during dehydration to help preserve cuticle. **A**, Root from –Si plant (Bar = 5 μm). **B**, Root from +Si plant, Cell wall darkly deposits on inner surface of wall. (Bar = 10 μm). **C**, Stem from +Si plant, Silicate bodies aggregated in the cell wall (Bar = 100 μm).

treatments. The mechanisms responsible for a growth stimulation by silica are unclear (Jones and Handreck, 1967). Horst and Marschner (1978) and Marschner et al. (1990) suggested that these effects could be related to increased tolerance to high manganese concentrations or to an imbalance in phosphorus and zinc supply. Yoshida et al. (1969) and Adatia and Besford (1986) concluded that improvement in plant growth resulted from a higher mechanical stability of stems and leaves and thus better light interception and higher photosynthetic capacity. From a nutritional standpoint, however, no reports link Si with nutritional properties (Voogt, 1989). On the other hand, many other reports are in agreement with the idea that the beneficial effect of Si is related to an increased resistance to fungal infection (Menzies et al., 1991; Miyake and Takahashi, 1983a; Miyake and Takahashi, 1983b). According to our results, the former explanation seems implausible, since the silicon-free nutrient solution used was not deficient in phosphorus or zinc and because no sign of manganese toxicity was observed on control plants. To our knowledge, this is the first study to report the beneficial effect of Si on the control of root rot diseases caused by *P. capsici*. Several other studies have associated the presence of Si with a decreased severity of disease caused by foliar pathogens. For instance, supplemental silicate treatments resulted in a significant reduction of powdery mildew infection in barley (Jiang et al., 1989), wheat (Leusch and Buchenauer, 1989), and cucumber (Menzies et al., 1991). A beneficial effect of Si also was reported for Helminthosporium blight (Akai, 1953) and for blast disease of rice caused by *Pyricularia oryzae* (Aleshin et al., 1986). Most of these studies reported the effect of Si on pathogenic fungi that infect aerial parts of different monocotyledonous and some dicotyledonous plants, where silica ($SiO_2 \cdot nH_2O$) accumulation is very high. By contrast, no information is available on the effects of Si on the receptibility of Si non-accumulating plant tissues to fungal pathogens. Our results strengthen the observations reported by Miyake and Takahashi (1983b) concerning Si effects on Fusarium wilt disease in naturally infected cucumber; an application of silicate fertilizer considerably reduced the number of wilted plants. Miyake and Takahashi (1983a) and Adatia and Besford (1986) reported a consistent decline in silica content of tissues of cucumber plants progressing from leaves to roots. Although Si is present at relatively low amounts in cucumber roots, our results provide further evidence that it can reduce the severity of fungal infections. These results are in line with the idea that the total Si in plant tissues is not as important as the available, mobile Si at the time of infection. Samuels et al. (1991), studying the mobility and deposition of Si in cucumber leaves by means of scanning electron microscopy and energy dispersive X-ray analysis, observed that once deposited in cucumber tissues, Si could not be remobilized by the plant. They found that Si deposited in the leaves of cucumber plants previously treated with 100 ppm Si nutrient solution was not available to enhance disease resistance 24 hr after they stopped feeding the plants with the Si amended solution. These reports support the recommendation that Si applied in hydroponic nutrient solutions should be used on a constant basis at 100 ppm from transplanting onward. Intermittent Si supply may not control fungal infections effectively. The mode of action of foliar-, feed-, and soil-applied Si in reducing the severity of fungal infection is not known. However, from the results of our experiments, it appears that Si acts systemically in the outcome of the host-pathogen interaction in that it is able to enhance resistance in the aerial as well as the underground parts of the plant. The present study suggests that control of *P. capsici* could not be attributed to a direct detrimental effect of potassium silicate on zoospore germination of *P. capsici* because the pathogen was reisolated from all the Si-amended solutions, from the first week of inoculation to the end of the experiments. However, the mechanisms of pathogen suppression by the host are not known. At this time, two hypotheses have been proposed: 1) Si accumulation in plant cell walls inhibits fungal growth and penetration of plant tissues (Carver et al., 1987) and 2) Si stimulates and changes the timing of host natural defense mechanisms, e.g., phenolic production (Menzies et al., 1991). From the results of the present study, the first hypothesis appears less probable, since *P. capsici* was reisolated from all root samples of Si-treated plants. These two hypotheses may not be mutually exclusive. Therefore, ultrastructural and biochemical studies are needed to clarify the beneficial role of Si in the interaction of *P. capisici* with pepper plants.

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