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Screening of alternative products for integrated pest management of cucurbit powdery mildew in Sweden

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Abstract Cucurbit powdery mildew (CPM) is one of the most important plant protection problems in Swedish cucumber production. CPM fungi have developed resistance to the commonly used fungicide (Imazalil) rendering its current use less effective. We therefore screened a selection of alternative products, evaluating their ability to control CPM in seven small-scale, semi-commercial, greenhouse experiments. Products were screened for their ability to suppress CPM on a susceptible cultivar, in 2013. In 2015, the best treatments were tested in different combinations, in different intervals on a susceptible and a partially resistant cultivar. The treatment that gave the best CPM control was Sakalia in combination with wetting agent Yuccah, (based on *Reynoutria sachaliensis* and *Yucca schidigera* respectively) applied at 7-day intervals. This treatment was highly efficient on both cucumber cultivars. Sakalia mixed with Yuccah applied at 14-day intervals had an almost equally controlling effect on CPM. The microbial pesticides, Polyversum (*Pythium oligandrum*) and AQ10 (*Ampelomyces quiscalis*) and the fungicide Imazalil, provided no to poor control of CPM compared to control treatments. Hortistar (Silicon) partially reduced CPM infections and was more efficient on the partially resistant cultivar. We conclude that Sakalia in combination with wetting agent Yuccah could efficiently control the disease even under the severe conditions caused by artificial inoculation. Further testing of this combination in commercial greenhouses will enable evaluation of the potential effects on yield and beneficial or pest insects. The knowledge gained from this study can be used to develop IPM tools for commercial production systems.

Keywords Cucumber production · Plant protection · Cucurbit powdery mildew · *Podosphaera xanthii* · Integrated pest management · Biopesticides · Biological control · Greenhouse crops

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Introduction

Cucumber is the most widely grown greenhouse crop in Sweden. In 2015, cucumber was cultivated on 67 ha of greenhouse space, resulting in a total yield of 28,000 t (Persson 2015a). One of the most important foliar diseases of greenhouse cucumber is cucurbit powdery mildew (CPM), which causes yield losses and crop quality reductions. The infection often results in a shortened growing season (Cerkauskas and Ferguson 2014; Nuñes-Palenius et al. 2006; Sitterly 1978). The disease is considered to be the major cause of crop losses in cucumber worldwide (Lebeda et al. 2010).

CPM, in the northern hemisphere, is primarily caused by the two obligate biotrophic polyphagous fungi; *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff (formerly *Sphaerotheca fuliginea (*Schlechend.:Fr) Pollacci) and *Golovinomyces cichoracearum* (syn. *Erysiphe cichoracearum*) (DC.) VP Heluta (Braun 1995). The two species differ morphologically but can nevertheless be difficult to distinguish visually (Björling et al. 1991; Sitterly 1978; Kapoor 1967). Polymerase chain reaction (PCR) methods allow for more precise differentiation (Chen et al. 2008).

G. cichoracearum has a lower temperature optimum and therefore more often occurs in spring or early summer when temperatures are generally lower in the greenhouse (Aguiar et al. 2012; Vakalounakis et al. 1994; Sitterly 1978). *P. xanthii,* mostly occurs during the height of the summer and onward and causes more severe infections in greenhouse conditions (Braun 1995; Sitterly 1978). The two species can be concomitant on the same place on the plant (Bardin et al. 1997; Sitterly 1978). In general, CPM is more severe in greenhouses than in the field as the greenhouse climate and continuous cropping offers more favourable conditions for disease development (Sitterly 1978).

Lebeda et al. (2010), have noted that breeding of cucurbit crops for powdery mildew resistance has been very successful in many cases. However, due to pathogen adaptation, the degree of protection achieved with resistant cultivars is variable and not always adequate as a sole management practise. New, partially resistant cucumber cultivars have been introduced but often produce smaller yields than susceptible cultivars, specifically in spring and autumn. Growers and extension officers report a direct correlation between degree of partial resistance and the demand for light, and thus, also yield since light is limiting (H. Hermans, Innocrop Consulting, pers. comm.). The negative relationship between disease resistance and yield has also been reported by Staub and Grumet (1993).

From January 1, 2014, it is compulsory for EU member-state farmers to follow integrated pest management principles according to the EU Framework Directive 2009/128/EC on the sustainable use of pesticides. Finding efficient alternatives to chemical fungicides and examining how they can work together in an integrated pest management (IPM) system is of great importance and there is an urgent need to develop IPM practices to control CPM.

There are two fungicides currently registered for use against CPM in Sweden, under an off-label permit. These are Amistar® (a.i. azoxystrobin), a QoI fungicide and Fungazil® (a.i. imazalil), an imidazole fungicide (http://www.frac.info/home). However, field resistance of wheat powdery mildew to QoI fungicides was reported in 1998 and soon after also reported in CPM (Ishii et al. 2001). Since then, resistance has been reported in CPM populations (mainly *P. xanthii*) to six groups of single-site inhibitors: benzimidazole, DMI, morpholine, hydroxypyrimidine, phosphorothiolate, QoI, and Pyridine carboxamides (Lebeda et al. 2010; Miyamoto et al. 2010). Therefore, fungicide treatments may not have the expected protective effect in many cases.

There are no alternatives to chemical fungicides such as biopesticides or biological control agents that could be used for managing powdery mildew in Sweden today. Sulphur, which is categorised as a preventative fungicide, is permitted, but is commonly not used due to high risk of phytotoxicity in cucumber, especially at high temperatures in the greenhouse (Cerkauskas and Ferguson 2014; H. Hermans, Innocrop Consulting, pers. comm.). There have been other alternative control agents available in the past in Sweden e.g. the antimicrobial biopesticide Enzicur®. In this particular case, the manufacturer chose not to fulfil the registration process.

Several alternative products are registered, in other parts of the world. Some previously tested products include microbial products based on fungi, oomycetes or bacteria that produce antifungal substances and/or are mycoparastitic such as AQ10® (*Ampelomyces quiscalis*), Polyversum® (*Pythium oligandrum*), Sporodex® (*Sporothrix flocculosa*) and Serenade® (*Bacillus subtilis*). Other previously tested products are based on chitosan, plant extracts, silicon, mineral oil and potassium bicarbonate (Cerkauskas and Ferguson 2014; Giotis et al. 2012; Su 2012; Wolff et al. 2012; Gilardi et al. 2008; Benhamou et al. 1999; McGrath and Shishkoff 1999).

Swedish conventional cucumber growers today mainly rely on the use of Fungazil (Imazalil) due to the lack of effective alternatives. Organic growers rely on climate control, proper hygiene and sanitation measures. Sole dependence on one fungicide creates an increased risk of development of fungicide resistance in the CPM pathogens. The aim of this study was to screen for effective alternative products against CPM in greenhouses and evaluate their effect on CPM alone and in combination with the standard fungicide. Treatment combinations were also tested at different application intervals and using susceptible and partially resistant cultivars in order to find tools for incorporation into an IPM system. The experiments were conducted in collaboration with a group of cucumber growers.

Materials and methods

Experimental design and treatments

The treatments of each experiment are shown in Table 1. Experiments 1 to 3 were conducted in 2013 and experiments 4 to 7 were conducted in 2015. All experiments were conducted in a greenhouse using two adjacent compartments of 120 m^2 each. The plant density differed somewhat between experiments but was slightly higher than the standard density of Swedish commercial cucumber greenhouses (see Table 1).

Experiments 1 to 3 were arranged as randomized complete block designs and one chamber was used for each experiment. In experiments 4 to 7, two cultivars were used with a split-plot experiment design comprising three blocks. Experiment 1 included three blocks and seven treatments. Each experimental plot contained six plants of which four were scored. In total, 84 plants were scored in this experiment. Experiments 2 to 7 included five blocks and seven treatments. Each experimental plot contained three plants of which the middle plant was scored. In total, 35 plants were scored for each of these experiments.

In all experiments, seven treatments were applied (Table 1). They were selected primarily based on results from available literature at the time. As the experiments continued, treatments were selected based on their observed performance in previous experiments. A list of products tested, including dose rates is given in Table 2.

All treatments, except Fungazil, were applied for the first time one day after powdery mildew inoculation. A seven-day spraying interval was standard procedure except for in experiment 6 and 7 where effects of different application intervals were tested. Fungazil was only applied twice during each experiment, which is in line with Swedish regulations for commercial applications. Fungazil was applied for the first time when visible symptoms were observed and the second and final time one week after the first treatment. Kendal Cops® was applied three times consecutively according to the manufacturer's recommendation and at seven day intervals,

The treatments were applied with a 5-L, 6 bar, compression sprayer (Ferrox®, Mesto Germany). For each treatment, a different sprayer was used to avoid cross contamination. All treatments were prepared according to instructions from the suppliers. A list of products and suppliers is found in Table 1.

Some of the products (AQ10, Resistim® and Sakalia®) were used in combination with an additive according to recommendations from suppliers'. These

Table 1 Overview of experimental setup of the different experiments conducted in 2013 and 2015

	2013			**2015**	
Planting Date	July 1	July 19	2 August	June 16	5 August
Duration	4 weeks	8 weeks	8 weeks	7 weeks	7 weeks
Cultivar	Euphoria	Euphoria	Euphoria	Euphoria, Proloog	Euphoria, Proloog
Plant density*	1.95 plants/m^2	2.02 plants/m^2	2.02 plants/m^2	2.32 plants/m^2	2.32 plants/m^2
Treatment	**Experiment 1**	**Experiment 2**	**Experiment 3**	**Experiment 4 and 5**	**Experiment 6 and 7**
1.	Kendal Cops	Sakalia + Yuccah	Sakalia + Yuccah	Sakalia + Yuccah	Yuccah, 7 d
2.	AQ10 + Bioglans	Polyversum	Polyversum	Hortistar	Sakalia, 7 d
3.	Fungazil	Hortifain	Hortifain	Hortistar + Fungazil	Sakalia + Yuccah + Fungazil 14 d
4.	Sakalia + Yuccah	Fungazil	Fungazil	Sakalia + Yuccah + Fungazil	Sakalia + Yuccah 14 d
5.	Hortistar	Resistim + Zence	Kendal Cops	Fungazil	Sakalia + Yuccah + Hortistar 14 d
6.	Untreated ctrl	Untreated ctrl	Untreated ctrl	Untreated ctrl	Untreated (Pos. ctrl).
7.	Water ctrl	Water ctrl	Water ctrl	Water ctrl	Sakalia + Yuccah 7 d (Neg. ctrl.)

*The standard plant density of Swedish commercial cucumber greenhouses is 1.5 plants/m^2

Table 2 List of products included in the experiments with the corresponding active ingredients and the doses used. All doses given in percentage are volume by volume

Product name	Active ingredients	Dose
AQ10 (Intrachem Bio Italia S.p.A, Italy)	*Ampelomyces quisqualis*	0.07 g/L
Bioglans (Borregard Bioplant A/S, Denmark)	paraffin oil	0.20%
Fungazil 100 (Certis Europe B.V., Netherlands)	imazalil	0.075%
Hortifain (Oy Faintend Ltd., Finland)	surfactants	1.50%
Hortistar (Hortifeeds Direct Ltd., UK)	silicon	0.10%
Kendal Cops (Valagro S.p.A., Italy)	chitosan, copper	0.90%
Polyversum (Biopreparáty, spol. s r. o., Czech republic)	*Pythium oligandrum*	1 g/L
Resistim 0–7-11 (BrØste A/S, Denmark)	potassium phosphite	1%
Sakalia[a] (Syngenta Nordics A/S, Denmark)	*Reynoutria sachaliensis*	1%
Yuccah (Plant Health Cure B.V., Netherlands)	*Yucca schidigera*	1%
Zence 40 (Duxon, Borregard Bioplant A/S, Denmark)	soap (potassium fatty acids)	0.10%

[a] Sakalia (Syngenta Nordics A/S, Denmark) is the current name of the plant extract based on *Reynoutria sachaliensis*. At the beginning of the project it was named Sentry R® (Plant Health Cure B.V., Netherlands) but is referred to as Sakalia throughout the text

products were tank mixed in the sprayer. However, in experiment 6 and 7, Sakalia and Yuccah® were also tested separately to evaluate the effect of these two products individually.

In experiments 4 to 7, when specifically testing treatment combinations, the different product combinations were applied using separate sprayers. The products were sprayed individually on the same plants with some drying time between sprayings. This was done to avoid the possible risk of unwanted chemical reactions in the tank e.g. precipitation. In experiments 1 to 5, both untreated and water treated plots were used as negative controls. Fungazil, applied according to commercial practice, was intended as a positive control.

Water treated controls and the treatment with Fungazil were excluded in experiment 6 and 7. The reasons were that untreated and water controls did not significantly differ in relative area under the disease progress curve (rAUDPC) in any of the 2013 experiments and that Fungazil had already been tested in five previous trials. This also allowed testing of additional treatments. Sakalia and Yuccah® applied every seven days were considered as a positive control in these experiments. Growing conditions and disease pressure were similar in both greenhouse compartments.

Plant material and growing conditions

Long English cucumber plants (*Cucumis sativus L.*) were used in all experiments. In experiments 1 to 3, the susceptible cultivar 'Euphoria' was used. In experiments 4–7, the susceptible cultivar 'Euphoria' and the partially resistant cultivar 'Proloog' were used. In all experiments, a cultivar with a high level of partial resistance ('Stelvio'), was planted between blocks and in edge rows in order to keep disease pressure down.

The plants were cultivated in a commercial nursery and transplanted to our experimental greenhouses at the four to five leaf stage onto rock wool slabs. The training system was adjusted, from the commonly used renewal umbrella system, in order to facilitate scoring of infection.

Plants in experiments 1 to 5, were pruned to maintain only the main stem up to the fifteenth or sixteenth leaf. In experiments 6 and 7, one to two side shoots per plant were allowed to keep growing in order to promote plant vigour. Fruits were harvested approximately three times a week and discarded but the yields were not recorded. Biological control by commercially available natural enemies was used against insect pests.

Climate regime

The climate regime was set up to resemble the standard regime of Swedish cucumber production systems. The greenhouse climate was controlled by a PRIVA® environmental computer which also recorded the climate data. The temperature in the greenhouse was set at 18 °C during the night and 21 °C during the day. No artificial lighting was used. The ventilation windows of the greenhouse were controlled by the temperature and level of relative humidity. The screens were closed at a radiation level of 500 W per square meter and from sunset till sunrise.

Supply of water and nutrients

The plants were grown in rock wool slabs without reuse of drainage water. A nutrient solution (WH Bouyant Rika T, NPK 3–1-5, Weibulls Horto AB) was supplied by a trickle irrigation system (Aquadrip AB) and was the same for all experiments. Irrigation was automatically adjusted to the radiation level. The basic composition of the undiluted nutrient solution in percentage (*w*/w) was 3.2 N (Total), 2.5 NO_3^-, 0.7 NH_4^+, 1.0 P, 4.7 K, 0.06 Mg, 0.8 S, 0.005 B, 0.002 Cu, 0.03 Fe, 0.02 Mn, 0.001 Mo and 0.005 Zn (Density: 1.15 kg/l). Calcium nitrate (Calcinit, Yara) was mixed with water and added from a separate container. The undiluted composition was 19% Ca, 14.4% NO_3^-, and 1.1% NH_4^+. Both solutions were mixed with irrigation water using a fertilizer injector (Dosatron™ D 25). In 2013, the average electrical conductivity (EC) in the root environment was 2.1 mS cm^{-1} and the average pH was between 5.8 and 6.0. In 2015, the average EC in the root environment was between 2.5 and 2.7 mS cm^{-1}. The average pH was between 5.9 and 6.2. During 2015, the drainage water was analysed every two weeks for macro and micro elements, EC and pH, in order to make adjustments of nutrient additions possible. When necessary, pH was adjusted with Phosphoric acid (53%).

Inoculation

Infected leaves were collected from different commercial greenhouses in the region with severe cucumber powdery mildew infection in 2013 and 2015. The morphological characteristics of the conidia from the sampled leaves were examined using light microscopy and the dominant CPM species was identified by PCR analysis. The sampled cucumber leaves from the commercial greenhouse were used to infect plants of a susceptible cucumber cultivar in order to maintain the fungi until it was time for inoculation of the experimental greenhouses. To ensure high viability and conidia roughly of the same age, the suspension used for inoculation was made from plants infected approximately two weeks before.

A spore suspension was made by rinsing infected leaves in water. The spore concentration was assessed with a haemocytometer and adjusted to 10,000 spores ml^{-1}. Tween 20 (20 μl L^{-1}) was added to the suspension to ensure even dispersal of spores. Within three hours of suspending the spores in water, all plants were sprayed with the suspension using a 5-L, 6 bar, compression sprayer (Ferrox®, Mesto Germany). The plants were inoculated at the four to five leaf stage one day after transplanting and all leaves of the plants were sprayed. Thus, all leaves above leaf number six were naturally infected.

Inoculation occurred one day prior to the first application of treatments in both years. To prevent contamination between treatments, thick, plastic curtains were put up between rows at the time of spraying. As often as possible, application was made in the afternoon in order to delay drying of plants and subsequent desiccation of the applied products.

Disease assessment/data collection

Disease severity was assessed on a weekly to biweekly basis by scoring the percentage leaf area covered with colonies in a similar manner to Longzhou et al. (2008). The first disease assessment was made after the first visible colonies were observed. Data was collected from every second leaf of every plant from crown to apex. The number of plants assessed per treatment in each experiment ranged between five and 12 depending on experimental design. Dead leaves and leaves damaged by pests and other diseases were also recorded.

Statistical analysis

The data was analysed by calculating the area under the disease progress curve (AUDPC) (Shaner and Finney 1977) based on the mean values of the percentage of leaf area covered with disease for each plant. Only leaves number six to twelve, counting from crown to apex, were included in the calculation of the means. The following standard formula was used:

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} \left[\left(Y_i + Y_{i+1} \right) \Big/ 2 \right] \left[X_{i+1} - X_i \right]$$

Where Y_i is disease severity in percent at the i^{th} observation, X_i is time (days) at the i^{th} observation and n is the total number of observations. The relative AUDPC (rAUDPC) was calculated by dividing AUDPC by the total area during the assessment period, assuming 100% disease from the start.

The rAUDPC values were subjected to analysis of variance and the significance of differences among treatments were analysed with Tukey's test at $p = 0.05$. In addition, the effect of cultivar was analysed by pooling data from experiment 4 with 5 and from experiment 6 with 7 using ANOVA. Furthermore, the effects of different intervals was also analysed by pooling data from experiment 6 with 7 using ANOVA. All statistical analyses were made using SAS version 9.4.

DNA extraction and species identification

Twelve Individual diseased leaf samples, from six individual plants, were harvested from the greenhouse, snap-frozen in liquid nitrogen and stored at −70 °C prior to DNA extraction. Total DNA was extracted from frozen leaf samples using a QIAGEN DNeasy® Plant Mini Kit (Qiagen, UK) following the manufacturer's protocol (Löbmann et al. 2016).

The internal transcribed spacer (ITS) of nuclear ribosomal DNA regions was amplified using the powdery-mildew specific ITS universal primer pair PN23 (5′-CAC CGC CCG TCG CTA CTA CCG-3′)/PN34 (5′-TTG CCG CTT CAC TCG CCG TT -3′). Pairs of primers specific to the ITS regions of *P. xanthii, G. cichoracearum, and L. taurica* were used for PCR amplification. These were: S1 (5′- GGATCA TTA CTG AGC GCG AGG CCC CG -3′)/S2 (5′- CGC CGC CCT GGC GCG AGA TAC A -3′), G1 (5′- TCC GTA GGT GAA CCT GCG GAA GGA T -3′)/G2 (5′- CAA CAC CAA ACC ACA CAC ACG GCG -3′), and L1 (5′-CCC TCC CAC CCG TGT CGA CTC GTC TC -3′)/L2 (5′- CTG CGT TTA AGA GCC GCC GCG CCG AA -3′), respectively (Chen et al. 2008).

PCRs were carried out using 10 ng DNA as described by White et al. 1990. Yield and integrity of the DNA was assessed using a NanoDrop Micro Photometer (NanoDrop Technologies, UK), and agarose gel electrophoresis, respectively. The expected size of the PCR products of PN23/PN34, S1/S2, G1/G2 and L1/L2 are around ~740 bp, 454 bp, 391 bp and 374 bp respectively. Both PN23 and S1 primers were used for sequencing at the GATC biotech AG sequencing facility (Germany). Resulting sequences were searched using the National Center for Biotechnology Information (NCBI) GenBank non-redundant nucleotide database. Sequences mapping to known species were determined with coverage and identity and the best NCBI accession were recorded.

Results

Climatic conditions

Temperature was normally in the range of 20–30 °C during daytime and in the range of 17–23 °C during night in both years. The relative humidity ranged between 25 and 70% during daytime and 66–100% during night. These conditions are close to those normally experienced in commercial cucumber greenhouses. Throughout the experiments, occasional extreme values were also recorded (Table 3).

Powdery mildew infection

Powdery mildew inoculations consistently resulted in visible infections 5–6 d.p.i on untreated plants. The disease pressure was fairly equal in all experiments indicated by similar disease progress curves for the untreated controls (Fig. 1). However, untreated controls of experiments 6 and 7 displayed a slower disease progression (Fig. 1d). Untreated and water controls did not significantly differ in any experiments in 2013, therefore, water control was excluded to allow testing of additional treatments.

The effect of foliar treatments

The plant extract based on *Reynoutria sachaliensis*, Sakalia, combined with a wetting agent, Yuccah, from Yucca palm tree (*Yucca schidigera*) applied at seven-day intervals, consistently had the most suppressive effect on powdery mildew disease severity (Fig. 1, Tables 4 and 5). This was shown by the significantly lower rAUDPC values compared to untreated control and most other treatments (Tables 4 and 5). Combining Sakalia and Yuccah with Fungazil or Hortistar did, however, not improve the disease control further. Fungazil, delayed the disease progress slightly in some experiments but in all of the experiments it had poor effect on disease severity, i.e. the disease severity was only slightly lower compared to untreated control (Fig. 1a).

The Fungazil treatment resulted in significantly less infection than the untreated control in all experiments conducted in 2013 (Table 4). However, in 2015, there was no significant difference between the Fungazil treatment and the untreated control in any of the experiments where Fungazil was included alone. Kendal Cops also showed good disease suppressing ability as indicated by low rAUDPC values (Table 4). However, this treatment

Table 3 Average, minimum, maximum temperature and relative humidity in greenhouse experiments conducted during 2013 and 2015 in experimental greenhouses of the Swedish University of Agricultural Sciences, Alnarp, Sweden

	2013			2015			
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7
Temperature (°C)							
Min	18.9	17.5	18.5	17.5	17.5	17.4	16.8
Max	31.9	33.4	34.5	34.9	37.7	34.6	33.8
Average	**23.1**	**22.7**	**21.9**	**21.8**	**22.1**	**22.3**	**22.2**
Humidity (%)							
Min	30.1	27.2	33.0	33.1	32.0	39.4	30.0
Max	95.9	95.0	100.0	100.0	100.0	100.0	100.0
Average	**70.9**	**74.3**	**78.2**	**78.7**	**73.5**	**85.2**	**87.7**

resulted in chlorotic leaves indicating phytotoxic effects in spite of following recommendations to only do three applications.

Experiments 2013

The lowest average infection in experiment 1 was found in plants treated with a combination of Sakalia and Yuccah but Kendal Cops and Hortistar also performed well indicated by rAUDPC values that were not significantly different from the best treatment. However, Kendal Cops caused phytotoxic effects. Fungazil supressed the disease to a higher degree than AQ10 with Bioglans as indicated by significantly lower rAUDPC values (Table 4). AQ10 combined with Bioglans was only included in experiment 1. This treatment only had a slight suppressing effect on disease development but still the rAUDPC values were significantly lower than untreated control. In general, the rAUDPC values were lower in experiment 1 compared to the other experiments due to shorter duration of this experiment.

All treatments, except Sakalia and Yuccah showed very poor disease suppression in experiment 2. Nevertheless, all treatments, except Polyversum, had significantly lower rAUDPC values compared to the controls. Hortifain had significantly lower rAUDPC values than the untreated and water control and Fungazil and Resistim combined with Zence but the plants still had very high levels of infection (Table 4, Fig. 1a). The effect of Resistim combined with Zence was equal to that of Fungazil in experiment 2 Table 4, i.e. substantial infection still developed. Sakalia combined with Yuccah was the best treatment in experiment 3. Kendal Cops was significantly more suppressive against CPM than Fungazil, however, as in experiment 1, it caused phytotoxic effects and was therefore excluded from further experiments. As in experiment 2, Polyversum had no significant effect on disease severity.

Experiments 2015

In every experiment, the highly efficient combination of Sakalia and Yuccah, significantly reduced the disease severity in both cultivars to a similar degree (Fig. 1b). The general disease suppression caused by all treatments in experiments 4 and 5, was rather similar.

Treatments with Hortistar resulted in significantly lower rAUDPC values compared to untreated control in both experiment 4 and 5 on cultivar 'Proloog'. Analysing the suppressive effect of Hortistar more closely by pooling the data of experiments 4 and 5, revealed that there were significant effects of treatment (P = <0.0001) and of cultivar (P = <0.0001). There was also a significant interaction between treatment and cultivar ($P = 0.0028$). Thus, treatment with Hortistar was more efficient on the partially resistant cultivar ('Proloog') compared to the susceptible cultivar ('Euphoria'). Combining Hortistar with Fungazil seemed to slightly improve the suppressive effect. However, the combination was only significantly different from Hortistar in cultivar 'Euphoria' in experiment 4. Unfortunately, combining Hortistar with Fungazil resulted in phytotoxic effects seen as small necrotic spots.

We analysed the cultivar effect in all experiments from 2015 by pooling experiment 4 and 5 as well as 6 and 7. The pooled experiments were identical except for being

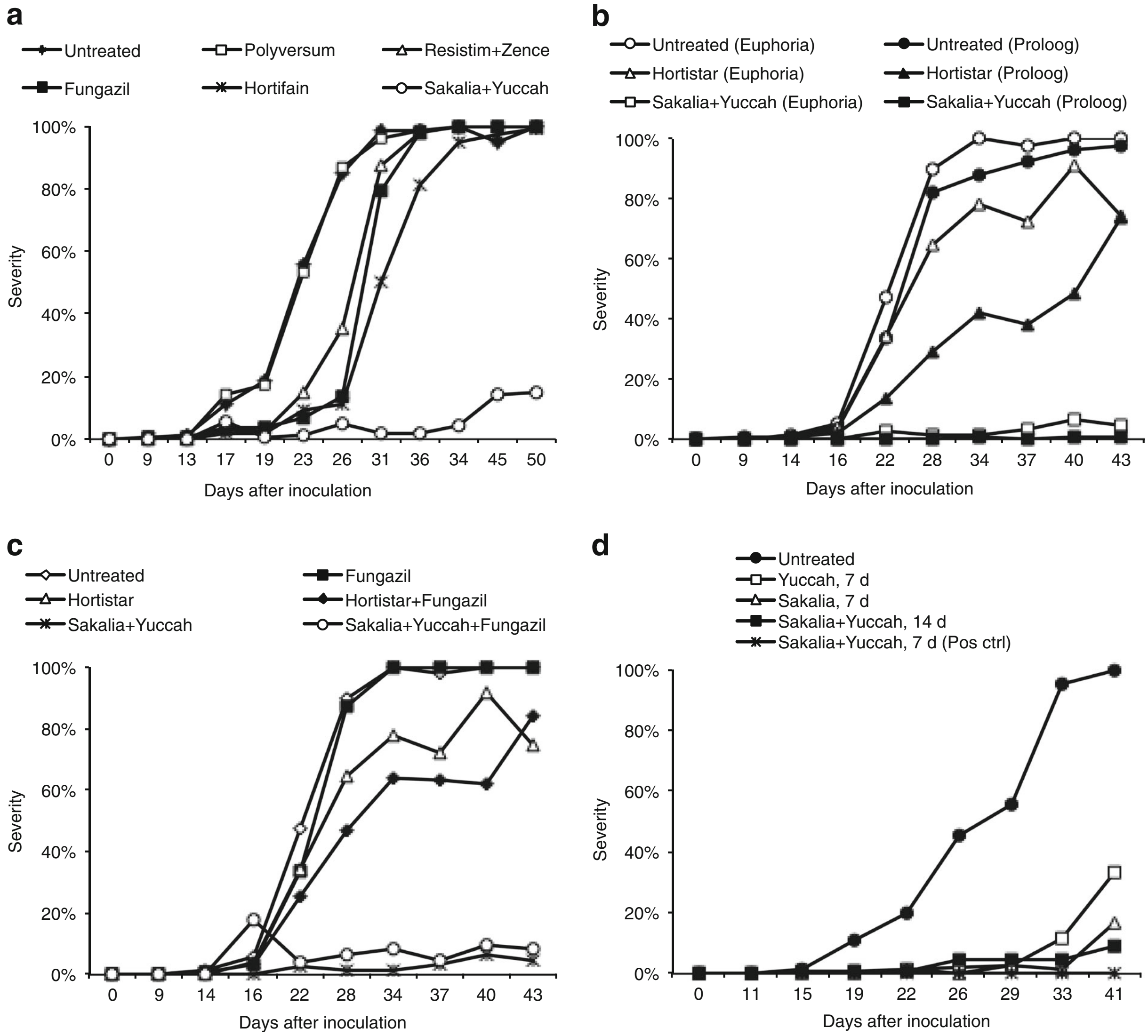

Fig. 1 Examples of disease progress curves of powdery mildew on cucumber in greenhouse experiments showing effect of treatments on mean disease severity (percentage of leaf area covered by powdery mildew) different days after inoculation with *Podosphaera xanthii*. Unless otherwise specified, all treatments were applied at seven-day intervals. **1a** Exp. 2, 2013. Effect of treatments on the susceptible cultivar 'Euphoria'; **1b** Exp. 4, 2015. Effect of treatments on the susceptible 'Euphoria' vs. the partially resistant cultivar 'Proloog'; **1c** Exp. 4 2015. Effect of the most efficient treatments combined with the Fungazil on the susceptible cultivar 'Euphoria'; **1d** Exp 6 2015. Effect of the most efficient treatment applied in seven or fourteen-day intervals and effect of Sakalia and Yuccah applied separately tested on the susceptible cultivar 'Euphoria'

conducted in separate compartments. In both cases there were significant differences between the two cultivars 'Euphoria' and 'Proloog' (P = <0.0001). Furthermore, when results from experiment 6 and 7 were pooled, a significant difference in rAUDPC values between seven- and fourteen-day intervals on the susceptible cultivar ($P = 0.032$) but not on the resistant cultivar ($P = 0.238$) was found. When the results from the two different cultivars were pooled, the seven-day interval treatment gave a slightly higher level of suppression indicated by significantly lower rAUDPC ($p = 0.021$).

The disease progress in the untreated controls was slower as indicated by lower rAUDPC values from untreated controls in experiment 6 and 7 compared to experiments 4 and 5 (Table 5). In both experiments, all treatments were highly effective against CPM on both cultivars. The rAUDPC values were low and did not significantly differ from each other. As in all of the earlier

Table 4 CPM disease severity expressed as relative area under the disease progress curve (rAUDPC) after different treatments in experiments conducted in 2013 with the cultivar 'Euphoria'. Combinations were tank mixed (mixed in sprayers)

	Experiment 1	Experiment 2	Experiment 3
Treatments	rAUDPC	rAUDPC	rAUDPC
Kendal Cops	0.027 d	–	0.394 c
AQ10 + Bioglans	0.192 b	–	–
Fungazil	0.111 c	0.523 b	0.482 b
Sakalia + Yuccah	0.018 d	0.043 d	0.066 d
Hortistar	0.054 d	–	–
Polyversum	–	0.674 a	0.644 a
Hortifain	–	0.463 c	0.421 bc
Resistim + Zence	–	0.558 b	–
Untreated	0.285 a	0.677 a	0.702 a
Water	0.257 a	0.689 a	0.671 a

ANOVA followed by Tukey's ($p < 0.05$) test was used for comparison of the treatments. Means with the same letter are not significantly different. For vertical comparision only

experiments, Sakalia and Yuccah applied at seven-day interval was the most efficient treatment. Additionally, Sakalia and Yuccah combined and applied at fourteen-day intervals provided a similar level of control (Table 5).

The separate effects of Sakalia and Yuccah, was also examined (Fig. 1d). Sakalia applied alone at seven-day intervals efficiently reduced the disease severity and the effect was similar to that of Sakalia mixed with Yuccah applied at seven-day intervals. However, applying Sakalia without Yuccah as an additive caused formation of brown run-off residues on the plants and fruits. Clearly, mixing Sakalia with Yuccah helped the droplets to spread more evenly, reducing the amount visible residues on leaves. Yuccah applied alone at seven-day intervals also proved to have a significantly suppressive effect compared to the untreated control.

Inoculum and species identification

Polymerase chain reaction (PCR) assays followed by sequence analysis determined *P. xanthii* as the only species present in both 2013 and 2015.

Discussion

Treatment with a combination of Sakalia, an extract of giant knotweed, *Reynoutria sachaliensis* (formerly marketed as Milsana and Regalia) and Yuccah (a wetting

Table 5 CPM disease severity expressed as relative area under the disease progress curve (rAUDPC) after different treatments in experiments conducted in 2015. The susceptible cultivar 'Euphoria' and the partially resistant cultivar 'Proloog' were used. Combinations were not tank mixed (except for Sakalia and Yuccah) and applied separately

	Experiment 4		Experiment 5		Experiment 6		Experiment 7	
Cultivar Treatments	Euphoria rAUDPC	Proloog rAUDPC	Euphoria rAUDPC	Proloog rAUDPC	Euphoria rAUDPC	Proloog rAUDPC	Euphoria rAUDPC	Proloog rAUDPC
Sakalia + Yuccah	0.020 c	0.003 d	0.072 c	0.029 d	-	-	-	-
Hortistar	0.461 a	0.244 bc	0.489 ab	0.201 c	-	-	-	-
Hortistar + Fungazil	0.366 b	0.200 cd	0.355 abc	0.147 cd	-	-	-	-
Sakalia + Yuccah + Fungazil	0.067 c	0.028 d	0.247 bc	0.120 cd	-	-	-	-
Fungazil	0.571 a	0.451 ab	0.591 ab	0.409 b	-	-	-	-
Yuccah. 7 d	-	-	-	-	0.077 b	0.014 b	0.052 b	0.035 b
Sakalia. 7 d	-	-	-	-	0.031 b	0.001 b	0.004 b	0.001 b
Sakalia + Yuccah + Fungazil 14 d	-	-	-	-	0.054 b	0.014 b	0.022 b	0.006 b
Sakalia + Yuccah 14 d	-	-	-	-	0.035 b	0.029 b	0.039 b	0.010 b
Sakalia + Yuccah + Hortistar 14 d	-	-	-	-	0.057 b	0.040 b	0.043 b	0.021 b
Sakalia + Yuccah 7 d	-	-	-	-	0.001 b	0.001 b	0.002 b	0.002 b
Untreated	0.602 a	0.467 a	0.644 a	0.520 ab	0.480 a	0.358 a	0.548 a	0.341 a
Water	0.612 a	0.548 a	0.620 a	0.590 a	-	-	-	-

ANOVA followed by Tukey's ($p < 0.05$) test was used for comparison of the treatments. Means with the same letter are not significantly different. For vertical comparison only agent derived from the palm *Yuccah schidigera*), consistently gave the best control of cucurbit powdery mildew (CPM) across all experiments. This treatment was most effective when applied at seven-day intervals in both the susceptible cultivar and the partially resistant cultivar. The combined Sakalia-Yuccah treatment was also effective at fourteen-day intervals. Particularly on the partially resistant cultivar CPM was controlled equally well with this treatment regardless of the application interval. To our knowledge, this is the first time the effect of Sakalia in combination with Yuccah has been tested scientifically in semi-commercial cucumber trials.

Extracts of *R. sachaliensis* have been used to control a variety of diseases such as powdery mildew of cucurbits, downy mildew of lettuce (*Bremia lactucae*), grey mold on grapes and strawberries (*Botrytis cinerea*), bacterial spot of tomatoes and peppers (*Xanthomonas campestris* pv. *vesicatoria*), Cercospora leaf blight on soybeans (*Cercospora kikuchii*) and bacterial canker on citrus (*Xanthomonas axonopodis* pv. *citri*), (Konstantinidou-Doltsinis and Schmitt 1998; Petsikos-Panayotarou et al. 2002; Su 2012). Induced resistance responses such as the accumulation of phytoalexins, toxic phenolic compounds, PR proteins and lignification of the plant cell wall occur after treatment with *R. sachaliensis* extracts (Konstantinidou-Doltsinis and Schmitt 1998; Wurms et al. 1999; McNally et al. 2003; Su 2012), and it is this induced resistance that likely explains the broad spectrum disease control reported. *R. sachaliensis* is also reported to have systemic effects against CPM (Konstantinidou-Doltsinis and Schmitt 1998). We did not investigate systemic effects during our experiments. However, leaves that were folded at the time of spraying, thus not receiving full coverage, had higher levels of infection than leaf surfaces evenly covered with the extract fluid, indicating that even dispersal of the extract is important for maximum efficiency of Sakalia for disease control.

Sakalia and the wetting agent, Yuccah, were also tested separately. Using Sakalia resulted in brown spraying residues on plants. The residues were more abundant when Sakalia was used alone than when combined with Yuccah, which may be explained by an improved dispersion when the products are combined. Since problems with spraying residues have not been described in previous studies, we also assume it could be related to the low pressure (6 bar) spraying equipment used. In commercial settings, high-pressure sprayers (50–100 bar) which improve dispersal of the products, are used. For possible implementation of this disease control strategy in commercial cucumber production, testing this product combination with high-pressure, commercial spraying equipment would be important.

Fungazil performed poorly compared to Sakalia and Yuccah combined in our experiments. This could be seen as an indication that the CPM isolates used in our experiments had developed some level of resistance towards imazalil. Azole fungicides have been leading agents for the control of fungal crop diseases since their introduction in the 1970s. However, there are increasing reports of resistance to many fungicides including azoles in plant pathogenic fungi (Hahn 2014). Therefore, our findings of reduced efficacy of Fungazil are not surprising, but rather underscore the importance of further research into alternative plant protection products for horticultural crops such as cucumber.

Products based on *R. sachaliensis* have previously been reported to act synergistically with synthetic fungicides, including azoles for the control of fungal and bacterial diseases (Su 2012). The lack of synergy between Sakalia and Fungazil observed in our study may therefore be due to the loss of efficacy of imazalil against Swedish CPM isolates rather than a lack of synergy between *R. sachaliensis* extracts and synthetic fungicides. It may also reflect the highly effective control obtained with Sakalia.

A cultivar effect has previously been reported with treatment of cucumber with *R. sachaliensis* extracts (Konstantinidou-Doltsinis and Schmitt 1998) and in our study, all treatments were consistently slightly more effective on the partially resistant cultivar. The treatments where we saw the biggest cultivar effect were Hortistar alone and Hortistar in combination with Fungazil. These treatments were significantly more effective on the partially resistant cultivar than on the susceptible cultivar. On the partially resistant cultivar, the infection in untreated controls was initially delayed a few days but by the end of experiments, the powdery mildew infection generally seemed equally severe in both cultivars.

The two biological control agents tested in 2013, did not efficiently control powdery mildew. Polyversum, formulated from the mycoparasitic oomycete *Pythium oligandrum*, was included in two experiments (Table 4) and it did not significantly suppress CPM compared to controls. AQ10, formulated from the hyperparasitic fungus *Ampelomyces quiscalis* and applied with the paraffin based additive Bioglans was included in the short-term experiment 1 (Table 4) and had a suppressive effect significantly different from the controls but was less efficient than

Fungazil. *A. quiscalis,* which is a well-known mycoparasite of CPM, is often found to co-occur at the same site as CPM fungi, and has previously been shown to be an effective treatment against some CPM fungi (Elad et al. 1998; Sedlakova and Lebeda 2010; Romero et al. 2007; Kristkova et al. 2009). Our results are in line with previous reports on the highly variable effect of *P. oligandrum* and *A. quiscalis*, often explained by their demands for high humidity or commercial formulation issues (Giotis et al. 2012; Benhamou et al. 1999).

Since we used an unconventional training system for the cucumber plants and were not able to add carbon dioxide into the cultivation system, we did not examine effects of the different treatments on yield in this study. Previous studies have demonstrated either no effect of *Reynoutria* plant extracts on cucumber yield, or a small increase in yield (Petsikos-Panayotarou et al. 2002; Wurms et al. 1999; Konstantinidou-Doltsinis and Schmitt 1998), indicating that this product may be suitable for commercial control of CPM.

Inoculating plants artificially provides a homogenous disease pressure, which is crucial for reliable and unbiased experiments, but usually results in a higher disease pressure than a commercial production system. Infection of the untreated and water control treated plants occurred uniformly throughout all experiments. However, in the final two experiments of 2015, the disease progression was slower and disease suppression greater in all treatments. Since the water control treatment was removed and these experiments only included the most efficient CPM treatments, inoculum levels and thus disease pressure were reduced. It is important to note that treatments which efficiently control the disease under our high disease pressure conditions must be considered highly efficient and thus have an even greater impact in less severe conditions, such as the natural infection levels of a commercial greenhouse.

In conclusion, treatment of cucumber plants with a combination of Sakalia and Yuccah provided the most efficient control of CPM in this study. This combined treatment applied at 7- or 14-day intervals was sufficient to control CPM in both the partially resistant and susceptible cultivar. Further testing of this treatment mixture in commercial cucumber production will allow evaluation of relevant side effects, such as spraying residues and the effects of these treatments on other diseases, beneficial organisms, insect pests and cucumber yield. Furthermore, it will allow the determination of the potential of these treatments to be used as part of an IPM program in a commercial setting.

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Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest All the Authors declare that they have no conflict of interest.

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