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Comparison of three biological control agents against cucumber powdery mildew (*Sphaerotheca fuliginea*) in semi-commercial-scale glasshouse trials

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Abstract

The effect of three reported biological control agents, *Ampelomyces quisqualis*, *Verticillium lecanii* and *Sporothrix flocculosa*, was tested against cucumber powdery mildew (*Sphaerotheca fuliginea*). Two glasshouse experiments, one in the summer and one in winter/spring were conducted on a semi-commercial scale. In both experiments, a susceptible and a partially resistant cultivar were used. In the second experiment, the additional effect of integration of biological control and silicon amendments to the nutrient solution was also assessed. In both experiments, *A. quisqualis* did not control the disease. *V. lecanii* had a small effect on powdery mildew in the first experiment but not in the second. *S. flocculosa* gave the best control of powdery mildew in both experiments. In the first experiment, weekly application of *S. flocculosa* reduced disease in the partially resistant cultivar to the same level as a treatment in which the fungicides bupirimate and imazalil were each applied once. Addition of silicon in the nutrient solution in a concentration of 0.75 mM reduced disease by 10–16%, averaged over all treatments. There was no interaction between silicon and the biocontrol agents. Yield was recorded in the second experiment and was significantly increased by the fungicide treatment compared to the control in the partially resistant cultivar. Yield in the treatment with *S. flocculosa* was not significantly different from the fungicide treatment in this cultivar. Silicon had no effect on yield in either cultivar.

Introduction

Powdery mildew, caused by *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Polacci, is the most important disease in glasshouse-grown cucumbers in the Netherlands, requiring high inputs of fungicides for control. The intensive use of pesticides is regarded undesirable both for environmental reasons and for the risk of the development of resistance by the pathogen. Furthermore, biological control of insects, which has become common practice, may be adversely affected by powdery mildew fungicides. Therefore, alternative control measures for powdery mildew need to be developed in order to decrease the fungicide input and the dependance on these fungicides.

Biological control of various powdery mildew fungi has been studied quite extensively in the past and was recently reviewed for greenhouse crops (Elad et al., 1996; Menzies and Bélanger, 1996). A few microorganisms have been shown to give moderate to good control under experimental conditions. For instance, *Ampelomyces quisqualis* Ces., a hyperparasite of several powdery mildew fungi, was shown to control *S. fuliginea* in cucumber by a number of researchers (Jarvis and Slingsby, 1977; Philipp and Crüger, 1979; Sundheim, 1982; Sztejnberg et al., 1989). *A. quisqualis* penetrates and feeds on the hy-

phae of the powdery mildew fungus (Hashioka and Nakai, 1980). A strain of *A. quisqualis*, isolated in the Hebrew University of Jerusalem, Israel (Sztejnberg et al., 1989), has been formulated and commercialized by Ecogen Inc. under the trade name AQ10.

The fungus *Verticillium lecanii* (Zimm.) Viégas is another hyperparasite reported to reduce powdery mildew in cucumber both on leaf disks and in glasshouse experiments (Askary et al., 1997; Spencer and Ebben, 1983; Verhaar et al., 1993; Verhaar et al., 1996). In a comparative study in glasshouse-grown cucumber, *V. lecanii* showed better control of powdery mildew than *Sporothrix rugulosa* (Verhaar et al., 1996).

The yeast-like fungus *Sporothrix flocculosa* Traquair, Shaw and Jarvis (syn. *Pseudozyma flocculosa*) (Boekhout, 1995) was effective against both rose and cucumber powdery mildew (Bélanger et al., 1994; Hajlaoui and Bélanger, 1991; Jarvis et al., 1989). In comparative experiments under controlled conditions, *S. flocculosa* showed more rapid colonization of powdery mildew colonies than *S. rugulosa* or *Tilletiopsis washingtoniensis* and was less affected by unfavourable climatic conditions (Hajlaoui and Bélanger, 1991). The effect of *S. flocculosa* is not based on hyperparasitism, but on antibiosis (Benyagoub et al., 1996; Choudhury et al., 1994; Hajlaoui et al., 1992).

Other fungi, such as *Tilletiopsis* spp. have been reported to control powdery mildews in small scale experiments (Urquhart et al., 1994). However, the performance in glasshouse trials has been disappointing, probably due to low humidity conditions (Hijwegen, 1992).

The efficacy of biocontrol agents depends on the climatic conditions in the crop. Powdery mildew fungi can thrive under dry conditions, whereas most biocontrol agents require relative humidities above at least 70% (Hajlaoui and Bélanger, 1991; Phillip and Helstern, 1986). Furthermore, the rate of development of the powdery mildew may influence the reduction achieved by biocontrol agents, especially in the case of hyperparasites. This means that the efficacy of biocontrol agents may differ from season to season, from cultivar to cultivar and may be influenced by other control measures.

The objective of this work was to compare the efficacy of *A. quisqualis*, *V. lecanii* and *S. flocculosa* on *S. fuliginea* on glasshouse-grown cucumber under semi-commercial conditions. In order to test the robustness of the biocontrol agents, experiments were run in a summer crop and a winter/spring crop of cucumber, both with a susceptible and a partially resistant cultivar. In the second experiment, the additional effect of integration of biological control and silicon amendments to the nutrient solution was also assessed. The objective was to determine if silicon amendments, reported to reduce the rate of development of powdery mildew (Adatia and Besford, 1986; Menzies et al., 1991; Dik and Voogt, unpublished results) influenced the performance of the biocontrol agents.

Materials and methods

Plant material

Long English cucumber plants (*Cucumis sativus* L.) of a susceptible cultivar (Jessica in exp. 1, Ventura in exp. 2) and a partially resistant cultivar (Flamingo) were grown in a commercial nursery and transplanted at the four to five leaf stage. The plants were grown in rockwool slabs and trained using the umbrella system (Jarvis, 1992). Fruits were harvested three times per week. Insects were controlled biologically.

Climate regime

Heating temperature in the glasshouse was set at 21–22 °C. Ventilation temperature was set at 0.5 °C above the heating point. Screens in the top of the glasshouse were closed from sunset until sunrise, in order to prevent heat loss and to increase relative humidity during the night. Extra CO_2 was added to each compartment. Data on temperature, relative humidity and vapour pressure deficit (VPD) were collected in each compartment at one-minute intervals. Averages of 60 min were stored in a VAX mainframe computer (Digital, Utrecht, The Netherlands).

Supply of water, nutrients and silicon

The plants were grown in rockwool with reuse of drainage water. The nutrient solution was supplied by means of trickle irrigation. The amount was automatically adjusted to the irradiation level. Approximately 30% of the nutrient solution supplied was drained off and reused. The basic composition of the nutrient solution was 1.0 NH_4, 6.5 K, 2.75 Ca, 1.0 Mg, 11.75 NO_3, 1.0 SO_4 and 1.25 H_2PO_4 in mM and 15 Fe, 10 Mn, 5 Zn, 25 B, 0.75 Cu and 0.75 Mo in μM. The EC in the root environment was kept between 3.0 and 3.5 $dS.m^{-1}$ and the pH between 5 and 6. In experiment 2, additional Si in a concentration of 0.75 mM was added to half of the plots as potassium metasilicate (9.1% Si, 25.4% K, Sikal, Hydro Agri, Vlaardingen,

Table 1. Dates of applications of treatments for the two cultivars in both experiments

Treatments	Experiment 1 (1994)		Experiment 2 (1995)	
	Susceptible cv.	Resistant cv.	Susceptible cv.	Resistant cv.
Control[a]	June 14,20,27, July 5	June 14,20,27, July 5,13,19	March 3,14,21,28	March 3,14,21,28, April 5,11,19,26, May 3
A.quisqualis	June 14,20,27, July 5	June 14,20,27, July 5,13,19	March 3,14,21,2,	March 3,14,21,28, April 5,11,19,26, May 3
V.lecanii	June 14,20,27, July 5	June 14,20,27, July 5,13,19	March 3,14,21,28	March 3,14,21,28, April 5,11,19,26, May 3
S.flocculosa	June 14,20,27, July 5	June 14,20,27, July 5,13,19	March 3,14,21,28	March 3,14,21,28, April 5,11,19,26, May 3
Fungicide[b]	June 20 (bupirimate)	June 20 (bupirimate), July 13 (imazalil)	March 17 (bupirimate), March 30 (bitertanol)	March 30 (bitertanol), April 18 (bitertanol)

[a] in exp. 1: Tween 80 and paraffin oil, in exp. 2: paraffin oil; [b] concentrations of fungicides were 200 ml per 100 l for bupirimate (Nimrod), 100 ml per 100 l for bitertanol (Baycor) and 25 ml per 100 l forimazalil (Fungaflor).

The Netherlands), for which nitric acid was added in a molar ratio of 2 mol H^+ to 1 mol Si to adjust the pH. This resulted in plots with no additional silicon (Si^-) and plots with silicon (Si^+). The increase in K and N supply by the Si and nitric acid application was equally settled by reduction of the K and N supply by the fertilisers. Drainage water was analysed every two weeks for macro- and micro- elements, Si, EC and pH. If necessary, adjustments to the basic composition of the nutrient solution were carried out.

Biocontrol agents and preparation of suspensions

The biocontrol agents tested in both experiments were prepared as follows. *V. lecanii* strain F88.1 was supplied by Verhaar as a fresh liquid suspension and was diluted to $5 * 10^6$ spores ml^{-1} in 0.3% light white oil (Sigma). *A. quisqualis* was used as the formulated product AQ10, provided by Ecogen Inc. (Langhorne, PA, U.S.A.). It was suspended at a rate of 6 g l^{-1} in 0.05% Tween 80 in exp. 1 and in 0.3% light white oil (Sigma) in exp. 2. *S. flocculosa* was provided by Bélanger as a formulation of dry spores. It was suspended in water with 0.02% Aqua Aid (Ken Crowe Ltd., Montreal, Canada), stirred in a blender and diluted in 0.3% light white oil (Sigma) to a final concentration of ca. $1 * 10^6$ colony forming units (CFU) ml^{-1}. The oil, Aqua Aid and Tween 80 amendments were used to enhance survival of the biocontrol agents and to improve homogeneous distribution of the spray solution on the leaves.

Inoculation with powdery mildew

Cucumber plants cv. Jessica were grown in a separate small glasshouse compartment and infected with *Sphaerotheca fuliginea*. Spores were blown off the leaves 36 h before the leaves were used for inoculation of the large glasshouse experiments, in order to ensure that all the spores in the suspension were fresh and of the same age. The source leaves were picked and washed in water. The spore concentration was assessed with a haemocytometer and adjusted to 100 spores ml^{-1}. Within two hours of suspending the spores in water, leaf 5 of all the plants in the glasshouse was inoculated with 5–10 ml per leaf. Floors in the glasshouse were wetted and inoculation took place late in the day in order to ensure sufficiently high relative humidity. Inoculation took place 6 days after planting in exp. 1 and 38 days after planting in exp. 2. Inoculation dates were chosen according to the expected first natural infection by powdery mildew in each season.

Experimental design and treatments

Both experiments were carried out in a glasshouse with ten compartments of 156 m^2 each, five compartments on each side of a corridor. The first experiment was planted on June 2, 1994, and the second experiment on January 17, 1995. One half of each compartment was planted with the susceptible cultivar, the other half with the partially resistant cultivar. Plant density was 192 plants per compartment in exp. 1 and 240 plants per compartment in exp. 2.

In both experiments, five treatments were applied, each replicated in two compartments: 1. control treatment; 2. fungicide according to commercial practice (Table 1); 3. *V. lecanii*; 4. *A. quisqualis*; 5. *S. flocculosa*. In exp. 1, the control consisted of two treatments, each applied to half the plants of each cultivar, i.e.

Tween 80 (0.05%) as control for *A. quisqualis* and light white oil (Sigma, 0.3%) as control for the other biocontrol agents. In exp. 2, the control consisted only of the oil (0.3%), since in this experiment *A. quisqualis* was mixed with paraffin oil instead of Tween 80. All treatments except the fungicide treatment were applied weekly. Spraying dates are shown in Table 1. The treatments were applied with a 10-l knapsack sprayer (Gloria 172RT, Gloria - Werke, Wadersich, Germany) at 3 atm. and a rate of 1500 l ha^{-1} for full-grown plants. A different sprayer was used for each treatment to prevent cross contamination. Applications were done during the last 4 h before sunset (evening in exp. 1, afternoon in exp. 2) in order to prevent excessive drying of plants after application and subsequent desiccation of biocontrol agents.

Each compartment contained two blocks of six different nutrient solutions, one complete block per cultivar. In exp. 1, all nutrient solutions were the same and the experiment was designed as a randomized complete block with two compartments per treatment as replicates. In exp. 2, silicon in the form of potassium metasilicate was added to three of the six nutrient tanks as described above. The experiment was set-up as a split-plot experiment with two randomized blocks with five treatments and three replicates of two nutrient solutions per compartment per cultivar.

Disease assessment

Powdery mildew infection was assessed on 12 plants per compartment per cultivar. Each nutrient tank supplied one plot of four rows of five plants per cultivar. In both experiments, the two middle plants of the six plots per cultivar were used for disease assessments. In exp. 2, six of the 12 plants were from plots with extra silicon and six plants from plots with standard nutrient solution. Infection was assessed as percentage leaf area covered with powdery mildew on all the leaves by position, with leaf 1 as the first full leaf. A set of drawn examples of which the exact percentage infected area was calculated with a semi-automatic image analyser (Videoplan, Carl Zeiss B.V., Weesp, The Netherlands) was used to calibrate assessments. Dead leaves and other diseases were also recorded.

The average percentage dead and diseased leaf area was calculated per plant and added to give the percentage not-green leaf area. Since severe powdery mildew infection can result in death of entire leaves, percentage not-green leaf area gives a better estimate of powdery mildew severity than percentage diseased leaf area. The percentage not-green leaf area was also calculated for three different leaf layers separately, i.e. a base leaf (leaf 6 in both experiments), a leaf in the middle of the canopy (leaf 13 in exp. 1, leaf 10 in exp. 2) and a leaf at the top of the canopy (leaf 20 in exp. 1, leaf 14 in exp. 2), in order to establish the effect of the biocontrol agents on different parts of the canopy. Data for whole plants and per leaf layer were averaged for the sampled plants per plot.

The first disease assessment was done before the first application of the treatments in order to assess possible differences between compartments. Disease was assessed twice per week in the susceptible cultivar and once per week in the partially resistant cultivar.

Assessment of silicon in the nutrient solution and in the leaves

On days 52, 83 and 113 after planting in the fungicide treatment in exp. 2, 10–15 leaves were sampled at different levels in the canopy (base, top and side shoots). The leaves were dried and ground and dry weight was assessed. Silicon was extracted according to Walinga et al. (1989) and measured by atomic absorption at 251.6 nm in a nitrous oxide-acetylene flame.

Populations of biocontrol agents

In both experiments, populations of the biocontrol agents were assessed at different times after spraying, on different leaf layers and cultivars to determine survival on the leaves. For *V. lecanii*, 12 leaf disks per cultivar per replicate were sampled at two heights in the canopy and incubated at high humidity. Subsequent growth of the fungus was observed using a stereo microscope. In exp. 2, germination of *V. lecanii* spores both before and after incubation at high humidity, was assessed microscopically for 100 spores per leaf disk.

On several sampling dates in exp. 1, leaf disks were examined microscopically for the presence of *A. quisqualis* and parasitization of the powdery mildew. Colonization of powdery mildew by *A. quisqualis* was assessed on whole plants on one sampling date in exp. 2 as percentage leaves on which powdery mildew was visibly colonized by the hyperparasites in three classes: no parasitization, $< 50\%$ of powdery mildew parasitized, and $> 50\%$ parasitized.

Population densities for all three biocontrol agents were assessed by sampling leaf disks at different heights in the canopy, washing the samples in sterile Tween 80 (0.01%) and dilution plating on PDA plates. Plates were counted after incubation at 21 °C for 2–8 days and the population density, expressed as

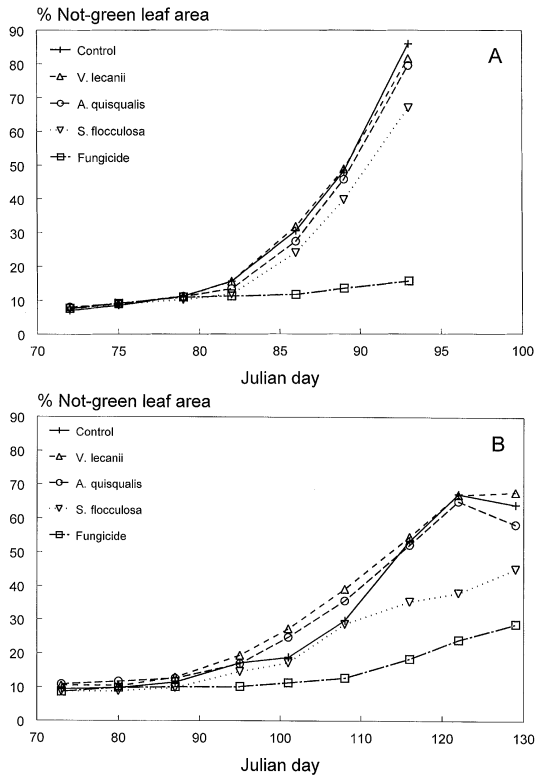

Figure 1. Disease progress of powdery mildew on cucumber in exp. 2 for all treatments without silicon in the nutrient solution for the susceptible cultivar Ventura (A) and the partially resistant cultivar Flamingo (B).

CFU cm^{-2}, was calculated. Samples were taken both in the compartments where the biocontrol agents were sprayed and in the control compartments to assess possible cross-contamination.

Yield

The number, weight and quality of the harvested fruits were recorded. Quality was recorded as first or second class, depending on size, colour and shape of the fruits using the same criteria as the auction. Total weight and number of fruits per plant and the percentage fruits of first class quality were calculated at the end of the experiments. In exp. 1, yield was assessed for one row of 12 plants per cultivar per compartment. In exp. 2, yield was assessed for 5 plants for each of the six nutrient plots for each cultivar in each compartment.

Statistical analysis

The Area Under the Disease Progress Curve (AUDPC) for percentage not-green leaf area was calculated, both for whole plants and for the three different leaf layers. Exp. 1 was analysed as a complete block, exp. 2 as a split-plot experiment with the treatments as main factor and Si level in the nutrient solution as factor within the treatments. AUDPC values and yield data were subjected to analysis of variance followed by Fisher's protected LSD test, at P = 0.05.

Percentage inhibition in AUDPC compared to the control treatment was calculated both for whole plants and for the three separate leaf layers. Linear regression analysis was performed on the percentage inhibition in AUDPC against leaf layer for each biocontrol agent and cultivar combination. Differences in elevations and slopes of the regression lines were analysed according to Snedecor and Cochran (1980) at P=0.05. All statistical analyses were done with Genstat (Genstat 5 Committee, 1992).

Results

Climatic conditions in the glasshouse

In the summer of 1994 (exp. 1), conditions in the glasshouse were warm and dry. The 12 h average temperatures ranged between 19 and 32 °C and 12 h average relative humidity (R.H.) was between 30 and 80%, resulting in VPD's between 0.4 and 3.0 kPa.

In exp. 2 (spring 1995) 12 h average temperatures ranged between 19 and 26 °C. The 12 h average R.H. was between 55 and 90%, resulting in VPD's between 0.2 and 1.5 kPa.

In both experiments, differences between compartments were within 0.3 °C and 0.1 kPa.

Powdery mildew epidemics

Inoculation of leaf 5 resulted in a few powdery mildew colonies on this leaf within one week in both cultivars in exp. 1 and the susceptible cultivar in exp. 2 and within two weeks in the partially resistant cultivar in exp. 2. Before the first application of the biocontrol agents, no differences were observed among treatments in both experiments. In both seasons, the experiments were terminated earlier for the susceptible cultivar than for the partially resistant one by spraying the susceptible plants in all treatments with a fungicide (imazalil in exp. 1, bupirimate in exp. 2) when disease became very severe. Since the plants of both cultivars were grown in the same compartments, the susceptible plants were providing an unnaturally high inoculum level for the partially resistant plants. This application of chemical fungicide to all susceptible plants was done on July 13 in exp. 1 and on

Table 2. AUDPC for powdery mildew, expressed as percentage not-green leaf area in the susceptible cultivar

Treatment	AUDPC [a] (% - days)		
	Exp. 1[b]	Exp. 2[b]	
		- Si	+ Si
Control Tween	289	*	*
Control paraffin oil	425	582	468
A. quisqualis	300	582	521
V. lecanii	348	545	496
S. flocculosa	306	477	383
Fungicide	141	241	184
LSD[c]	114	64	64

[a] AUDPC = Area Under the Disease Progress Curve.
[b] The total number of days in the disease assessment period was 27 in exp. 1 and 21 in exp. 2.
[c] LSD = Least Significant Difference at P =0.05.

Table 3. AUDPC for powdery mildew expressed as percentage not-green leaf area in the partially resistant cultivar

Treatment	AUDPC [a] (% - days)		
	Exp. 1[b]	Exp. 2[b]	
		- Si	+ Si
Control Tween	1206	*	*
Control paraffin oil	1517	1688	1572
A. quisqualis	1071	1753	1638
V. lecanii	1072	1618	1481
S. flocculosa	787	1226	1204
Fungicide	522	742	685
LSD[c]	503	221	221

[a] AUDPC = Area Under the Disease Progress Curve.
[b] The total number of days in the disease assessment period was 45 in exp. 1 and 56 in exp. 2.
[c] LSD = Least Significant Difference at P = 0.05.

April 4 in exp. 2, 35 and 39 days after inoculation with powdery mildew, respectively.

The effect of the biocontrol agents and silicon on powdery mildew and other diseases

In general, the development of the epidemic was delayed by *S. flocculosa* in both experiments and by *V. lecanii* in exp. 1. This was especially noticeable in the partially resistant cultivar. Eventually however, disease reached a very high level in all treatments except the fungicide treatment. Disease progress in all treatments without silicon in exp. 2 is shown in Figure 1. In exp. 1, there was a distinct inhibitory effect of Tween 80 on powdery mildew development in both cultivars compared to paraffin oil. Compared to Tween 80, *A. quisqualis* did not reduce powdery mildew severity. In exp. 2, *A. quisqualis* mixed with paraffin oil instead of Tween 80, had no effect on powdery mildew in either cultivar.

The Area Under the Disease Progress Curve (AUDPC) for not-green leaf area is given for both experiments in Tables 2 and 3. *V. lecanii* and *A. quisqualis* did not cause a significant reduction in the AUDPC in either experiments compared to the appropriate control. Only *S. flocculosa* significantly reduced the AUDPC. For the partially resistant cultivar, the AUDPC in the *S. flocculosa* treatment was not significantly different from the fungicide treatment in exp. 1.

The effect of silicon in exp. 2 was significant but not very strong (Figure 2). On average for all treatments, addition of silicon gave 16 and 11% reduction of powdery mildew for the susceptible and the partially resistant cultivars, respectively. There was no interaction with the biocontrol agents, indicating that reduction of powdery mildew by biocontrol and silicon act independently and the biocontrol agents are not influenced by the silicon in the leaves.

The percentage inhibition obtained by the treatments compared to the control is shown for both cultivars for 1994 and 1995 without silicon and 1995 with silicon in Figure 2. The effect of all three biocontrol agents was stronger on the partially resistant cultivar than on the susceptible cultivar, especially in exp. 1. For most cultivar/treatment combinations, the effect without silicon was stronger in 1994 than in 1995. In 1995, silicon increased inhibition for all treatments.

In exp. 1, the inhibition of powdery mildew was clearly correlated to the position of the leaves. For *V. lecanii* and *S. flocculosa*, the percentage inhibition in exp. 1 compared to the control is plotted against leaf layer in Figure 3. *A. quisqualis* showed no control in any of the leaf layers analysed. Better control was achieved by *V. lecanii* and *S. flocculosa* on the lower leaves than on the top leaves. For both these biocontrol agents, the slopes of the regression lines were significantly different from horizontal. No significant differences between slopes of regression lines occurred among the cultivar- treatment combinations. In exp. 2, only *S. flocculosa* showed an effect on powdery mildew. The inhibition achieved in this experiment was not significantly influenced by leaf layer, indicating that the climatic conditions in exp.

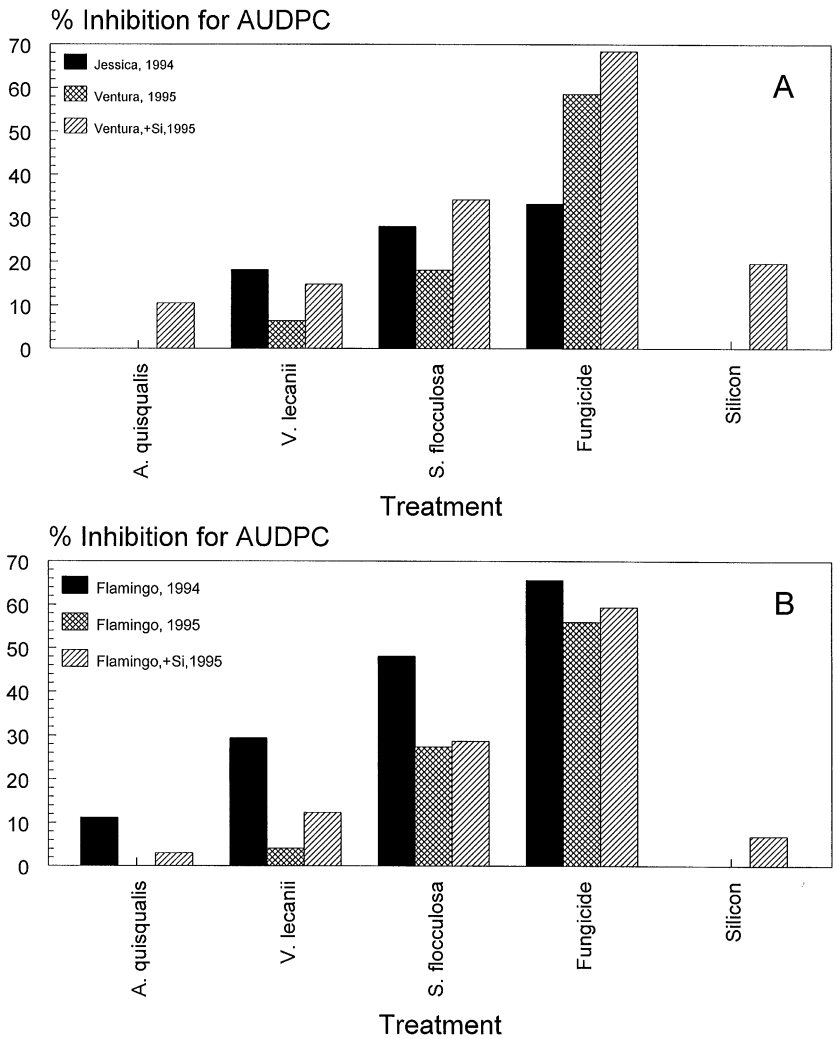

Figure 2. Percentage inhibition in the Area Under the Disease Progress Curve (AUDPC) of cucumber powdery mildew compared to the control treatments in the susceptible cultivars (A) and the partially resistant cultivar (B) in two experiments.

2 were not negatively influencing the performance of *S. flocculosa* on the upper leaves.

In exp. 1, infection by *Pythium aphanidermatum* occurred early in the experiment. No effect of any of the treatments was observed. In exp. 2, *Botrytis cinerea* stem infection began to appear in April. At the end of the experiment, the number of dead plants was not influenced by any of the treatments.

Silicon levels in nutrient solution and leaves

In exp. 2, the amount of silicon in the recirculating drain solution in the tanks with additional silicon decreased steadily from 0.80 mM in January to 0.44 mM in April. In the control tanks, silicon levels ranged throughout the experiment between 0.09 and 0.12 mM. Differences between tanks with the same silicon treatment were negligible.

The amount of silicon in the leaves of both cultivars is shown for different leaf positions and several sampling dates in Table 4. Silicon levels clearly increased with time, resulting in substantial differences in silicon content of the leaves between Si^+ and Si^- plants. There were no differences in silicon uptake between the two cultivars.

Population dynamics of the biocontrol agents

All three biocontrol agents were only found in the compartments in which they had been applied.*V. lecanii* was present on more than 90% of the samples up to one week after spraying in both experiments. At

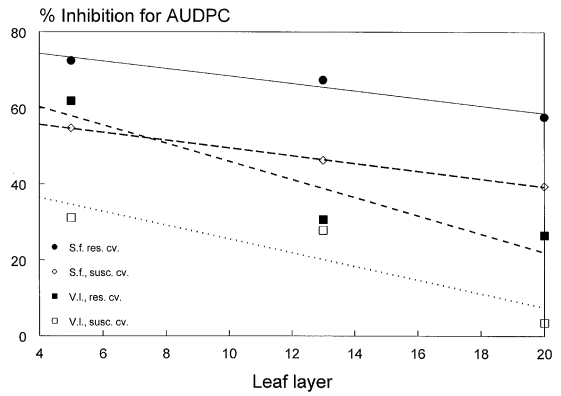

Figure 3. Percentage inhibition in the Area Under the Disease Progress Curve (AUDPC) of cucumber powdery mildew by *S. flocculosa* and *V. lecanii* compared to the control treatments plotted against leaf layer in exp. 1. V.l. = *Verticillium lecanii*, S.f. = *Sporothrix flocculosa*. Leaf layer 1 is the first full leaf. Equations for regression lines were (Y = percentage inhibition): Y = 46.4 - 1.97 * leaf layer (R^2 = 0.84) for *V. lecanii* in the susceptible cultivar; Y = 72.5 - 2.53 * leaf layer (R^2 = 0.84) for *V. lecanii* in the partially resistant cultivar; Y = 61.1 - 1.1 * leaf layer (R^2 = 1.00) for *S. flocculosa* in the susceptible cultivar; Y = 79.6 - 1.1 * leaf layer (R^2 = 0.97) for *S. flocculosa* in the partially resistant cultivar.

the end of exp. 1, population densities of *V. lecanii* were up to 4 times higher on leaf disks in the middle of the canopy than on the top leaves. The germination rate of *V. lecanii* on sampled leaf disks as assessed in exp. 2 was around 7% on fresh samples and around 38% after incubation under humid conditions and 20 °C for 24 h. Germination was not influenced by leaf layer, cultivar or silicon treatment.

A. quisqualis was present on most of the samples in exp. 1 and mostly on samples in the middle of the canopy in exp. 2. Dilution plating showed, that population densities in exp. 1 were quite stable up to one week after spraying. However, in most of the samples observed microscopically, spores of *A. quisqualis* were found but no parasitism was observed on the leaf disks. In exp. 2, assessment of the parasitism of powdery mildew by *A. quisqualis* on all the leaves of selected plants showed that on 69% of the leaves, powdery mildew was not parasitized, on 23% of the leaves, less than 50% of the powdery mildew colonies and on 8% of the leaves, more than 50% of the powdery mildew colonies were parasitized. No differences between the two compartments were observed.

S. flocculosa was recovered from all samples. There was no consistent effect of cultivar, leaf layer or silicon treatment (in exp. 2) on population density. However, the population density of *S. flocculosa* was always higher on leaves with powdery mildew infection than on disease-free leaves.

Table 4. Silicon levels in leaves (mmol/kg dry matter) of cucumber plants fed with or without potassium silicate in the fungicide treatment in exp. 2

Position of leaf	Cultivar	March 10		April 10		May 5	
		+ Si	− Si	+ Si	− Si	+ Si	− Si
Base	Flamingo	658	148	n.d.	n.d.	n.d.	n.d.
	Ventura	671	133	n.d.	n.d.	n.d.	n.d.
Top	Flamingo	425	104	714	170	864	173
	Ventura	424	108	705	175	859	199
Side-shoot	Flamingo	n.d.[a]	n.d.	620	151	738	146
	Ventura	n.d.	n.d.	602	164	730	166

[a] n.d. = not determined.
Samples of 10–15 leaves were taken per leaf layer in plots with and without silicon amendments to the nutrient solution.

Yield

In exp. 1, *P. aphanidermatum* infection interfered with reliable yield assessments.

In exp. 2, total yields in the susceptible cultivar were not significantly influenced by any of the biocontrol agents or the silicon amendment and ranged from 4.8 to 5.5 kg plant^{-1}. In the partially resistant cultivar, only the fungicide treatment significantly increased total yields compared to the control. However, in the treatment with *S. flocculosa*, yields were not significantly different from the fungicide treatment. Silicon had no effect on yield.

For both cultivars, the percentage of first class quality fruit was not influenced significantly by the biocontrol agents, the fungicide or silicon and ranged between 74 and 81%.

Discussion

To our knowledge, our experiments are the first to compare several biocontrol agents of cucumber powdery mildew in semi-commercial scale experiments. Based on the experimental design which prevented cross contamination and the similar conditions in all compartments of the experiments in terms of nutrition, climate and initial disease pressure, we were able to obtain an unbiased and reliable assessment of the relative efficacy of the biocontrol agents tested.

In both experiments, *A. quisqualis* did not control powdery mildew compared to the controls. *V. lecanii* gave some control in exp. 1 but not in exp. 2. In both

experiments, *S. flocculosa* gave the best control of the biocontrol agents, even to the point where the effect was not significantly different from the fungicide treatment. Some of our results contrast with previous reports of efficacy obtained in small-scale experiments. For example, with *A. quisqualis* Jarvis and Slingsby (1977) and Sztejnberg et al. (1989) obtained approximately 50% reduction in severity of powdery mildew and an increase in yield in glasshouse-grown cucumber compared to the control. Verhaar et al. (1996) found that *V. lecanii* could maintain powdery mildew on an artificially inoculated cucumber crop below 15% infected leaf area for 9 weeks on the partially resistant cultivar Flamingo. The AUDPC was not significantly different from the fungicide treatment in their first experiment. However, in the replication of the experiment, *V. lecanii* showed no effect on powdery mildew compared to a control treatment with water. The discrepancy between results reported in the literature and our results may be attributed in part to the drier conditions that prevailed in our experiments.In this context, *S. flocculosa* seemed to be the least affected by dry conditions, which is in accordance with findings of Hajlaoui and Bélanger (1991). The climatic conditions during daytime in exp. 1 were quite severe for the biocontrol agents. Irradiation, temperature and vapour pressure deficit were very high and this probably caused the leaf layer effect on performance of both *V. lecanii* and *S. flocculosa*. During the more moderate conditions in exp. 2 this effect of leaf layer was not as clear. Nonetheless, control in the first experiment was at least as good as in the second experiment. This indicates that the biocontrol agents can survive periods of unfavourable conditions, provided that these periods are alternated with periods of more moderate temperatures and higher humidities, as generally occur during the night. The population studies confirm that all three biocontrol agents survived on the leaves in both experiments. However, activity of *A. quisqualis* and *V. lecanii* was very limited.

Apart from a better tolerance to dry conditions, the more consistent performance of *S. flocculosa* compared to the two other agents may also be attributed to the mode of action of the biocontrol agents. Both *A. quisqualis* and *V. lecanii* are hyperparasites (Askary et al., 1997; Sundheim and Tronsmo, 1988; Yarwood, 1932) and their growth needs to be as fast as that of the pathogen in order to give sufficient control. It was our distinct impression that the powdery mildew colonies in our experiments developed faster than the hyperparasites. Parasitism was observed in the centre of the colonies, but at the edges the pathogen was growing away from the hyperparasites. On the other hand, *S. flocculosa* is not a hyperparasite, but a fungus which excretes antibiotics (Benyagoub et al., 1996; Choudhury et al., 1994; Hajlaoui et al., 1992). The advantage is that the biocontrol agent does not have to be in direct contact with the pathogen, because the molecules will diffuse over the leaf surface.

Additives are commonly used with biocontrol agents to promote survival and to ensure a homogeneous coverage on the plant surface. Glycerol, Tween and different kinds of oil have been shown to improve the performance of biocontrol agents (Hijwegen, 1992; Philipp and Hellstern, 1986; Philipp et al., 1990; Spencer and Ebben, 1983; Verhaar et al., 1996). However, these additives may directly affect powdery mildew (Hijwegen, 1992; Verhaar et al., 1996). In our first experiment, the treatment with only Tween 80 gave significant control of powdery mildew compared to paraffin oil. Since *A. quisqualis* was applied together with Tween 80 and gave similar control as Tween 80 by itself, the relative control of this treatment in this experiment was attributable to the additive only. This is confirmed by the lack of control by *A. quisqualis* in the second experiment, in which it was applied in an oil mixture. We have tested the oil that we used for its effect against powdery mildew in several smaller scale experiments. The results showed, that this oil in all concentrations tested (up to 5%) did not control powdery mildew in cucumber (Dik and Bélanger, unpublished results). Bélanger et al. (1994) found that *S. flocculosa* mixed with 1% paraffin oil controlled rose powdery mildew slightly better than the biocontrol agent alone. They ascribed this result to increased survival of the biocontrol agent rather than to a direct effect of the oil on powdery mildew. The use of additives as controls in our experiments has ensured that we can separate the effect of the biocontrol agents themselves from that of the additives.

In both experiments, control was generally better in the partially resistant cultivar than in the susceptible cultivar. This integration of cultivar and biocontrol should therefore be considered in a general management scheme of powdery mildew. Also, silicon amendments to the nutrient solution provided some additional reduction in powdery mildew severity. Biocontrol may also be integrated with chemical control. The performance of *A. quisqualis* was much better when used in combination with low level fungicide applications (Sundheim, 1982). For *A. quisqualis*, the compatibility with fungicides has been assessed by

Philipp et al. (1982, 1984) and Sztejnberg et al. (1989). For *S. flocculosa* and *V. lecanii*, more information on compatibility with fungicides is needed. In a very susceptible cultivar, integration with chemical fungicides may prove to be necessary.

In spite of their inefficacy in the experiments reported here, both *A. quisqualis* and *V. lecanii* were present on the leaves and started to grow immediately after placing sampled leaf disks under humid conditions. This confirms that the humidity conditions prevailing in the glasshouses limited their growth. No differences occurred in the presence of the biocontrol agents in treatments with different silicon levels, confirming that the silicon and biocontrol agents acted independently. The applied biocontrol agents were only found in the compartments where they were sprayed, so no cross-contamination occurred between compartments. It has been suggested that biocontrol agents may spread with the powdery mildew spores and therefore results may be difficult to interpret (Philipp et al., 1984); in our experiments the separate compartments apparently formed a sufficient barrier.

In general, it can be concluded that of the three biocontrol agents tested, *S. flocculosa* shows the best potential for efficient biocontrol of cucumber powdery mildew under the conditions prevailing in Dutch glasshouses. The experiments were run in the two seasons in which powdery mildew is most severe and under conditions that were similar in all aspects to commercial glasshouse conditions. The control in our experiments was not sufficient for commercial growers, with the exception of the partially resistant cultivar in exp. 1, but in this respect it must be noted that our experiments aimed at testing the performance of the biocontrol agents under severe infection pressure. Artificial inoculation of all plants provided a homogeneous, but at the same time unnaturally high disease pressure quite early in the growing season. The fact that even in this situation significant control occurred allows optimism with respect to the possibilities of biocontrol under more moderate infection pressures. Further experiments will be needed to provide more information on this aspect. Furthermore, integration with yet other methods, for example induced resistance by means of plant extracts and with chemical control will be the topic of future research.

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