



Utveckling av icke-kemiska bekämpningsmetoder mot gurkmjöldagg i samverkan med odlare

Development of non-chemical greenhouse control methods against cucumber powdery mildew in collaboration with growers

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Photo: Ramesh Vetukuri

Sammanfattning

Den mest odlade växthusgrödan i Sverige är gurka. Omfattningen av ekologiskt odlad gurka är dock ganska begränsad och en av de viktigaste anledningarna till detta är svårigheter att bekämpa gurkmjöldagg utan kemiska medel. Angrepp av gurkmjöldagg kan orsaka stora skördeförluster och försämrad kvalitet. Det är därför viktigt att utveckla biologiska och andra icke-kemiska metoder för att bekämpa denna sjukdom. Gurkmjöldagg orsakas av två obligata biotrofa svampar, *Podosphaera xanthii* och *Golovinomyces cichoracearum*, som är svåra att skilja åt morfologiskt men som kan identifieras med PCR-metoder. Från högsommaren och framåt brukar *X. xanthii* vara den art som dominerar. Det finns sorter av gurka med partiell resistens men dessa är också mer ljuskrävande och ger vanligen lägre skörd.

Vi har tidigare undersökt effekten av ett antal alternativa medel mot gurkmjöldagg i forskningsväxthus (Rur et al., 2017). Vi fann då att ett medel (Sakalia®) baserat på extrakt av växten *Reynoutria sachaliensis* hade mycket god effekt liksom ett vätningsmedel (Yuccah®) baserat på extrakt av en annan växt, *Yucca schidigera*. I detta projekt har vi testat dessa medel i praktisk kommersiell odling av gurka under två år, 2017 och 2018. Vi testade även det biologiska medlet Serenade®, ett kiselmedel (Hortistar®), apelsinolja samt en laboriestam av *Pythium oligandrum*. *P. oligandrum* är en algsvamp som verkar antagonistiskt mot svampar och som använts inom biologisk bekämpning.

I det kommersiella växthuset där vi utförde försöken kunde vi inte ha en helt obehandlad kontroll eftersom det skulle innebära för stor risk för sjukdomsspridning till den omgivande kommersiella kulturen. Under det första året, 2017, fann vi att Yuccah hade mycket svag effekt och i detta försöksled blev angreppen kraftiga. Däremot hade Sakalia mycket god effekt och angreppen där blev ganska små. Även i försöksled med Serenade alternerat med apelsinolja var effekten mot gurkmjöldagg god. Den sämre effekten av Yuccah ledde till drygt 25% lägre skörd. I de andra försöksleden var skörden likartad.

I försöken 2018 kunde vi inte ha med behandlingen med Yuccah p.g.a. risken för kraftiga angrepp i omgivande kultur. Vi jämförde sjukdomsutvecklingen efter applicering av följande medel: Sakalia applicerat varannan vecka, Serenade/apelsinolja alternerat med applicering varje vecka, Hortistar applicerat varje vecka samt *P. oligandrum* applicerat varje vecka. Vi hade även med försöksled där Hortistar och *P. oligandrum* alternerades med apelsinolja. Detta år gav Sakalia den klart bästa effekten mot gurkmjöldagg trots att detta medel bara applicerades varannan vecka. Plantorna såg också, enligt både odlaren och oss själva, något mer vitala ut efter applicering av Sakalia. Även de andra behandlingarna höll angreppen på relativt låg nivå. Generellt var angreppen mindre och började senare under säsongen under 2018. Skörden var ganska likartad mellan försöksleden och generellt högre än under 2017.

Sammanfattningsvis finns flera biologiska eller andra icke-kemiska medel som har effekt mot gurkmjöldagg under realistiska förhållanden. Det växtextraktbaserade medlet Sakalia hade bäst effekt med det råder osäkerhet om detta kan komma att bli registrerat. EFSA har identifierat flera kunskapsluckor vad gäller toxicitet av medlet och det kunde inte uteslutas att komponenter i medlet hade mutagena egenskaper. Det återstår att se om detta utgör hinder för framtida godkännande.

Resultaten har underhand kommunicerats och diskuterats med jordbruksverket rådgivare och med odlaren och hans kollegor.

SLU Ekoforsk project, Final Report

Development of non-chemical greenhouse control methods against cucumber powdery mildew in collaboration with growers

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Background

The most widely grown greenhouse crop in Sweden is cucumber and is cultivated on 65 ha greenhouse area with a total yield of 28.000 tons. However, the organic production of cucumber is limited to a few growers and only one of at large scale. One of the most important reasons for the limited organic production is cucurbit powdery mildew disease (CPM), which can cause severe yield losses and problems with quality. It is very difficult to control CPM without traditional fungicides and control has been increasingly more difficult also in conventional cultivation due to a limited number of products available and the development of fungicide resistance. Therefore, there is a great need for the development of alternative biological methods for the control of CPM that can be applied both in organic and conventional cucumber production.

CPM is primarily caused by two obligate biotrophic polyphagous fungi, *Podosphaera xanthii* and *Golovinomyces cichoracearum* (Braun 1995). Both species can occur at the same time but are difficult to distinguish morphologically. However, PCR methods allow for precise differentiation. *G. cichoracearum* has a lower temperature optimum and therefore more often occurs in spring or early summer (Vakalounakis et al. 1994; Aguiar et al. 2012; Sitterly 1978). *P. xanthii*, mostly occurs from the height of the summer onward and causes more severe infections under greenhouse conditions (Sitterly 1978; Braun 1995). CPM is in general more severe under greenhouse conditions than in the field.

Partially resistant cucumber cultivars have been developed but they are more light demanding and since light is often limited in Scandinavia cucumber production, these cultivars produce lower yields (Staub and Grumet, 1993). Also, due to pathogen adaptation, the protection achieved with resistant cultivars is variable and most often not enough as a sole management practice (Lebeda et al., 2010).

Recently Serenade® has been approved as plant protection agent in cucumber (Jordbruksverket, pers. comm.). However, there are no other biopesticides or biological control agents available in Sweden that could be used against CPM (Jansson, 2016). The exception is sulphur which is permitted but commonly not used due to high risk of phytotoxic effects in cucumber (Cerkasas and Ferguson 2014; H. Hermans, Innocrop Consulting, pers. Comm.). In other countries several alternative products are registered including biocontrol agents based on fungi, oomycetes or bacteria, e.g. AQ10 (*Ampelomyces quisqualis*), Polyversum (*Pythium oligandrum*), Sporodex (*Sporothrix flocculosa*) and Serenade (*Bacillus subtilis*). Only two fungicides were previously registered for use against CPM in conventional production in Sweden. These are Amistar® (a.i. azoxystrobin), a QoI fungicide and Fungazil® (a.i. imazalil, later changed name to Diabolo), an imidazole fungicide (Jansson 2016; FRAC 2016) but recently more fungicides has been approved in greenhouse cucumber, i.e. Flexity® (a.i. metrafenone), OrtivaTop® (a.i. difenoconazole and azoxystrobin) and Topas® (a.i. Penconazole) (Johanna Jansson, Swedish Board of Agriculture, pers. comm.).

However, fungicide resistance has been reported in CPM populations (mainly *P. xanthii*) to six groups of single-site inhibitors e.g. QoIs to which azoxystrobin belongs (Lebeda et al. 2010; Miyamoto et al. 2010). Organic growers could use Serenade but for the rest rely on climate control, partially resistant cultivars, proper hygiene and sanitation measures only.

We have during recent years investigated the effect of a number of biocontrol agents and biopesticides based on plant extracts or inorganic compounds such as silicon or copper. The investigations were carried out as a participatory action research project where growers, advisors and scientists were involved in the planning of the project. We have earlier screened alternative products against CPM in medium-scale greenhouse experiments and their effect was compared with a common fungicide regime. We found that 'Sakalia®' based on extract of *Reynoutria sachaliensis* combined with a wetting agent based on *Yucca schidigera* provided efficient control of CPM. Some of the other products, e.g. Hortistar® also had a significant effect against CPM (Rur et al. 2017).

This study aimed at investigating if Sakalia would perform equally good in a commercial greenhouse where the agents were applied with professional high-pressure robot sprayers. The overall aim is to develop efficient integrated plant protection strategies against CPM for Swedish greenhouse cucumber production in collaboration with growers. We also aimed at studying possible side effects on other pest and disease problems and on the quality of the harvested cucumbers.

An original aim was also to perform laboratory studies of the mechanisms of control by the plant extract based biopesticide Sakalia. These studies were based on finances of the Department of Plant Protection Biology during another year. However, since the PhD-student found another employment and stopped the PhD education the co-funding from the Department was withdrawn. However, still those studies might still be conducted at later stage.

Initially we were in contact with two commercial growers, one organic grower and one conventional grower. Due to practical reasons and cost reasons we decided to perform all trials at the conventional cucumber grower. Another reason was that also the conventional grower had stopped using conventional fungicides and instead controlled the powdery mildew with alternative methods including the now approved biocontrol agent Serenade.

Materials and methods

Large scale trials were performed at Tomas Isberg, Sånagården, Kvidinge during 2017 and 2018. The first year Sakalia and Yucca (a wetting agent) that both showed very good performance in research greenhouse studies (Rur et al., 2017) were tested and compared with a weekly applications strategy involving orange oil (every second week) alternated with application of Serenade® (every second week) (Table 1).

In the treatments with Sakalia and Yucca applications were carried out only every second week since that gave good protection in our earlier research greenhouse trials (Rur et al., 2017). In 2017 the treatment with orange oil and Serenade had two replicates but due to practical and cost reasons the other treatments could not be replicated (See greenhouse trial plan Fig. 1a). However, each treatment consisted of 128 plants in 64 pots. One of the plants in each pot was individually scored for diseases.

The second year, 2018, Sakalia but not Yuccah was tested again and compared with the strategy with Serenade and orange oil alternated. This year we also tested the effect of Hortistar® and a biocontrol agent we work scientifically with (*Pythium oligandrum*) (Table 1). Sakalia was applied every second week and in the other treatments applications were carried out every week. Furthermore, we investigated if the effect of these agents could be improved if they were alternated with orange oil. The same numbers of plants as in 2017 were used and weekly scored for diseases (Fig1b).

Table 1. List of products/ microorganisms used in the trials.

Product name/Isolate	active ingredient	Dose/concentration*
Sakalia(Syngenta Nordics A/S, Denmark)	Extract of <i>Reynoutria sachaliensis</i>	1%
Yuccah (Plant Health Cure B.V., Netherlands)	Extract of <i>Yucca schidigera</i>	1%
Serenade (Bayer Crop Science)	<i>Bacillus subtilis</i> /lipopeptides	4%
Orographic (ORO AGRI, Netherlands)	Orange oil	1%
Hortistar (Hortifeeds Direct Ltd., UK)	Silicon	0.1%
<i>Pythium oligandrum</i> (Lab-strain, SLU, Sweden)	<i>Pythium oligandrum</i> spores	15000 spores ml ⁻¹

In all treatments the volume of spray liquid applied was 0.2 L m⁻².

Due to the risk of severe infections and disease spread in the commercial cultivation we could not have an untreated control. All applications were carried out with the grower's spray robot with a pressure of 20 bar. The inoculum of *P. oligandrum* was prepared as follows and applied with the same sprayer. Two day old mycelial plugs of *P. oligandrum* were inoculated in 10% liquid V8 and cultured for five days with gentle shaking on a shaker. Just before spray application, V8 liquid media was replaced with water by filtration, followed by maceration in blender for 5 seconds.

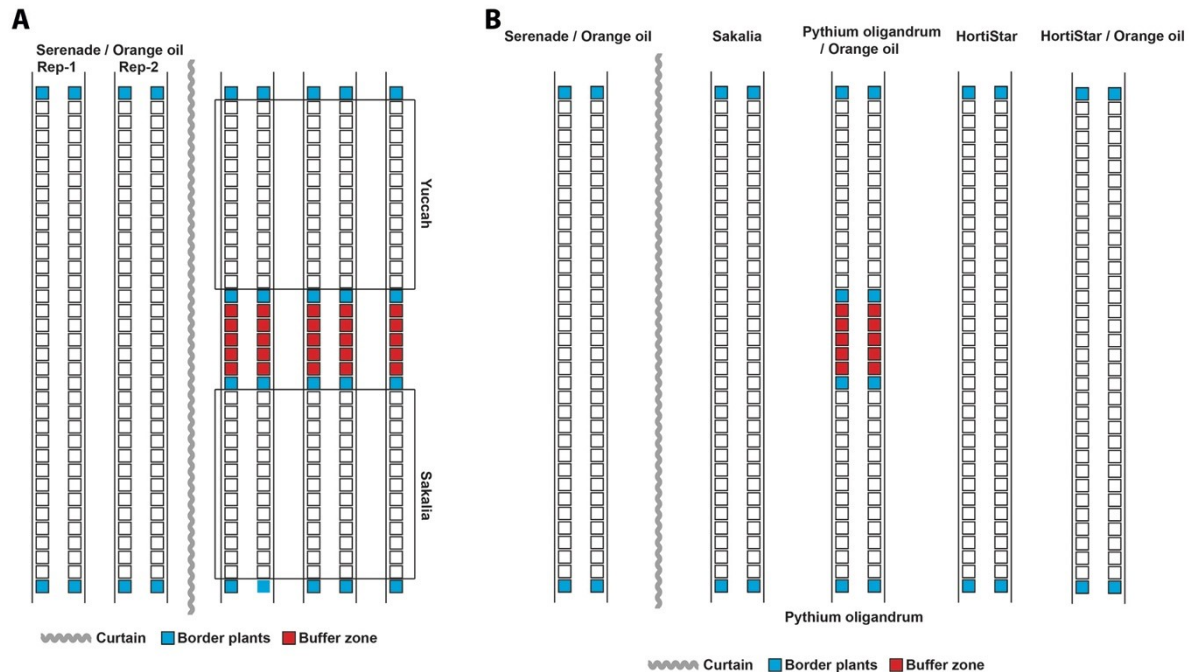


Figure 1. Design plan for the greenhouse trials 2017 (A) and 2018 (B).

Disease assessments

After first observation of disease symptoms each plant was weekly scored for cucumber mildew disease severity (% infected leaf area). On each plant 4 individual leaves, representing the whole plant, were scored and the mean percentage infection was calculated for each plant. Based on the weekly scoring of disease severity the area under the disease progress curve was calculated using the following formula (Shaner & Finney 1977):

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

where Y_i is disease severity at the i^{th} observation, X_i is time (days) at the i^{th} observation and n is the total number of observations. Relative AUDPC (rAUDPC) was calculated as follows: $\text{rAUDPC} = \text{AUDPC}/\text{maxAUDPC}$. Data collected between July 15 and October 15 were used for the calculation both years.

Yield

The yield of both first- and second-class cucumber fruits was weighed at each harvest occasion and summarized as total yield per crop season for each treatment or treatment replicate. The yield was obtained per row and not per individual plant. The total numbers of harvested cucumbers were also counted for each treatment.

Other observations

The presence of other diseases, like greymold, and insect or spider mite infestations were also recorded at each disease-scoring occasion.

Statistical analysis

Each row of plants within each treatment had 30 pots with 2 plants in each that were individually scored for disease severity every week. For each pot, except for the border pots the rAUDPC during the infection period was calculated (July 15 until october 15, 2018). We considered each plant Number in east-west direction as a block. Thus, the experiment was considered to have 28 blocks when the border plants were excluded. The problem is then that the treatments were not randomized within each block. Therefore, we investigated if there were any gradient in the infection rate within the trial area both in east-west direction and in north-south direction. First, for each treatment ANOVA was carried out with plantNo as block and rowNo as treatment. Since all p-values were above 0.18 we concluded that there was no gradient in infection rate in the east-west direction of the trial area. To investigate if there were any gradient in the north-south direction the correlation between plant number and rAUDPC was calculated for each treatment. For two of the treatments no significant correlation was found but for the other two the correlation was significant. However, the change with plant number was much smaller than the differences between the treatments. Based on that we concluded that there was no gradient in infection rate within the experimental area and therefore performed an analysis of variance to investigate the significance of differences in infection rate among the treatments. We did three analysis, first with all 28 plants as blocks, then we grouped the plants into 7 (with 4 plants in each block) or 4 (with 7 plants in each block) blocks. All three analysis gave the same result.

Results

Powdery mildew infection rate

In 2017 the infections started already in the beginning of July, while in 2018 the first symptoms of CPM were observed in the beginning of August. Both years the second cultivations planted in the beginning of June were used for the experiments. The infection pressure was thus higher 2017 compared to 2018 as is evident from the disease progress curves (Fig 2). That was also evident from the calculated area under the disease progress curve.

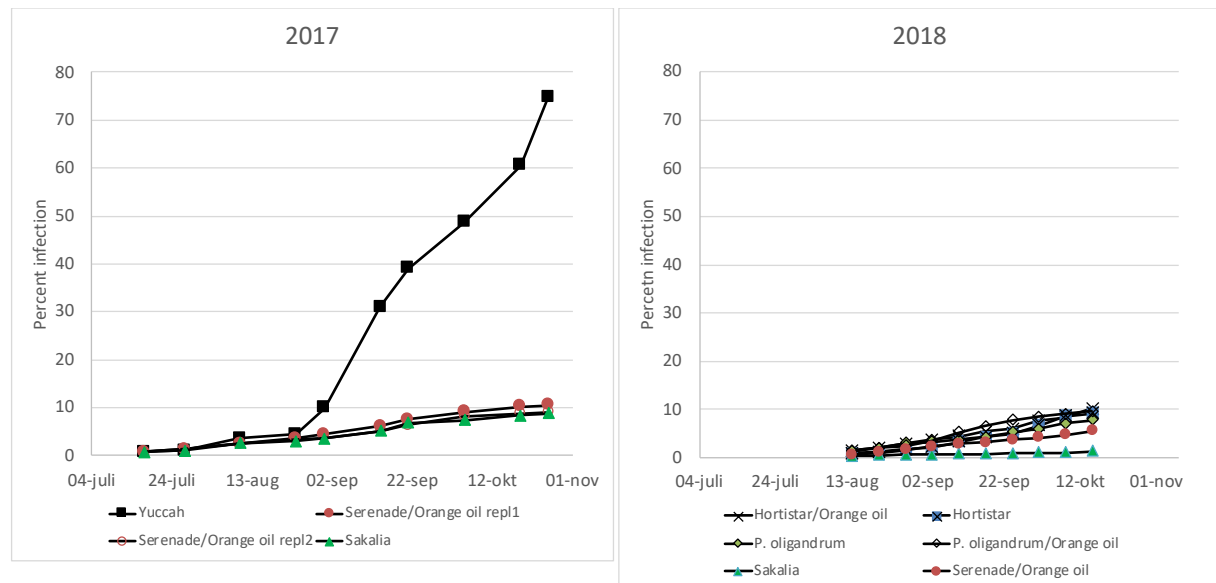


Figure 2. Disease progress curves of cucumber powdery mildew in a commercial greenhouse cultivation 2017 and 2018. Each point is the mean percent infection of 2 rows with 30 plants in each. The mean coefficient of variation between replicate rows was 8,2 % (2018).

We could not have an untreated control in the experiments because it would mean a too high risk for the commercial production in the rest of the grower's greenhouses. However, in 2017 we tested the wetting agent Yuccah alone and that resulted in very poor control with severe infections, indicating that the other treatments had a good effect against CPM. The efficacy of Sakalia seemed to be similar to the treatment with orange oil and Serenade alternated despite that Sakalia was applied only every second week. The grower's and our's general impression were that the plants looked somewhat more vigorous in the treatment with Sakalia. Due to the limitations in randomization of the trial and that the infection rate among the treatments, except for Yuccah, were quite similar we did not statistically analyse the data.

In 2018 we did not include Yuccah as a treatment but compared the effect of Sakalia with the strategy with orange oil and Serenade alternated, Hortistar, and Hortistar alternated with orange oil. In addition, we tested inoculum of the biocontrol labstrain of *Pythium oligandrum* alone or alternated with orange oil. The infection rate appeared to be lowest with Sakalia (Fig 3) followed by the strategy with orange oil and Serenade alternated. Thus, treatment with Sakalia had the lowest infection rate despite that applications were carried out every second week, while in all other treatments applications were done every week. Of practical reasons in the commercial greenhouse we neither in 2018 could obtain a randomized experimental design with replicates thus not allowing a common analysis of variance. However, we did an analysis of variance after excluding gradient effects in the greenhouse (see materials and methods). Four of the treatment (Orange oil/Serenade, Sakalia, Hortistar and Hortistar/Orange

oil) where all of the plants were treated similarly were included in the analysis. The rows in the middle where half of the plants were treated with *Pythium oligandrum* and the other half with *Pythium oligandrum*/Orange oil) was not included in the analysis. Differences in rAUDPC among the treatments were tested with Tukey's multiple range test and showed that the infection rate differed significantly among the treatments (Figure 4). The treatment effect in the ANOVA model had a F-value of 78 and a p-value <0.0001.

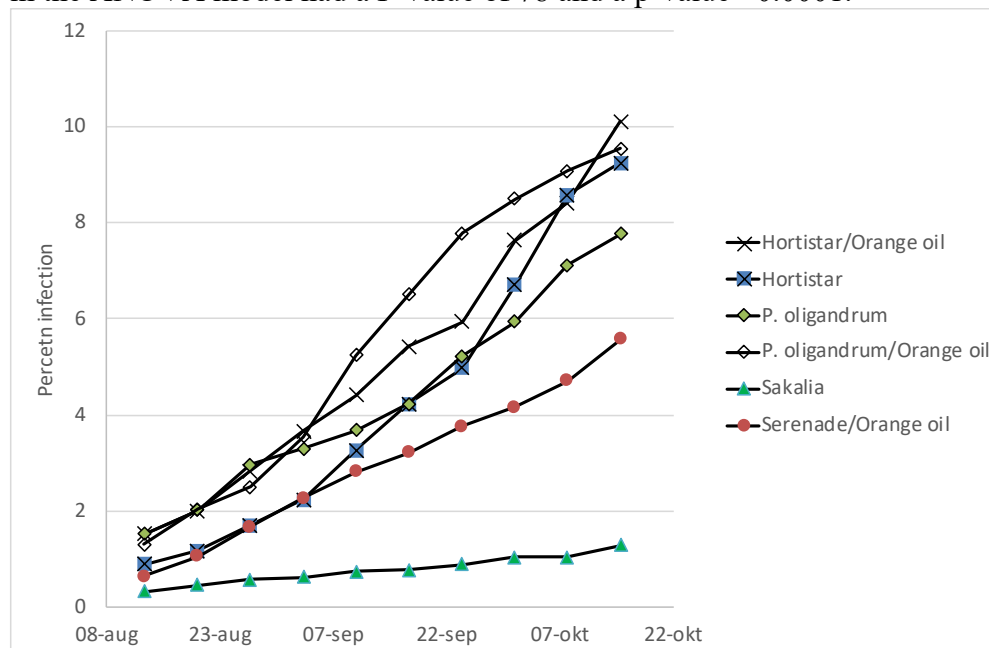


Figure 3. Disease progress curves of cucumber powdery mildew in a commercial greenhouse cultivation in 2018. Plants were treated with different biocontrol or other non-chemical agents. The mean coefficient of variation between replicate rows was 8.2 % (2018).

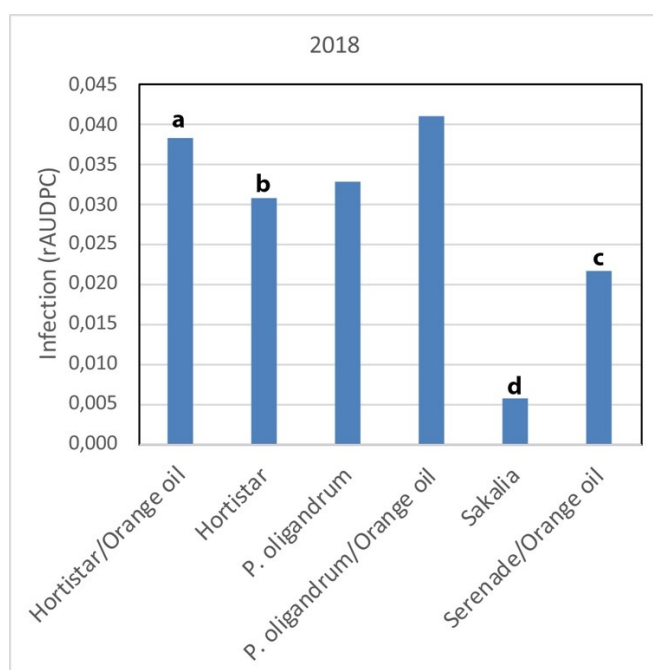


Figure 4. Infection rate cucumber powdery mildew after different treatments with biocontrol and other non-chemical agents expressed as area under disease progress curve (rAUDPC) in 2018. The mean coefficient of variation among plants within treatment was 6.7 %. Different letters indicate significant difference according to Tukey's test. The treatments with *P. oligandrum* was not tested due to that those treatments had fewer plants.

Both the biocontrol treatment with *P. oligandrum* and the treatment with Hortistar was also tested in a spraying regime that alternated these treatments with orange oil to see if that could improve the level of control (Fig. 3). However, the alterations did not result in improved control compared to the treatments on their own. Instead there was a tendency that using the agents alone gave better protection. Hortistar alternated with orange oil resulted in significantly higher rAUDPC compared to using Hortistar alone (Fig. 4). For *P. oligandrum* the comparison between the biocontrol agent alone or with orange oil treatments was not significantly different even if the trend observed was the same as with Hortistar.

Yield

The yield was pooled from each row, as it was collected by the staff employed by the grower, so we could not get yield data from each individual plant. The data presented here are accumulated yield over the season. Both years it was the second cucumber crop that was planted in June and finalized in the middle of October.

In 2017 the yield was substantially lower in the treatment with Yucca which seemed to be due to the much higher infection rate of CPM in that treatment. The total yield among the other treatments were similar, just over 10 kg/plant in average (Table 2). About 90% of the cucumber fruit yield were first class in all treatments.

Table 2. Cucumber yield in a commercial greenhouse after treatments with different biocontrol and other non-chemical agent against CPM.

Treatment	Yield (kg/plant)			No fruits/plant	Mean fruit weight (kg)
	1st class	2nd class	Total		
2017					
Yuccah	6.64	0.98	7.62	17.4	0,44
Serenade/Orange oil, Repl. 1	9.27	1.09	10.37	22.1	0,47
Serenade/Orange oil, Repl. 1	9.24	1.14	10.38	23.5	0,44
Sakalia	9.63	1.02	10.65	24.4	0,44
2018					
Sakalia	12.94	3.70	16.64	22.2	0,75
Serenade/Orange oil	13.09	2.49	15.58	23.8	0,66
Hortistar	13.65	2.33	15.98	24.2	0,66
Hortistar/Orange oil	12.52	4.57	17.09	21.8	0,78
<i>P. oligandrum</i>	13.20	n.d.	n.d.	n.d.	n.d.
<i>P. oligandrum</i> /Orange oil	14.75	n.d.	n.d.	n.d.	n.d.

n.d. = not determined

In 2018 the average total yields were higher ranging from 15,5 to 17 kg/plant over the whole season. Due to lack of replications we cannot conclude if there were any significant differences in yield between the different treatments. In 2018 the proportion 1st class yield seemed a bit lower, 75-85 %, than in 2017.

Relationship between infection rate and yield

The yield in the different treatments were substantially higher 2018 compared to 2017 and that could be for a variety of different reasons. The infection rate was lower in 2018 but probably the increased yield has other reasons, e.g. different climate due to the unusual warm summer in 2018. Therefore, we normalized the data by calculating the relative yield compared to the Orange oil/Serenade treatment within each year and then plotted it against

the infection rate (rAUDPC)(Fig 5). Even though there was only one point with very high infection pressure the correlation between yield and infection was significant ($R^2=0,88$, $p<0,01$). This indicated that during severe infection the yield loss can be 25% or more, while it did not seem to be a significant relationship between yield and infection if the rAUDPC was lower than 0,05.

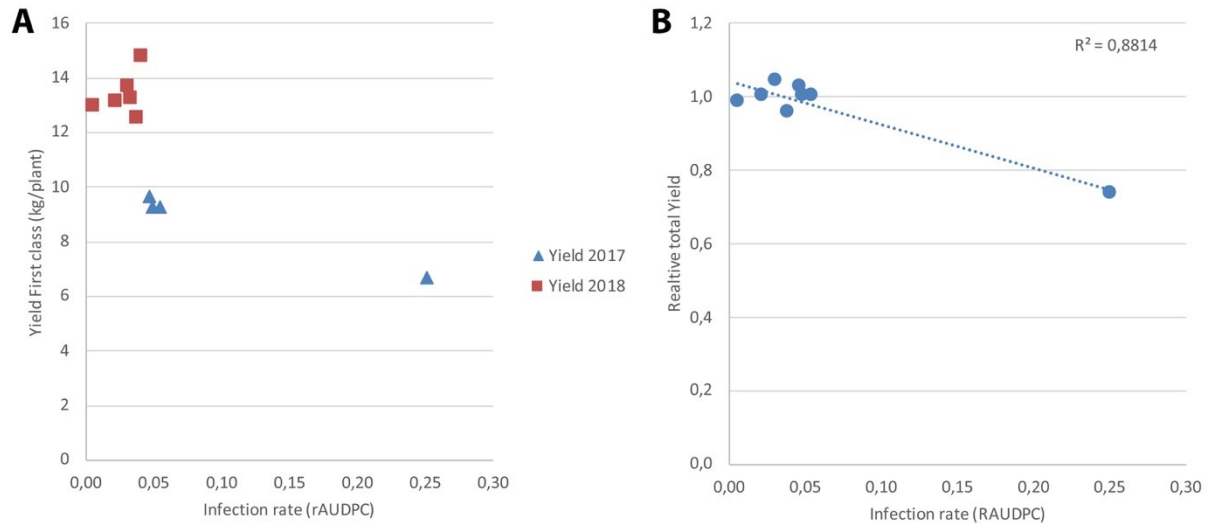


Figure 5. Relationship between yield and infection rate of CPM. A: First class yield 2017 and 2018. B: Relative total yield within year for 2017 and 2018.

Other observations

Grey mold or other fungal diseases were only occasionally observed at very low rate. Infestations of insect pests were also only found at low rate and did not seem to differ among the treatments. In 2018 a spider mite infestation was observed but the severity did not differ among the treatments. The grower's and our impression were that the plants treated with Sakalia looked somewhat healthier than in the other treatments both years. In the earlier published studies in research greenhouse, application of Sakalia (Rur et al., 2017) sometimes lead to brownish precipitation on the leaves. However, in these trials that problem was not observed probably due to the application with the professional high-pressure robot sprayer resulting in a better atomization of droplets.

Discussion/Conclusions

The trials within a commercial greenhouse production of cucumbers confirmed earlier results on efficient control of cucumber powdery mildew by the plant extract-based product Sakalia. However, in the earlier greenhouse study we also found good effect of a wetting agent Yuccah but in these trials that resulted in severe infections. Also, Hortistar (a silicon agent), orange oil and Serenade seemed to control CPM at an acceptable level. Further we tested a labstrain of *Pythium oligandrum* that also protected against CPM. However, Sakalia had by far the best efficacy followed by Serenade and orange oil in an alternated treatment regime. Thus, there are potentially several non-chemical products that could control cucumber powdery mildew in organic production. These are important also for conventional growers since the efficacy of the available fungicides have been impaired due to development of fungicide resistance in the pathogen populations (Rur et al. 2017) and since we wish to encourage conventional growers to take an IPM approach and reduce the use of synthetic pesticides.

It is often discussed whether combination or alternations of control agents with different mode of action will perform better than using the same control agent all through. We tested that by comparing Hortistar and *P. oligandrum* alone with treatment where they were alternated with orange oil. The tendency was that the level of control was better when the agents were applied alone. I might be a different situation if they can be combined at the same application time and that also needs to be tested. Still, an advantage of alternating agents with different mode of action is that it is less likely that the fungus evolve strains that can overcome the effect of the control agent.

We could not have an untreated control in our trials due to the risk of heavy infections in the commercial crop. However, the first year on of the treatments (Yuccah) had very poor effect. The much higher infection rate in that treatment resulted in more than 25% yield loss. With an untreated control maybe even higher yield losses could have been noted.

Using Sakalia and the other products did not result in any visible side effects, like phytotoxic effects, quality issues on the harvested cucumber fruits or changed patterns of insect infestations. Instead the general impression was that the crop looked healthier and more vigorous after treatments with Sakalia. Another advantage was that similar or better protection was obtained with a longer application interval.

However, Sakalia is not approved for registration as a plant protection agent in EU and it is uncertain if that will be approved in the future. EFSA has published a peer review of risk assessment of *Reynoutria sachalinensis* extract (EFSA 2015). Concerns was reported regarding mammalian toxicology since several data gaps concerning e.g. acute toxicity studies was identified and a risk assessment for operator, worker, bystander and resident exposure could not be performed. EFSA further reported that a mutagenic effect of *Reynoutria sachalinensis* ethanolic extract cannot be excluded. Thus, the future of using extracts of *Reynoutria sachalinensis* for plant protection purposes is uncertain.

For conventional growers a few more fungicides have been approved in cucumber as mentioned in the introduction. For organic production there is still too few products that are approved for use against CPM. However, besides Serenade (a.i. *Bacillus subtilis*) there is recently a new product available (Amylo-X) based on another *Bacillus* species (*Bacillus amyloliquefaciens* subsp. *Plantarum*) (Johanna Jansson, Swedish Agricultural Board, pers. comm.) and it would be interesting to test its efficacy against CPM in similar trials.

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