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Foliar applications of silicon fertilisers inhibit powdery mildew development in greenhouse cucumber

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Abstract

Powdery mildews (*Podosphaera xhantii*) remain among the most important pathogenic fungi in greenhouse vegetable production, and are the cause of extensive fungicide use in European horticulture. Although the underlying mechanisms are not known, the ability of soluble silicon (Si) to reduce the impact of powdery mildew infection on several greenhouse crops has been amply described. Still, the use of Si in greenhouse production is very limited. One major reason for this is the challenge associated with the standard form of application, which is amendment to soil or nutrient solutions. Foliar applications could represent a more practical means of application, but have not been adequately tested. In the present study, foliar applications of two commercially available silicon (Si) based products were evaluated for their effect in reducing powdery mildew development in commercial greenhouse cucumber (*Cucumis sativus*) production. The products Carbon Silpower® and Carbon Defense® were supplied to the mildew susceptible cultivars 'Euphoria' and 'Jessica', one or two times per week in two different concentrations. All treatments resulted in a significantly lower mildew infection development than untreated control plants (water only). In general, more frequent applications improved the fungal inhibitive effect. The most effective treatment was a high concentration of Carbon Silpower solution (with 56 mM Si) applied twice per week, reducing the disease severity by as much as 87% compared to the control.

Key words: *Cucumis sativus*, infection, powdery mildew, *Podosphaera xhantii*, *Sphaerotheca fuliginea*, Silicon, Si, foliar application, alternative disease management.

Introduction

Agriculture worldwide is focusing on eco-friendly and sustainable production in all sectors. Simultaneously, increasing national restrictions regarding the use of pesticides have led to a search for alternative methods of disease control. Foliar sprays containing silicon (Si) are being developed as a potential prophylactic treatment against fungal infection in greenhouse cultivars. Although Si is not listed among the essential elements for plant growth, its beneficial role in plant nutrition is well established [1-3]. Silicon is absorbed by plants as monosilicic acid (H_2SiO_4), and accumulates to higher concentrations in leaf epidermal cells than in any other cell type [4]. Beneficial effects of Si have been detected in plants exposed to both biotic and abiotic stresses [5-8], as well as in plant disease management [9-13]. However, and despite the documented beneficial effects of Si, its use in plant production is very limited. The principal reasons for this are the lack of established products on the market as well as the challenges associated with the standard form of application, which is supplying Si as an amendment to soils or nutrient solutions. Limited miscibility with other products and clogging of drip irrigation equipment has been reported problems when using Si fertilisers [14] (and Norwegian growers). Thus, there is commercial interest to develop more user-friendly means of application, and foliar application could be an attractive alternative.

Application of silicon (Si) has reduced the intensity of fungal diseases on important crops such as rice [14-17], grape [18], wheat [19, 20], zucchini [21] and cucumber [12, 21-25]. The interpretation of the mechanisms underlying the Si-induced resistance remains inconclusive. While some studies indicate that Si acts by providing a physical barrier in the epidermis, preventing penetration of fungal hyphae [17, 18, 23, 24], more recent studies link the enhanced resistance of Si-treated plants to specific plant defence mechanisms [8, 12, 16, 20, 25]. Powdery mildews (Erysiphaceae) remain among the most important pathogenic fungi in greenhouse vegetable production, and are the cause of extensive fungicide use in European horticulture [26, 27] (Vold, J. M. and Grimstad, S. pers. comm.). Cucumber is one of the most studied species with regard to Si amendments, and several studies have proven the effectiveness of Si in reducing the severity of powdery mildew (*Podosphaera xhantii*) on greenhouse cucumber [7, 12, 21]. However, among previously reported results on Si-induced inhibition of fungal development, the focus has been on application to roots and only limited information is available on foliar Si applications.

Thus, the objective of the present study was to determine the effect of two foliar Si-amendments, under commercial development, on powdery mildew infection development in greenhouse grown cucumber in Norway.

Materials and Methods

Plant material, treatments, and disease development measurements: Two experiments were carried out in an acrylic greenhouse at Frostagrønt AS, which is the largest commercial grower of cucumber in Norway, located at 63°30‘N. Seeds of *Cucumis sativus* cvs. ‘Euphoria’ (Experiment 1) and ‘Jessica’ (Experiment 2) were sown in rock wool cubes. After three weeks of growth, when plant height was 40-50 cm, the plants were transplanted to peat boards in a greenhouse at 25/20°C and a photoperiod of 20 h with a photosynthetic photon flux of ≈300 $\mu mol\ m^{-2} s^{-1}$. Fertilisation was provided through drip irrigation with a commercial complete nutrient solution (Tailor Made Superex, Kekkilä, Finland). The electrical conductivity of the feeding solution ranged from 3.0 to 3.5 $mS\ cm^{-1}$ for the first four weeks and was gradually subsequently lowered to a range of 2.0-2.5 $mS\ cm^{-1}$, depending on solar irradiance.

The treatments consisted of two different commercially available foliar sprays (Floratine Biosciences, Memphis, USA) (Table 1), supplied in two concentrations. Treatments were applied to upper leaf surfaces, to the drip point, as a fine mist using an electric aerosol spray container (Teknoma XL 200). Plants were treated in groups of three (Experiment 1) or five (Experiment 2) plants growing in the same peat board. Each group was separated by two untreated buffer plants to equalize infection pressure.

Since the experiments were performed in a large-scale commercial greenhouse, plants were not inoculated with powdery mildew. The selected greenhouse experienced frequent mildew infections, and at the start-up of both experiments, the first signs of natural infection had already appeared on the plants. The treated plant location was randomly arranged in the greenhouse.

In both experiments, the severity of mildew infection was scored for each treatment group of three or five plants on a scale of 0 – 5, denoting proportions of infected leaf area of 0, <5 %, 5-10 %, 10-30%, 30-50% and >50%, respectively. Data were analyzed by analysis of variance, with a term for individual treatment group effects to control for repeated measures over two or more dates. Although parametric analysis of ordinal data increases the risk of Type I error, this approach allows assessment of both main and interacting effects while controlling for the repeated measures designs of the experiments, and in many cases probabilities were sufficiently low to support robust conclusions.

Experiment 1: Powdery mildew susceptible cucumber cv. ‘Euphoria’ was treated with foliar applications of the Si-based product Carbon Silpower. Two concentrations of Carbon Silpower (solutions with 28 mM and 56 mM Si) and one control with pure water were sprayed on the leaf blade upper surfaces. All leaves on each plant were sprayed and allowed to dry. Plants sprayed once were compared to plants sprayed twice. Spraying was performed on day one and five (the plants receiving one treatment were sprayed only on day one) and disease development was investigated five and ten days after the first treatment. The treatment and experimental setup is shown in Table 2. The treatments were replicated three times with three plants in each treatment group for a total of nine plants per treatment. The data were analyzed as a 3 treatment (control and two concentrations) × 2 spray frequency × 2 date full factorial, with a term for group within treatment and spray frequency to account for repeated measures over the three dates. Treatment × spray frequency × date means were compared using Tukey’s HSD using the experiment wise mean square error.

Experiment 2: Mildew susceptible cucumber plants of the ‘Jessica’ cultivar were supplied with foliar applications of the Si-based products Carbon Silpower and Carbon Defense. Treatments were started three weeks after sowing, on the day before they were transplanted in the greenhouse. Two concentrations of each product (solutions with 28 mM and 56 mM Si, mixed with greenhouse tap water) and one control with only tap water were applied at two frequencies; once or twice per week (Table 2). All leaves on each plant were sprayed and allowed to dry. The treatments were replicated three times with five plants in each treatment group for a total of 15 plants per treatment. Disease development was recorded on day 4, 7, 10, 14 and 17 after the first day of treatment (Table 2). On the fourth day after the onset of treatments, the plants sprayed with both concentrations of Carbon Defense developed symptoms of severe leaf burn. The symptoms worsened by day seven so the Carbon Defense treatments were suspended. Three days later the plants had recovered and the Carbon Defense treatments were continued at reduced concentrations (16 mM and 8 mM solutions). The data were analyzed as a 5 treatment (control and two concentrations of two products) × 2 spray frequency × 4 date full factorial, with a term for group within treatment and spray frequency to account for repeated measures over the three dates.

Table 1. Composition of Si-containing nutrient solutions from Floratine Biosciences.

Carbon Silpower®		Carbon Defense®	
Potassium oxide (K_2O)	12.0%	Potassium oxide (K_2O)	18.0%
Potassium	4.6%	Phosphoric acid (H_3PO_4)	5.0%
Silicon (Si)	3.7%	Silicon (Si)	3.7%
Derived from:		Derived from:	
Potassium silicate		Potassium silicate	
Potassium sulphate		Potassium sulphate	
		Potassium phosphate	

Table 2. Treatments and experimental setup for experiments 1 and 2. Spraying was performed one or two times per week (indicated by x), and infection scores were recorded (r) two times per week during the experiment.

Experiment 1						
Day	1	5	10			
CarbonSil 28 mM	x					
CarbonSil 28 mM	x	x				
CarbonSil 56 mM	x					
CarbonSil 56 mM	x	x				
Scoring	r	r				
Experiment 2						
Day	1	4	7	10	14	17
CarbonSil 28 mM	x		x		x	
CarbonSil 28 mM	x	x	x	x	x	
CarbonSil 56 mM	x		x		x	
CarbonSil 56 mM	x	x	x	x	x	
CarbonDef 28 mM	x		x		x	
CarbonDef 28 mM	x	x	x	x	x	
CarbonDef 56 mM	x		x		x	
CarbonDef56 mM	x	x	x	x	x	
Scoring	r	r	r	r	r	

Results

Experiment 1: Foliar applications of Carbon Silpower significantly reduced powdery mildew infection on greenhouse cucumber plants when measuring the severity of infection on day 5 and day 10 after treatment initiation (Fig. 1 and Table 3). Ten days after the first treatment, the plants receiving two treatments had significantly lower powdery mildew development than the plants given only one treatment per week. Orthogonal comparisons indicate a significant but relatively weak difference between the two concentrations used, but it is worth noting that infection development increased in the second week in all treatments except the higher concentration applied twice, where there was a slight decrease; this effect is reflected in the significant three-way interaction term (Table 3).

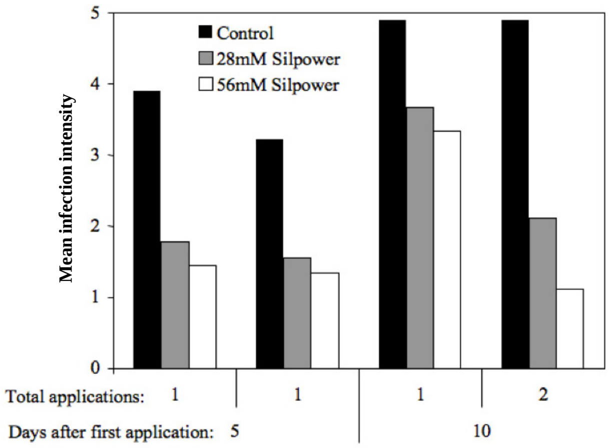

Figure 1. Mean mildew infection severity scores for cucumber plants sprayed with water (control) and 28 mM or 56 mM Carbon Silpower once or twice. Plants were assayed five and ten days after first application. The groups receiving two applications were sprayed a second time on day five after first application.

Experiment 2: All Si treatments significantly reduced powdery mildew severity compared to the DI water control, and more frequent spraying also significantly reduced infection (Table 4). Orthogonal comparisons within the treatment effect (Silpower vs. Defense, then low vs. high concentration within these) found no significant differences between treatments across all dates and the two spraying frequencies. The non-significant two and three-way interaction involving spray frequency indicate that the mean difference attributed to spray frequency (xx intensity score units) was generally consistent across all treatments and dates. As in the previous experiment, the higher concentration of Carbon Silpower kept infection development at or below 5% of leaf area over the 17 days of the experiment, as compared to slightly higher infection development in other Si treatments and more than 50% of leaf area infected in controls at the end of the experiment (Fig. 2). Plants sprayed with Carbon Defense at both 28 and 56 mM Si developed symptoms of leaf burn by three days after spraying, and these symptoms worsened by day six, resulting in the decision to apply the product at reduced concentrations. This apparently relieved leaf burn symptoms but may also explain the somewhat higher infection development in plants receiving Carbon Defense as compared to Carbon Silpower (Fig. 3).

Table 3. Analysis of variance of mildew infection severity scores for cucumber plants sprayed with water (control) and 28 mM or 56 mM Carbon Silpower one or two times, including orthogonal comparisons for the treatment effect.

Source	DF	SS	MS	F	Prob >F
Date	1	34.45	34.45	154.12	<0.0001
Treatment	2	34.36	17.18	76.86	<0.0001
Control vs. Si treatment	1	33.19	33.19	148.49	<0.0001
28 mM vs. 56 mM Si	1	1.16	1.16	5.22	0.041
Date*Treatment	2	1.24	0.62	2.78	0.068
Frequency	1	4.98	4.98	22.28	0.0005
Date*Frequency	1	5.79	5.79	25.89	<0.0001
Treatment*Frequency	2	0.94	0.47	2.11	0.1642
Date*Treatment*Frequency	2	9.24	4.62	20.67	<0.0001
Group [Treatment,Frequency]	12	6.54	0.55		
Error	84	18.78	0.22		
Total	107	217.21			

Table 4. Analysis of variance of mildew infection severity scores for cucumber plants sprayed with water and two commercial foliar sprays at two different concentrations and two frequencies.

Source	DF	SS	MS	F Ratio	Prob > F
Treatment	4	40.31	10.08	41.22	<0.0001
Control vs. all Si Treatments	1	38.98	38.98	159.47	<0.0001
Date	3	28.07	9.36	38.27	<0.0001
Frequency	1	2.51	2.51	10.29	0.0044
Date*Treatment	12	17.85	1.49	6.09	<0.0001
Frequency*Treatment	4	0.67	0.17	0.69	0.6090
Date*Frequency	3	0.47	0.16	0.64	0.5945
Date*Frequency*Treatment	12	2.45	0.20	0.84	0.6145
Group [Frequency,Treatment]	20	4.44	0.22		
Error	60	14.67	0.24		
Total	120	155.87			

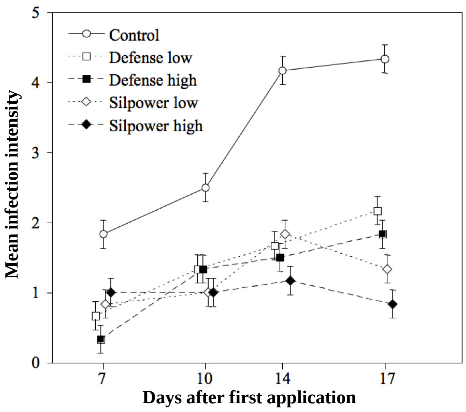

Figure 2. Mean mildew infection development for cucumber plants sprayed with water and two commercial foliar sprays at two different concentrations each. Values are the mean of five treatment groups x two spray frequencies, error bars ± 1 SE based on experiment wise mean square error. Values for the Si treatments offset ±0.1 or 0.3 days for clarity.

Figure 3. Mildew infection on cucumber leaves in Experiment 2. Untreated leaf (left) compared to leaf supplied with foliar sprays of a Carbon Silpower 28 mM solution (right).

Discussion

Despite the differences in cultivar and sampling days between experiment 1 and 2, which were due to practical considerations in the commercial production, the Si-based foliar sprays Carbon Silpower and Carbon Defense were effective in controlling mildew infections. In both experiments, more frequent spraying improved the fungal inhibitive effect. There was also a tendency towards better mildew restraining effect from the higher concentration of Carbon Silpower compared to the lower concentration of the same product (56 mM vs. 28 mM Si). This tendency was not found for Carbon Defense. Moreover, and even though not statistically different, a somewhat higher infection development was recorded among the plants receiving Carbon Defense as compared to Carbon Silpower. When comparing the two products, however, it is important to notice that the plants sprayed with Carbon Defense at both concentrations developed symptoms of leaf burn by three days after the first treatment, resulting in a three day pause in the treatments and continuation of product application at reduced concentrations. This relieved leaf burn symptoms but may also explain the somewhat higher infection development in plants receiving Carbon Defense as compared to Carbon Silpower.

Among previously reported results, some authors included amendments to both soil and leaves [12, 21, 22], while others focused on root supply only [7]. In the studies including foliar Si applications, the results are somewhat contradictory. While Menzies *et al.* [21] demonstrated that foliar applications of potassium silicate were as effective as root applications in reducing powdery mildew on cucurbit leaves, Liang *et al.* [7] found no mildew suppressing effects of foliar applied Si. These differences, however, could be explained by different methods for application and inoculation; while Menzies *et al.* [21] performed the inoculation with fungal conidia on the same leaves that had been supplied with Si sprays, Liang *et al.* [7] inoculated leaves beneath the leaves that had been given foliar Si amendments. Hence Liang *et al.* [7], along with other authors [18, 28], suggest that the antifungal effects from foliar amendments are due to a surface effect or mechanical barrier due to Si accumulation in the leaf epidermis, rather than a systemic effect. In the aspect of a local effect, the strong mildew-inhibiting effect observed in the present study could be attributed to spraying of all leaves. As the product on the leaf surfaces may be gradually washed off by routine mist application, a predominantly local effect could also explain why more frequent applications are more effective than occasional applications.

As an alternative to the mechanical barrier hypothesis, Si in the form of silicic acid could act both locally by inducing defence mechanisms in elicited cells, and by contributing to systemic acquired resistance by enhancing plant signalling [8, 12, 16, 20, 25]. In a whole-transcriptome study of responses to Si treatment and fungal inoculation in Arabidopsis, Fauteux *et al.* [9] found that, while root-applied Si alone has almost no effect on metabolism as indicated by transcript up- or down-regulation, it does actively modulate transcriptional responses to fungal attack. However, there seems to be little evidence that Si can be directly absorbed by leaves [14, 29], thus it is unclear whether foliar application can have similar effects. Some studies indicate that mildew inhibition effects could be caused by phosphate or potassium salts that are components of nutrient sprays with Si [30-32]. However, when studying the effect of foliar applications of potassium silicate, Menzies *et al.* [21] included P and K control treatments, with K and PO_4 sprays equivalent in concentration to that in the Si spray. Since none of the controls reduced the development of powdery mildew, Si appeared to be the active ingredient [21]. Future studies should include chemical analysis with emphasis on Si absorption by leaves, as well as P and K control treatments to account for the possible impact of these additional nutrient components of the applied Si-solutions. Another aspect that deserves closer attention is the comparison of the effect of Si-amendment to leaves versus soil. Nonetheless, with good Si-based products and effective and practical means of application, the use of Si-based foliar sprays for controlling powdery mildew development could permit reductions in the traditional fungicide use.

Conclusions

Foliar application of potassium silicate and other Si-based fertilisers reduces the development of powdery mildew in greenhouse-cultivated cucumber, although the basic mechanisms are not known. Procedures for application and inoculation seem to influence on the results; strengthening the theories suggesting a more local protective effect of foliar applied Si. On the whole, our results strengthen previous reports on the mildew restraining effects of Si amendments to cucumber plants, and represent foliar applications of Si-based fertilisers as an attractive alternative for powdery mildew control in cucumber production.

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