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ORIGINAL ARTICLE

Reduced efficacy of biocontrol agents and plant resistance inducers against potato early blight from greenhouse to field

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Abstract

Early blight in potato, caused by *Alternaria solani*, is mainly controlled by frequent applications of synthetic fungicides. Reducing the use of synthetic fungicides in agriculture is desired to reach an overall sustainable development since the active components can be harmful for humans and for the ecosystem. In integrated pest management, IPM, the idea is to combine various measures, including optimized crop management, crop rotation, use of resistant cultivars, biological control agents (BCAs), plant resistance inducers, and fertilizers, to decrease the dependence on traditional chemical fungicides. In this paper, we present the results from greenhouse and field trials where we evaluated the effect of strategies aimed at reducing our reliance on synthetic fungicides including treatments with biological control agents (BCAs) (*Pythium oligandrum*, Polygandron®, and *Bacillus subtilis*, Serenade®) and plant resistance inducers (silicon products HortiStar® and Actisil®) for early blight in potato. The agents were applied separately or in combination with each other or with synthetic fungicides. In the greenhouse, trials application of these agents resulted in 50–95% reduction of infection by *A. solani*, but their combination did not generally improve the outcome. However, the effects were much smaller in the hand-sprayed field trials, 20–25% disease reduction and almost disappeared in full-scale field trials where application was done with tractor sprayers. In this article, we discuss possible reasons behind the drop in efficacy from greenhouse trials to full-size field evaluation.

Keywords *Alternaria solani* · Biological control · Biocontrol agents · Plant resistance inducer · Potato disease · Field trials

Introduction

The fungus *Alternaria solani* is a soil-borne pathogen causing early blight in several Solanum species including potato (*Solanum tuberosum* L.). *A. solani* overwinters in the soil and causes infection when the right climate is obtained for development of disease. Early blight affects tuber yield globally, and yield losses of up to 50% have been reported (Leiminger and Hausladen, 2012). Besides late blight, caused by *Phytophthora infestans,* early blight is one of the most important foliar diseases in potato (Abuley et al., 2019). Early blight affects starch potato yield in southern Sweden, causing earlier defoliation of the plants (Andersson and Wiik, 2008). Starch potato cultivars are harvested later in the season than ware potato cultivars since the starch is stored in the tubers later in the summer. Most of the Swedish table potato is already harvested when the early blight infection strikes in Sweden, while the yield of potato starch can be significantly reduced. Therefore, starch potato cultivars are more affected by the pathogen *A. solani* in southern Sweden.

To control early blight infection, synthetic fungicides are traditionally used, but to reach a more sustainable agriculture it would be beneficial to exchange some of these chemical treatments with biological equivalents. According to the EU Directive (2009/128/EC), the dependence on chemical pesticides should be reduced by combining alternative

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measures. Biological control agents (BCAs) in this case bacteria or oomycetes are natural antagonists to the pathogens and are thus used to control diseases. The BCAs can either parasitize or in other ways, through antibiosis or nutrient competition, outcompete the unwanted pathogen (Gao et al., 2017). Additional nutritional supplements or plant resistance inducers (PRIs) that are not classified as synthetic fungicides may also replace or complement traditional chemical treatment strategies in order to develop more sustainable disease management methods in agriculture. Another important reason to search for alternative disease control methods is that fungicide resistance is developing quickly in the *A. solani* population in response to fungicide applications (Odilbekov et al., 2019; Mostafanezhad et al., 2021) resulting in a vulnerable crop production. There are only a limited number of efficient fungicides against early blight currently available for farmers. This causes a vulnerability in Swedish potato cultivation and increases the risk of fungicide resistance development in the pathogen population.

There are several alternatives to synthetic fungicides that have shown effectiveness against early blight in greenhouse and field trials. The biocontrol agent *Pythium oligandrum* has been shown to have effects on a wide variety of plant pathogens in different crops, like damping-off of sugar beet caused by *Pythium ultimum* (Martin and Hancock, 1987), bacterial wilt of tomato caused by *Ralstonia solanacearum* (Hase et al., 2008), *Verticillium* wilt in pepper caused by *Verticillium* spp. (Rekanovic et al., 2007), and grapevine trunk wood disease caused by *Phaeomoniella chlamydospora* (Yacoub et al., 2016). Ikeda et al. (2012) reported that treatment of potato seed tubers with *P. oligandrum* oospores significantly decreased black scurf disease severity index on stolons caused by *Rhizoctonia solani* in field conditions. This decrease was at a same level as that caused by Flutolanil®, a chemical fungicide commonly used to treat black scurf. Kurzawińska and Mazur (2009) showed that potato tuber dressing and/or plant spraying with Polyversum® (a commercial formulation of *P. oligandrum*) significantly decreased late blight disease infection caused by *P. infestans* in the field, at the same level as the chemical pesticide Vitavax 2000 FS (Active components karboxin and thiuram).

Abbasi and Weselowski (2014) studied the effect of weekly foliar sprays of commercial formulations of *Bacillus subtilis* in the form of dried (Serenade MAX®, 1 kg/ha) and aqueous suspension (Serenade ASO®, 4 L/ha) on foliar early blight disease of tomato during 2008–2010.

Their field trials during 2008-2010 showed that Serenade ASO had a significant effect on early blight development based on both rAUDPC (relative area under disease progress curve) values and final disease severity rating in 2008. Treatments with Serenade MAX also significantly reduced early blight infection in field trials for tomatoes conducted in 2009. Egel et al. (2019) studied the effect of Serenade® in the management of *A. solani* on tomato plants in greenhouse and two field sites, where the field sites had different climatic conditions. In the greenhouse studies, Serenade® was used alone as a treatment and it significantly decreased early blight disease levels in two out of three greenhouse trials. In the field studies, Serenade® was alternated with botanical product Regalia® (a commercial formulation of the plant *Reynoutria sachalinensis*). The treatment regime in which Serenade® and Regalia® were applied alternatively did not significantly decrease disease levels compared to the untreated control (Eget et al., 2019).

In addition to BCAs, there are other low risk alternatives for possible use against plant pathogens like PRIs. Silicon, the second most abundant element on earth (Kumaraswamy et al., 2021), has been used against different pathogens in potato as well as other crops. Silicon may strengthen plant cell walls or induce defense responses in plants (Wang et al., 2017). Gulzar et al. (2021) showed that treating tomato plants with silicon (in the form of potassium silicate, 1.7 mM), increased resistance to *A. solani*. Spraying silicon (in the form of Na_2SiO_3, 100 mM) on potato leaves enhanced potato resistance against another common potato disease, late blight, caused by *Phytopthora infestans,* in a detached leaf assay (Xue et al., 2021). However, the effectiveness of silicon to protect potato in the field from either late blight or early blight has not been thoroughly investigated in the literature.

In the present study, biocontrol agents (BCAs) and plant resistance inducers (PRIs) were tested against early blight in greenhouse and field studies. To be able to practically integrate BCAs and/or PRIs into IPM strategies, reliable data showing efficacy under agricultural relevant field situations are needed. Even though it is known that alternative products have lower efficacy than fungicides, direct comparisons between greenhouse and field settings such studies are rare in the literature. Based on the previous promising studies, several treatments including the BCAs Serenade® (*B. subtilis*), Polygandron® (*P. oligandrum*) and an oospore suspension of *P. oligandrum* (prepared in the laboratory), and the PRIs/silicon fertilizers HortiStar® and Actisil® were used in the present study. The aim was to evaluate their efficacy against early blight disease in greenhouse experiments and field trials to conclude if results from the greenhouse can help predict the efficacy under field conditions. Since knowledge about how to combine or alternate these alternative products with traditional synthetic fungicides is also needed, traditional fungicide treatments and combinations of fungicides and alternative treatments were included in the trials. The experiments were conducted in three phases, greenhouse trials, small scale field trials with manual application of the treatments and large field trials with tractor sprayer applications. The main questions were: 1) Is the efficacy against early blight disease consistent between the greenhouse and the field, or between field trial plots of different sizes? 2) Do combinations of different alternative treatments improve efficacy against early blight disease? 3) Can traditional fungicide treatments applied in lower amounts be combined with alternative treatments give sufficient disease control?

Materials and methods

In this study different alternative measures are evaluated against early blight infection. Four organisms/products; *P. oligandrum* (also as Polygandron®), Serenade®, Actisil® and HortiStar® are tested alone or in combinations in three different settings; Greenhouse, small plot field trials and large plot field trials (Table 1). In greenhouse experiments, the products were diluted in distilled water, while in all field trials non-chlorinated water from a well at the experimental farm was used.

Preparation of *Alternaria solani* inoculum

Alternaria solani isolate AS112, isolated from a field in Sweden, was used in the greenhouse experiments. To obtain a spore suspension, the fungi was grown on 20% strength potato dextrose agar medium (PDA) supplemented with 12 g L^{-1} Bacto Agar in 9 cm petri dishes and incubated at 25 °C for 7 days in darkness. To increase sporulation, the plates were incubated for another seven days under UV-C light (254 nm dominant wavelength) for 5–6 h per day. Conidia were harvested by flooding the plates with Milli-Q distilled water containing 0.01% (v/v) Tween 20, while conidia were dislodged using a sterile L-shape cell spreader. The final concentration of the conidial suspension was adjusted to 10^4 conidia per mL using a hemocytometer. To ensure the adherence of conidial suspension at the inoculation site on the leaves surface, the conidial suspension was supplemented with 0.1% Bacto Agar (Odilbekov et al., 2014).

Pythium oligandrum preparation

Slightly different formulations of *P. oligandrum* were used in the greenhouse and field trials over the three years, due to new registration of a formulated product to the Swedish market in March 2019. To produce inoculum for the greenhouse and field trials in 2018 and 2019, solid agar plates of V8 media were inoculated with one agar plug of *P. oligandrum* (CBS-strain 530.74) and allowed to grow for seven days at 20 °C. From the solid *P. oligandrum* cultures, five agar plugs were inoculated into 1L bluecap bottles containing 300 mL clarified V8 broth. The bottles were put into a rotary incubator, shaking at 120 rpm at 20 °C for seven days. To harvest the oospores from the liquid cultures, the mycelia were macerated using a high-speed blender and 200 mL of sterile water was amended. The inoculum was then filtered. A final concentration of 2.5×10^4 oospores/mL was obtained. In 2020 field trials,

Table 1 Overview of all the treatments performed in different settings

Treatment	Active ingredient	Type	Green house	Small field	Large field	Field trial year
P.oligandrum lab formulation		BCA	x	x		2018–2019
Polygandron	*P.oligandrum*	BCA	x	x		2020
Serenade	*B.subtilis*	BCA	x	x	x	2018–2020
Actisil	Silicon + Calcium + Cholinechloride	PRI	x		x	2016–2017
HortiStar	Silicon	PRI	x	x	x	2018
Combinations:						
P. oligandrum + Serenade	x	x				2018–2019
Polygandron + Serenade	x	x				2020
P. oligandrum + HortiStar	x	x				2018
Polygandron + HortiStar	x					
HortiStar + Serenade	x	x				2018–2019
Serenade + HortiStar + Polygandron	x	x				2020
Fungicide + Actisil					x	2016–2017
Alterations:						
Serenade/Fungicide					x	2019–2020
HortiStar/Fungicide					x	2018

the registered product from Biopreparaty, Polygandron WP, batch 08,022,020, with a concentration of 5×10^5 oospores per gram, or 200 g/ha, was diluted with well water at the trial site and applied according to the label corresponding to a dose of 300 L liquid/ha. A decision was made in 2020 to use the formulated product and not produce inoculum at SLU, since it would make the field trials easier to reproduce later and to handle during the season.

Serenade® preparation

The registered product Serenade® ASO from Bayer Crop Science containing *Bacillus subtilis*, strain QST 713, containing a minimum of 1.05×10^{12} cfu/L according to the label was used. For the greenhouse trials, 12.5 mL of Serenade® was diluted with tap water resulting in a concentration of 0.5% Serenade®. The same process was done using well water for the small trials. For the large trials, Serenade® was applied in a concentration of 2.0–6.0 L/ha diluted with well water to a total liquid dose of 300 L/ha. Slightly different doses were used for the large field trials. The dose was increased the second season from 2.0 L/ha in 2019 to 4.0 L/ha in 2020. Also, five treatments in 2019 were decreased to three in 2020 for the Serenade® only treatment, and the first application, T0, was done earlier in 2020 to enable earlier colonization of soil and lower leaves (Table 3a and b and supplementary files Table 2). The treatment consisting of reduced fungicide, with two full-dose sprays instead of four, was only present in the trial in 2020.

HortiStar® preparation

HortiStar® is a product containing silicate foliar fertilizer from Hortifeeds with a silicon content of 19%. 2.5 mL of HortiStar® was diluted with tap water resulting in a concentration of 0.10% HortiStar® for using in the greenhouse trials. The same process was done using well water for the small trials. For the large trial in 2018 HortiStar® was added at a dose of 0.5 L/ha.

Actisil® preparation

YaraVita Actisil® is a silicon containing fertilizer from Yara marketed as a plant strengthener. The silicon is present in the available form of stabilized orthosilicic acid. Actisil® also contains choline and calcium. According to the label, Actisil® will increase the cell wall stability and further increase the natural resistance. For greenhouse experiments, Actisil® was evaluated for two different potato cultivars, Désirée and Matilda. Actisil® was sprayed 24 h prior to

Table 2 rAUDPC and rAUC results from year 2018–2020 in the small hand-sprayed trial testing disease reduction from different substances and combinations. The analysis was done both separately for each year and pooled across years. Different letters indicate statistical differences ($p < 0.05$) according to the post hoc Tukey test for all years pooled, except for the treatments only present in some of the years where the analysis was done separately for the years that treatment was present

Treatment	Brand name	Active component	rAUDPC				rAUC			
			2018	2019	2020	mean	2018	2019	2020	mean
Untreated control			0.028 a	0.118 a	0.019 a	0.055 a	0.036 a	0.191 a	0.033 a	0.087 a
P. oligandrum	Polygandron WP (2020)*	*Pythium oligandrum*	0.019 ab	0.093 ab	0.016 a	0.043 b	0.028 a	0.156 ab	0.034 a	0.073 b
Serenade	Serenade ASO	*Bacillus subtilis*	0.015 b	0.093a b	0.011 a	0.040 b	0.027 a	0.156 ab	0.030 a	0.071b
HortiStar	HortiStar	Silicate	0.018 ab	0.093 ab	0.015 a	0.042 b	0.032 a	0.157 ab	0.031 a	0.073b
Serenade + *P. oligandrum*			0.013 b	0.087 ab	0.019 a	0.040 b	0.026 a	0.151 b	0.032 a	0.070b
P.oligandrum + hortistar			0.017 ab				0.030 a			
Serenade + hortistar			0.017 ab	0.090 ab			0.030 a	0.147 b		
Serenade + hortistar + *P.o ligandrum*					0.011 a				0.026 a	

*The formulated product Polygandron WP® was used in 2020. In 2018 and 2019, the inoculum was produced in laboratory inoculation with *A. solani* (as a 0.1% solution on the foliage). The effect of YaraVita Actisil® was evaluated in 2016 and 2017 large field trials. Actisil® was used in a dose of 0.4 L/ha diluted with well water to a total liquid dose of 300 L/ha.

Greenhouse experimental design

The greenhouse experiments were performed at the Swedish University of Agriculture, in Alnarp, Sweden. Five separate greenhouse trials were conducted to examine the efficacy of different treatments (Table 1). The experiments had a randomized complete block design with 4–6 replicate blocks.

Plant material preparation and growth conditions

Solanum tuberosum cv. Désirée and cv. Matilda was grown by subculturing of 3-week-old stems cutting to around 2 cm with one leaf on Murashige and Skoog (MS) media (30 g/L sucrose, 8 g/L phyto agar, 4.4 g MS, pH 5.8), in tissue culture boxes. The boxes remained in a phyto chamber with 16 h of light (140 µE) per day for 21 days. After that, the in vitro plantlets were transferred to 2.5 L plastic pots in a greenhouse chamber with adjusted temperature to 22 °C with 16 h of natural day light supplemented with artificial light. In all greenhouse experiments, only the cultivar Désirée was used except in the first greenhouse experiments where both Désirée and Matilda cultivars were used.

Greenhouse treatments

Forty-five days after transferring the plants into the greenhouse, plants were sprayed with Serenade®, *P. oligandrum*, Polygandron®, HortiStar® or Actisil® using a 600 mL hand sprayer until run-off. Oospore suspension of *P. oligandrum* lab strain (CBS-strain 530.74) was also added to the soil (20 mL) in the second and third greenhouse experiments as a separate treatment. Combined treatments (Table 1) were sprayed separately with 1–2 h interval for the foliage to dry. After 48 h (24 h for Actisil® experiment), the plants were inoculated by placing a drop of 10 µL *A. solani* conidial suspension on the surface of 10 chosen leaflets (5 leaflets per leaf) in the middle part of the plant. A tent was constructed to maintain high humidity (around 95%) during the first 24 h after inoculation. Then, relative humidity was stabilized at 85% using a misting system within the chamber.

Disease assessment

Ten days after inoculating the plants with *A. solani*, disease development was estimated by measuring the diameter of the lesions in two perpendicular directions using a vernier caliper supposing an oval area. Then the lesion area, LA, was calculated as the following equation:

$$LA = 0.25 \times \pi \times D1 \times D2$$

where D1 and D2 are the diameters in millimeters.

Synergy calculation

In the combined treatments, the synergy factor (SF) was calculated according to the Abbott method (Abbott, 1925):

$$SF = C_{obs}/C_{\exp}$$

where C_{obs} is the observed disease protection ratio and C_{exp} is the expected disease protection ratio. A SF value greater than 1 indicates synergistic interaction, and a SF value smaller than 1 indicates antagonism interaction between the compounds of a treatment. C_{exp} was calculated as:

$$C_{exp} = A + B - A \times B$$

for two-compound treatments, and as:

$$C_{exp} = A + B + C + A \times B \times C - A \times B - B \times C - A \times C$$

for three-compound treatments. A, B and C in the above equations denote the observed disease protection ratios of the single compounds.

Field trial experimental design

Field trials were conducted from 2016 to 2020 in southern Sweden at two different sites. In all the field trials, the starch potato cultivar Kuras was used since it is the most common cultivar used in this part of Sweden for potato starch production. For the trial with Actisil in 2016, there was also an additional starch cultivar, Stayer, included. Kuras and Stayer have been noted to be susceptible for early blight (unpublished data). The seed tubers were obtained from Lyckeby, SSF. Both the large and the small field trials were fertilized, and managed following standards set by the Swedish Rural Economy and Agricultural Societies, as seen in the supplementary material, in Mosslunda, south of Kristianstad, Sweden. The potatoes were planted with a row distance of 75 cm and a planting distance of 38 cm. No inoculations with pathogens were done in any of the trials. Natural early blight infection did occur every year (Fig. 2). Standard treatments to control late blight, insects, and weeds were implemented throughout the season according to the supplementary files. Irrigation was done when needed.

Small plot field trials

The small field trials were placed in Helgegården (56.018696, 14.064942) in 2018, 2019 and 2020. Hand sprayers of the brand Ferrox 5L model 3565 were used for application and the formulations were diluted with well water to correspond to 300 L liquid/ha. The applications were done at a pressure of three bars five times over the season with two-week intervals starting in the beginning of July. The combined treatments were sprayed twice without the solutions being mixed. In between the two treatments, enough time passed for the foliage to dry. The layout consisted of four blocks with a randomized complete block design. Each plot consisted of four 2.0 m long rows of potatoes where the two middle rows were treated and visually scored.

Large plot field trials

The large-scale field trials were carried out in 2016, 2017, 2018, 2019 and 2020 at two locations, Helgegården (56.018696, 14.064942) and Nymö (56.024848, 14.335998), in southern Sweden. The setup was a randomized complete block design with four blocks with plots of 10 m in five rows where the three central rows, 18 m^2, were harvested. The yield and starch content were measured, and total yield and starch yield were converted to yield/ha. The starch content of the tubers was calculated from measurements of specific weight (International Starch Institute Denmark, 1986). A tractor sprayer (Lechler IDKT Purple 0,25) with a flat fan nozzle medium droplet size was used for application at 300 L liquid/ha at a pressure of three bars.

Field trial treatments

Four different treatments were evaluated from which two are classified as BCAs, *P. oligandrum* and *B. subtilis*, and two as fertilizers/PRIs, HortiStar® and Actisil® (Table 1). The date of each treatment is presented as T1, T2, T3, etc., where the exact date for each year's T1 can be found in the supplementary files. The treatments following T1 were applied with 1 week intervals.

Field disease assessment

In the field trial assays, the level of early blight infection and defoliation was visually scored weekly according to Duarte et al. (2013). Infection was defined as the percentage of green leaf area covered by typical dark early blight spots, and defoliation was defined as the percentage of the total canopy that was dead or defoliated. The relative area under the disease progress curve, rAUDPC, as well as the area under the defoliation curve, rAUC, was calculated according to Shaner and Finney (1977) by using the formula:

$$AUDPC = \sum_{i=1}^{n} \left[\left(Y_{i+n1} + Y_i \right) / 2 \left[X_{i+1} - X_i \right] \right]$$

where Y_i is the level of early blight infection in percentage at observation number *i*. X_i is the date of the scoring, and *n* the total number of observations. The scoring was done weekly from the beginning of August to mid-September, and rAUDPC/rAUC was calculated from AUDPC/AUC by dividing the AUDPC/AUC value with the total area of the graph by multiplying the number of days with 100% infection. Leaves with lesions from the field were collected, and the presence of the early blight causal agent *A. solani* was confirmed both in microscope and with PCR (Landschoot et al., 2017).

Statistical Analysis

Differences in lesion sizes for plants in the greenhouse experiments across treatments were tested with ANOVA (PROC GLM) using SAS 9.4 (SAS Institute, Cary, USA). To investigate effects of the treatments in the field trials, R-studio (version 1.1.456–© 2009–2018 RStudio, Inc) ANOVA was also used, with sum of squares type III for both trial settings. For post hoc comparisons of means, Tukey's test (*p*-value < 0.05) was used.

The ANOVA series for the small field trial consisted of the two response variables rAUDPC and rAUC, as a function of the fixed variables: treatment, year and block (nested within year) and of the interactions of these variables. For the large field trials, the same methods were used with additional response variables for tuber yield, starch content and starch yield.

Results

Greenhouse experiments

All treatments, except for adding *P. oligandrum* to the soil, including foliar sprays with Polygandron®, Serenade®, HortiStar®, Actisil® and combined treatments gave significantly decreased lesion sizes caused by *A. solani* on the greenhouse potato plants (Fig. 1). Treatment with *P. oligandrum* on foliar parts of the plants resulted in a significant decrease in lesion size compared to untreated control in three out of four experiments. In the experiment with Actisil®, the cv. Matilda had larger lesions than cv. Désirée (Fig. 1, exp 1; Anova $F = 5.9$, $p = 0.038$). The experiment with Actisil® was repeated once with cv. Désirée and similar results were obtained.

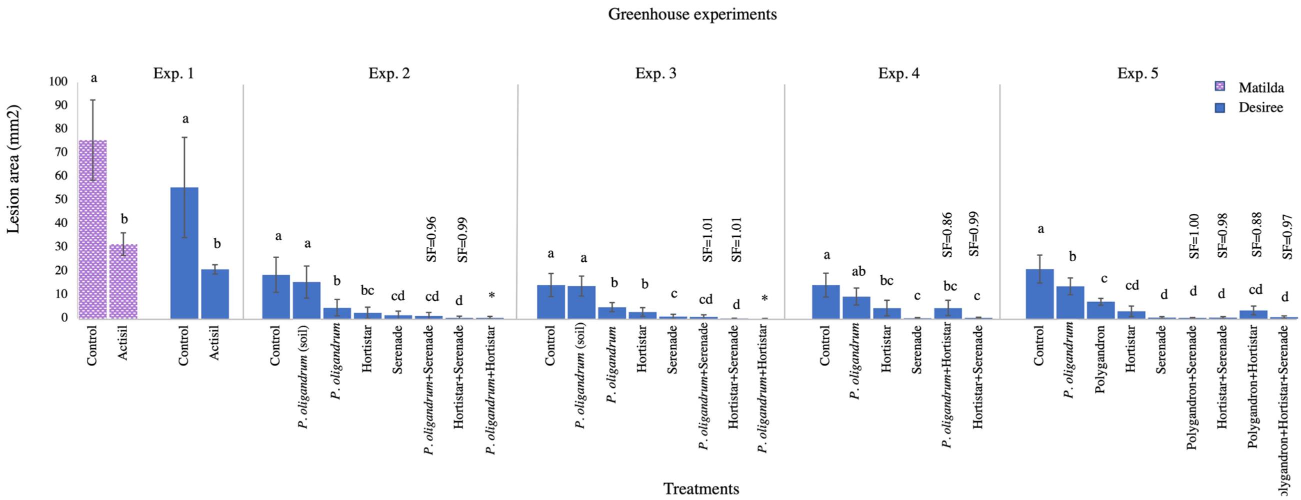

Fig. 1 Control of early blight disease of potato (cultivar Désirée and Matilda) caused by *Alternaria solani* using *Pythium oligandrum*, Polygandron®, Serenade®, Actisil® and HortiStar® in greenhouse experiments. Treatments were applied 48 h (Exp. 2–5) or 24 h (Exp. 1) before inoculation of plants with *A. solani*. All treatments were sprayed on the plants, while *P. oligandrum* was added to the soil (20 mL) in the second and third experiments as a separate treatment, marked as *P. oligandrum* (soil). Different letters show statistically significant differences between treatments in each experiment according to Tukey's test (p-value < 0.05). Vertical bars show standard deviation. SF = Synergy factor calculated according to the Abbott method. Control: plants only inoculated with *A. solani*. *: Excluded from the statistics due to foliage falling off

On average, application of Serenade® (alone or in combination with other treatments) resulted in the largest reduction of lesion sizes. A 90% reduction in lesion size was seen in these treatments (1.5 mm^2 average lesion size) compared to untreated controls (14–21 mm^2 average lesion size). In the second and third experiments, there were a lot of dropped leaves in plants treated with the combination of *P. oligandrum* and HortiStar® (Fig. 1) indicating a possible phytotoxic effect.

As shown in Fig. 1, the Synergy Factor (SF) values were generally close to one ($0.96 \leq SF \leq 1.01$) except for *P. oligandrum* + HortiStar® (SF = 0.86 in exp 4) and Polygandron® + HortiStar® (SF = 0.88 in exp 5). Thus, no synergistic effects between different agents were observed in the greenhouse studies, implying that combining agents did not increase their efficacy.

Field trials

In the field trials, the potato plants were naturally infected by *A. solani* during all years. However, the onset of infection and the disease pressure varied among years as indicated by the infection rates in untreated controls in the large field trials (Fig. 2). 2020 was notable since the infection came late in the season and did not cause as much visible damage compared to the other years. The difference is likely due to climatic differences between the years. The disease pressure was overall higher at Nymö than at Helgegården (Fig. 2).

Small plot field trials

Relative area under disease progress curve (rAUDPC) and the relative area under defoliation curve (rAUC) were used for analyzing the effects of two BCAs, one PRI, and combinations of them on early blight (Table 2, Fig. 3). The rAUDPC and rAUC values were based on scoring data from mid-August to mid-September. Analysis of variance over all three years showed a general significant effect of treatment on rAUDPC ($F_{4, 36} = 6.48$, p-value 0.0005), but there was no significant interaction between treatment and year ($F_{8, 36} = 1.87$, p-value 0.095). However, the F value for the year-effect was large ($F_{2, 36} = 567$, p-value < 0.0001) and shows that the seasonal variations are much larger than the effect of treatments (Fig. 3). All the treatments resulted in significant reductions of rAUDPC according to a post hoc Tukey test (Table 2). For rAUC, the results were similar (Treatment: $F_{4, 36} = 4.46$, p-value 0.005, Year: $F_{2,36} = 869$, p-value < 0.0001, Interaction year treatment $F = 2.27$, p-value 0.044; Table 2). For the treatments that were not included in all years, a separate analysis per year was done that also showed a significant effect of the treatments compared to the controls according to Tukey test (Table 2). In 2018, the effect was only significant for Serenade® and for the combination Serenade® + *P. oligandrum*, and in 2019 only the effect of the combination Serenade® + P. *oligandrum* was significant. If the years 2018 and 2019 are pooled, the results are the same as for all three years (analysis not shown). The low infection pressure in 2020 coincides with

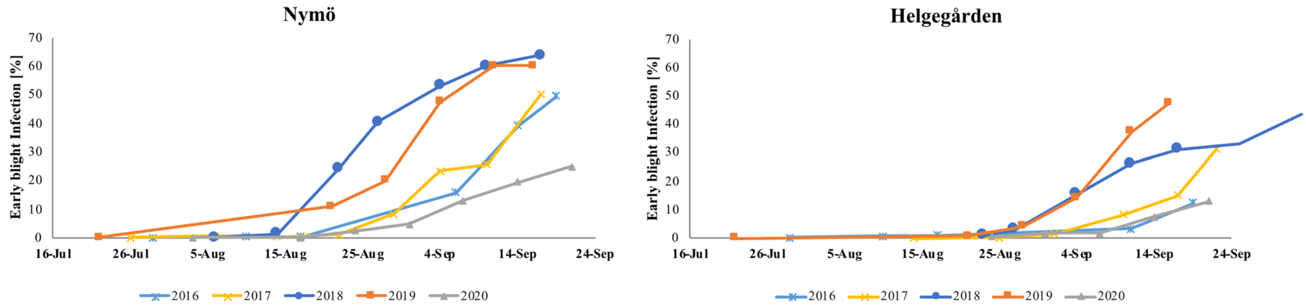

Fig. 2 Infection development disease progress curves for untreated plots during the different seasons and sites of the large trials

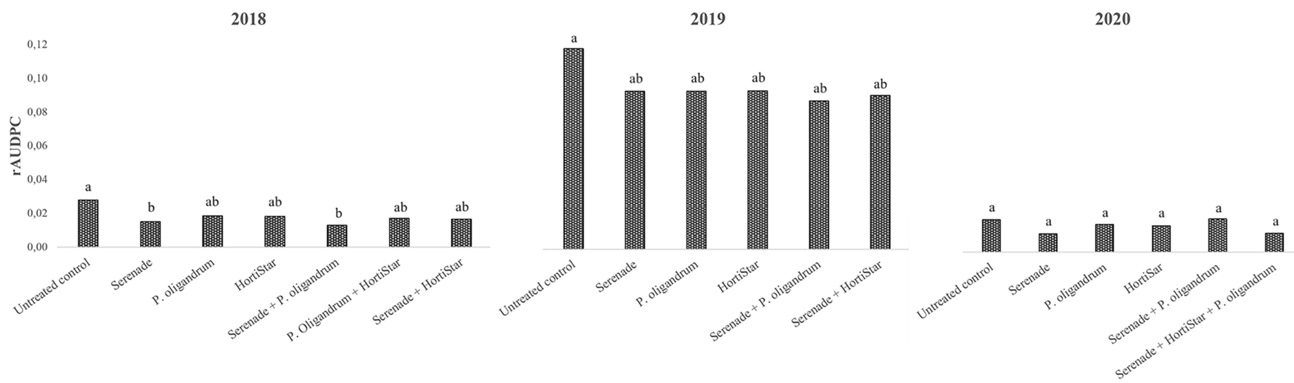

Fig. 3 rAUDPC values for the different years and treatments in the small trials

the lack of significant effect of any of the treatments in that year alone.

The disease reduction was numerically largest with Serenade® or with Serenade® combined with *P. oligandrum* (Table 2, Fig. 3). On average over all the years, the treatments resulted in a disease reduction, measured as rAUDPC, of about 28% for Serenade® and 27% for the combination Serenade® and *P. oligandrum*.

Large plot field trials

In the large field trials, the effects of Serenade®, Actisil® and HortiStar® alone or in combination/alteration with traditional fungicides were evaluated. These treatments were compared with a traditional fungicide application regime. Serenade® and Actisil® were evaluated for two seasons each and HortiStar® for one. The disease scoring and harvest data were recorded for all the large field trials.

Serenade® Serenade® was evaluated in 2019 and 2020. Analysis of variance over both years and sites indicated a general significant effect of treatment on rAUDPC ($F = 99.9$, p-value < 0.0001). The fungicide regime, reduced fungicide regime (evaluated only in 2020) and reduced fungicide regime combined with Serenade® all resulted in significantly lower infection compared to untreated control (Table 3). However, treatment with Serenade® alone did not result in any reduced infection rate in 2019. In 2020, there was a small but significant disease reduction compared to untreated controls at Nymö and when the two trial sites were analyzed together (Table 3).

There was no significant effect of Serenade® on tuber yield, starch content or starch yield in any of the years. However, there was significantly higher yield and starch yield seen as an effect of the fungicide treatments in 2019 at both trial sites and when both trial sites were analyzed together (Table 3a). Further, the starch content was significantly higher in the fungicide treatment at one of the trials site and when both sites were analyzed together.

Actisil® In all the treatments including fungicides, there are significantly lower infection rates compared to untreated controls in 2016 (Table 4a). However, treatments with Actisil® alone did not result in any significant disease reduction. Still, in 2016 one interesting observation was made. Combining half dose fungicides with Actisil® resulted in significantly lower infection than using half dose fungicides alone, and this com-

Table 3 a and b Results from Serenade® treatment in the large trials for 2019 (a) and 2020 (b), 2019: Nymö T1 = 17/6; Helgegården T1 = 19/6, 2020: T0 = 5/6, T1 = 16/6; Helgegården T0 = 10/6, T1 = 17/6, treatment dose in L/ha in parenthesis

Treatment	rAUDPC	rAUC	Yield (ton/ha)	Starchcontent%	Starchyield (ton/ha)
2019 (a)					
Helgegården					
Untreated control	0.183 b	0.1953 b	54.7 a	19.3 a	10.6 a
Narita (0.4)T5, T9; Propulse (0.45)T7, T11	0.008 a	0.0770 a	60.0 b	20.0 a	12.0 b
Serenade (2.0)T3, T5, T7, T9, T11	0.185 b	0.2023 b	54.1 a	19.5 a	10.6 a
Serenade (2.0)T3, T5; Narita (0.4)T7, T11; Propulse (0.45)T9	0.016 a	0.0788 a	61.6 b	20.4 a	12.5 b
Nymö					
Untreated control	0.233 c	0.455 c	63.6 a	20.4 a	13.0 a
Narita (0.4)T5, T9; Propulse (0.45)T7, T11	0.070 a	0.1080 a	70.3 b	21.9 b	15.4 c
Serenade (2.0)T3, T5, T7, T9, T11	0.227 c	0.436 c	65.2 ab	20.3 a	13.2 ab
Serenade (2.0)T3, T5; Narita (0.4)T7, T11; Propulse (0.45)T9	0.128 b	0.223 b	66.0 ab	21.2 ab	14.0 b
Mean					
Untreated control	0.207 c	0.325 b	59.2 a	19.9 a	11.8 a
Narita (0.4)T5, T9; Propulse (0.45)T7, T11	0.039 a	0.092 a	65.2 b	20.9 b	13.7 b
Serenade (2.0)T3, T5, T7, T9, T11	0.206 c	0.319 b	59.7 a	19.9 a	11.9 a
Serenade (2.0)T3, T5; Narita (0.4)t7, t11; Propulse (0.45) T9	0.072 b	0.151 a	63.8 b	20.8 b	13.3 b
2020 (b)					
Helgegården					
Untreated control	0.045 b	0.0888 a	56.2 a	23.3 a	13.1 a
Narita (0.4)T4, T8; Propulse (0.45)T6, T10	0.004 a	0.0463 a	54.3 a	23.8 a	12.9 a
Narita (0.4)T5; Propulse (0.45)T8	0.003 a	0.0366 a	57.9 a	23.8 a	13.8 a
Serenade (4.0)T0, T2, T6	0.035 b	0.0997 a	53.6 a	23.8 a	12.8 a
Serenade (4.0)T0, T2, T6;Narita (0.4)t5; Propulse (0.45) T8	0.005 a	0.0301 a	58.8 a	23.5 a	13.8 a
Nymö					
Untreated control	0.075 c	0.4745 c	74.4 a	18.4 a	13.7 a
Narita (0.4)T4, T8; Propulse (0.45)T6, T10	0.011 a	0.2670 a	74.0 a	19.0 a	14.0 a
Narita (0.4)T5; Propulse (0.45)T8	0.012 a	0.2823 a	73.4 a	18.8 a	13.8 a
Serenade (4.0)T0, T2, T6	0.056 b	0.4043 bc	72.7 a	18.4 a	13.4 a
Serenade (4.0)T0, T2, T6; Narita (0.4)t5; Propulse (0.45) T8	0.018 a	0.3168 ab	74.2 a	19.2 a	14.2 a
Mean					
Untreated control	0.060 c	0.2820 a	65.3 a	20.8 a	13.4 a
Narita (0.4)T4, T8; Propulse (0.45)T6, T10	0.008 a	0.1570 a	64.1 a	21.4 a	13.5 a
Narita (0.4)T5; Propulse (0.45)T8	0.007 a	0.1600 a	65.6 a	21.3 a	13.8 a
Serenade (4.0)T0, T2, T6	0.045 b	0.2520 a	63.2 a	21.1 a	13.1 a
Serenade (4.0)T0, T2, T6; Narita (0.4)T5; Propulse (0.45) T8	0.012 a	0.173 a	66.5 a	21.3 a	14.0 a

bination did not have more infection than the treatment with full dose of fungicides (Table 4a). This indicated that there was a combination effect between Actisil® and the fungicides in 2016. The fungicide regime this year was RevusTop® (T1 and T2) followed by Signum® (T3, T5, T7, T9). However, a similar strategy was investigated in 2017 using another fungicide regime: RevusTop® (T4, T8, T12) alternated with Signum® (T6, T10). This year no significant combination effect was found between reduced doses of fungicides and Actisil®.

When the Actisil® trial result was analyzed separately for the years 2016 and 2017, there was no significant effect from any treatments on the yield or starch yield (Table 4).

Table 4 a and b. Results from Actisil® treatment in the large trials 2016 (a) and 2017 (b) the letters show significance ($p<0.05$) obtained from a Tukey test within the years 2016: Nymö T1 = 15/6; Helgegården = 22/6. 2017: Nymö and Helgegården T1 = 15/6 treatment dose in L/ha in parenthesis

Treatment	rAUDPC	rAUC	Yield (ton/ha)	Starch content%	Starchyield (ton/ha)
Helgegården					
2016 (a)					
Untreated control	0.029 b	0.100 a	49.3 a	24.6 a	12.1 a
RevusTop (0.3)T1, 2; Signum (0.25)T3, 5, 7, 9	0.021 a	0.080 a	50.3 a	24.8 a	12.4 a
Actisil (0.4)T3-T9	0.027 ab	0.081 a	50.9 a	24.8 a	12.6 a
RevusTop (0.15)T1, 2; Signum (0.125)T3, 5, 7, 9	0.025 ab	0.094 a	49.6 a	24.8 a	12.3 a
RevusTop (0.15)T1, 2; Signum (0.125)T3, 5, 7, 9); Actisil (0, 4)T3-T9	0.019 a	0.074 a	50.0 a	24.8 a	12.4 a
Nymö					
Untreated control	0.108 b	0.239 b	80.2 a	21.7 a	17.4 a
RevusTop (0.3)T1, 2; Signum (0.25)T3, 5, 7, 9	0.071 a	0.190 a	81.3 a	22.1 a	18.0 a
Actisil (0.4)T3-T9	0.106 b	0.252 b	78.8 a	22.1 a	17.4 a
RevusTop (0.15)T1, 2; Signum (0.125)T3, 5, 7, 9	0.077 a	0.187 a	81.3 a	22.0 a	17.9 a
RevusTop (0.15)T1, 2;Signum (0.125)T3, 5, 7, 9); actisil (0.4)T3-T9	0.060 a	0.176 a	82.8 a	21.7 a	17.9 a
Mean					
Untreated control	0.068 c	0.169 c	64.7 a	23.2 a	14.8 a
RevusTop (0.3)T1, 2; Signum (0.25)T3, 5, 7, 9	0.046 ab	0.135 ab	65.8 a	23.4 a	15.2 a
Actisil (0.4)T3-T9	0.067 c	0.166 bc	64.9 a	23.4 a	15.0 a
RevusTop (0.15)T1, 2; Signum (0.125)T3, 5, 7, 9	0.051 b	0.141 abc	65.4 a	23.4 a	15.1 a
RevusTop (0.15)T1, 2; Signum (0.125)T3, 5, 7, 9); actisil (0.4)T3-T9	0.040 a	0.125 a	66, 4 a	23, 2 a	15, 1 a
Helgegården					
2017 (b)					
Untreated control	0.025 b	0.102 a	71.6 a	22.1 a	15.8 a
RevusTop (0.3)T4, 8, 12; Signum (0.25)T6, 10	0.004 a	0.088 a	73.5 a	22.7 a	16.7 a
RevusTop (0.15)T4, 8, 12; Signum (0.125)T6, 10	0.011 ab	0.090 a	72.1 a	23.0 a	16.6 a
Actisil (0.4)T4, 6, 8, 10, 12	0.026 b	0.100 a	69.6 a	22.8 a	15.8 a
RevusTop (0.3)T4, 8, 12; Signum (0.25)T6, 10; Actisil (0.4)T4, 6, 8, 10, 12	0.006 a	0.092 a	71.9 a	22.8 a	16.4 a
RevusTop (0.15)T4, 8, 12; Signum (0.125)T6, 10; Actisil (0.4)T4, 6, 8, 10, 12	0.005 a	0.087 a	71.1 a	23.3 a	16.6 a
Nymö					
Untreated control	0.104 c	0.283 ab	78.4 a	21.3 a	16.7 a
RevusTop (0.3)T4, 8, 12; Signum (0.25)T6, 10	0.041 a	0.222 ab	81 a	21.1 a	17.1 a
RevusTop (0.15)T4, 8, 12; Signum (0.125)T6, 10	0.054 ab	0.196 a	81.1 a	20.8 a	16.9 a

Table 4 (continued)

Treatment	rAUDPC	rAUC	Yield (ton/ha)	Starch content%	Starchyield (ton/ha)
Actisil (0.4)T4, 6, 8, 10, 12	0.098 c	0.294 b	77.1 a	21.0 a	16.2 a
RevusTop (0.3)T4, 8, 12; Signum (0.25)T6, 10; Actisil (0.4)T4, 6, 8, 10, 12	0.039 a	0.196 a	80.3 a	21.0 a	16.9 a
RevusTop (0.15)T4, 8, 12; Signum (0.125)T6, 10; Actisil (0.4)T4, 6, 8, 10, 12	0.063 b	0.258 ab	78.8 a	21.1 a	16.7 a
Mean					
Untreated control	0.064 b	0.193 b	75 a	21.7 a	16.3 a
RevusTop (0.3)T4, 8, 12; Signum (0.25)T6, 10	0.023 a	0.155 ab	77.2 a	21.9 a	16.9 a
RevusTop (0.15)T4, 8, 12; Signum (0.125)T6, 10	0.032 a	0.143 a	76.6 a	21.9 a	16.8 a
Actisil (0.4)T4, 6, 8, 10, 12	0.062 b	0,. 197 b	73.4 a	21.9 a	16.0 a
RevusTop (0.3)T4, 8, 12; Signum (0.25)T6, 10; Actisil (0.4)T4, 6, 8, 10, 12	0.022 a	0.144 a	76.1 a	21.9 a	16.6 a
RevusTop (0.15)T4, 8, 12; Signum (0.125)T6, 10; Actisil (0.4)T4, 6, 8, 10, 12	0.034 a	0.173 ab	75 a	22.2 a	16.6 a

Table 5 Results from HortiStar® treatment in the large trials. The letters are showing significant differences ($p < 0.05$) obtained when the two sites were analyzed together (Mean) and separately 2018: Nymö T1 = 15/6; Helgegården T1 = 21/6 treatment dose in L/ha in parenthesis

Treatment	rAUDPC	rAUC	Yield (ton/ha)	Starch content%	Starchyield (ton/ha)
2018					
Helgegården					
Untreated control	0.087 c	0.184 c	60.6 a	19.5 ab	11.8 a
RevusTop (0. 6)T4, 8, 12; Signum (0. 25)T6, 10	0.041 b	0.116 b	65.3 ab	20.4 b	13,. 3 ab
RevusTop (0. 3)T4, 8, 12; Signum (0. 125)T6, 10	0.046 b	0.113 b	62.9 ab	19.2 a	12.1 ab
Narita (0. 4)T3, 7; propulse (0. 45)T5, 9	0.010 a	0.073 a	67.4 b	20.2 ab	13.6 b
RevusTop (0. 3)T4, 8, 12; Hortistar (0, 5)T2, 6, 10	0.051 b	0.125 b	62.6 ab	20.1 ab	12.6 ab
RevusTop (0. 3)T4, 8, 12;Hortistar (0. 5)T2, 4, 6, 8, 10	0.041 b	0.120 b	65.8 ab	20.3 ab	13.4 b
Nymö					
Untreated control	0.301 e	0.433 b	62.2 a	18.6 a	11.6 a
RevusTop (0, 6)T4, 8, 12; Signum (0. 25)T6, 10	0.196 ab	0.385 ab	66.1 a	18.7 a	12.3 a
RevusTop (0. 3)T4, 8, 12; Signum (0. 125)T6, 10	0.240 d	0.428 b	67.2 a	19.1 a	12.4 a
Narita (0. 4)T3, 7; propulse (0. 45)T5, 9	0.181 a	0.328 a	67.5 a	19.1 a	12.9 a
RevusTop (0. 3)T4, 8, 12; Hortistar (0. 5)T2, T6, 10	0.236 cd	0.422 b	65.2 a	18.7 a	12.2 a
RevusTop (0. 3)T4, 8, 12; Hortistar (0. 5)T2, T4, T6, T8, 10	0.212 bc	0.381 ab	64.7 a	18.6 a	12.0 a
Mean					
Untreated control	0.194 d	0.308 c	61.4 a	19.1 ab	11.7 a
RevusTop (0. 6)T4, 8, 12; Signum (0. 25)T6, 10	0.119 b	0.251 b	65.7 b	19.5 ab	12.8 b
RevusTop (0. 3)T4, 8, 12; Signum (0. 125)T6, 10	0.143 c	0.270 bc	65 ab	18.8 a	12 2 ab
Narita (0. 4)T3, 7; propulse (0. 45)T5, 9	0.095 a	0.200 a	67.4 b	19.6 b	13.3 b
RevusTop (0. 3)T4, 8, 12; Hortistar (0. 5)T2, T6, 10	0.143 c	0.274 bc	63.9 ab	19.4 ab	12.4 ab
Revus Top (0. 3)T4, 8, 12; Hortistar (0. 5)T2, T4, T6, T8, 10	0.126 bc	0.250 b	65.3 ab	19.5 ab	12.7 ab

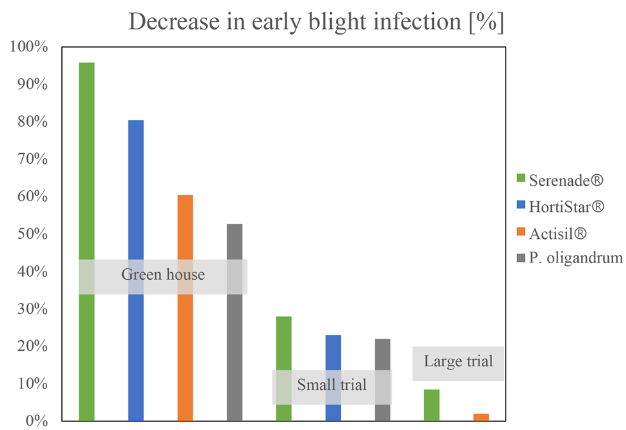

Fig. 4 Comparison between average reduction in early blight infection between the different trial setups for the four different agents. To compare the results from the different trial settings, the percentage of infection reduction from the treatments compared to control is used. For the greenhouse trial, this means reduction in the size of the lesion, and for the field trial the numbers come from the visual hand scoring

HortiStar® HortiStar® was evaluated only in 2018 (Table 5). We investigated the effect of the fungicide regime RevusTop® (T4, T8, T12) alternated with Signum® (T6, T10) and compared with treatments combining half dose fungicides with HortiStar®. A treatment with only HortiStar® was not included. Alternating/combining the fungicide RevusTop® with HortiStar® five times did not result in significantly lower infection rates than the same combination where HortiStar® was applied only three times. The combination treatments with HortiStar® did not have more infection than a similar treatment where RevusTop® was alternated with the fungicide Signum®.

Analyses over both trial sites showed that only fungicide treatments resulted in significantly higher yield and starch yield in average, while the two combinations with HortiStar® or the reduced fungicide regime did not.

Discussion

The EU Directive (2009/128/EC) concerning the sustainable use of pesticides proposes a reduced dependence on synthetic pesticides. Integrated pest management (IPM) should be implemented according to the directive, and BCAs, PRIs, and fertilizers could be part of future IPM strategies. Further, reduced fungicide applications have benefits including sustainability, cost efficiency, and a decreased risk of fungicide resistance development (Odilbekov et al., 2019). In the present study, we evaluated the effect of two forms of *P. oligandrum*, including a lab strain formulation and a commercial BCA product named Polygandron®, the BCAs Serenade® (based on *B. subtilis*), and the silicon fertilizers Actisil® and HortiStar® against early blight in potato. In general, we found good and promising effects of the investigated BCAs and PRIs in greenhouse experiments, small but significant effects in small hand-sprayed field trials but almost no effect in large-scale field trials where the agents were applied with a tractor sprayer (Fig. 4). The synthetic fungicides did, however, efficiently reduce the infection and generally increased the yield.

No effect on the tuber yield was observed in this study, except from the synthetic fungicides. If biological control agents or PRIs/fertilizers are to be used in traditional farming, the effect has to be comparable to that of traditional fungicide, also economically. In organic farming on the other hand, BCAs will only be compared to untreated; however, still they must result in yield improvement. The differences among the years in the field trial is reflecting the fluctuating efficacy of the alternative treatments. A dilemma of using BCAs or PRIs/fertilizers in conventional agriculture is the uncertainty of sufficient disease control that may depend on environmental conditions, disease pressure and microbial interactions.

The efficiency of BCAs for the control of early blight

The oomycete *P. oligandrum* does not only act directly through mycoparasitism, antibiosis, and competition for nutrients, but also interacts with plant roots and stimulates plant defense responses (Bělonožníková et al., 2020) related to the soil microbial community and direct and indirect pathogen inhibition. However, we did not observe any disease reducing effect when *P. oligandrum* was added to the soil in the greenhouse experiments as we did for foliar treatment. The more effective result in foliar treatment could be explained as a direct effect of *P. oligandrum* on the pathogen which is in the same part of the plants. When *P. oligandrum* was used in the soil, perhaps the interaction with roots and stimulation of the plant defense responses was limited due to an unsuitable environment, microbial competition, or a longer time may be required for the interaction to occur in the soil.

Earlier reports indicate that the BCA *B. subtilis* strains have inhibitory effects on *A. solani* in vitro (Zhang et al., 2020); however, little was known of the potential to reduce early blight infections in the field. In a study conducted in Germany, researchers evaluated Serenade® and *Trichoderma* in combination to control early blight in comparison with conventional fungicides. They found an average of 20% inhibitory effect of the biological control treatment, whereas the chemical control agent showed 78% protection (Metz, 2017). In this study, Serenade® also reduced early blight infection to a similar degree (28%) in the small plot trial both alone and combined with *P. oligandrum*. However, in the large plot trials the effect was much smaller or absent. Metz and Hausladen (2022) also made a large field evaluation 2016–2019 where they yet again experience a drop of efficacy when the trial is scaled up. The BCAs only showed a significant reduction in field in one year out of four.

The highest reduction in lesion size in greenhouse trials was observed in plants treated by Serenade® alone or in combination with other treatments. According to the literature, *B. subtilis* can control a wide variety of pathogens in different plants (Collins and Jacobsen, 2003; Lahlali et al., 2013; Abbasi and Weselowski, 2014; Egel et al., 2019). *B. subtilis* can colonize the leaf surface and compete with *A. solani* for space and nutrients and physically prevent penetration of the pathogen into the leaves (Abbasi and Weselowski, 2014) Secondary metabolites and lipopeptides have also been found to reduce the lesion size of *A. solani* in potato (Abbasi and Weselowski, 2014). The reduction of lesion size observed in the greenhouse plants treated by Serenade® can be the result of these direct mode of actions since the pathogen and Serenade® were in contact on the potato leaves. Induction of plant resistance by Serenade® (Lahlali et al., 2013) may also explain the disease reduction. In the greenhouse experiments, Serenade® alone was as effective as combined treatments including Serenade® and we did not observe any synergistic effects. This result may relate to the fact that when using Serenade® alone, the lesions were so small, they were measured at close to zero, so combined treatments showed no significant difference here.

The efficiency of PRIs for the control of early blight

Both Actisil® and Hortistar® contain silicon that can mechanically strengthen plant cell walls (Ma and Yamaji, 2006). Silicon can also enhance induced systemic resistance in potato plants (Xue et al., 2021). Actisil® was evaluated both in greenhouse and in large trials in 2016 and 2017. In the greenhouse Actisil® significantly decreased the lesion sizes after inoculation with *A. solani*, but in the large field trials there was no effect on the early blight development. Still, Actisil® treatment in combination with fungicides had significant effect on the infection rate in 2016 but this was not the case in 2017. In all greenhouse experiments, HortiStar® caused significant reductions of the lesion sizes and also reduced the disease in the small hand sprayed field trials. HortiStar® was only evaluated in one field season (2018) for the large trials. This season the fungicide Signum® alternated with RevusTop® was used as a reference fungicide regime. Replacing Signum® with HortiStar® gave the same result with respect to disease development rate. However, at that time fungicide resistance against boscalid (a.i. in Signum®) was widely spread and the efficacy of Signum® was strongly reduced (Mostafanezhad et al., 2021). The fungicide reference with Narita® and Propulse® was also included in this trial and would be better for comparison. HortiStar® was not efficient enough to affect yield or infection rate.

Positive effects in the greenhouse do not always translate to efficient disease control in the field

To be able to integrate alternative agents in IPM strategies, there is a need to unravel the reasons behind the discrepancy between the frequently reported successes in greenhouse studies and the poor and variable effects in field trials.

All the field trials were treated with late blight fungicides, which would presumably be toxic to *P. oligandrum* and might be one reason behind the limited effect of *P. oligandrum* in the field trials.

Another possible explanation to the results might be related to the durability of effect. In the greenhouse studies, the agents were applied 24–48 h before the inoculation with *A. solani*. In the field trials, the interval between the treatments was two weeks and it could be that the effect of the treatments diminished some days after treatments. Still, in the small hand-sprayed trials we found a significant effect although much smaller than in the greenhouse with a two-week interval. The durability of the effects of BCAs and PRIs needs to be studied in more detail. The timing of treatments may also be an important factor. In our field trials, we applied the BCAs and PRIs at the same times as chemical treatment would be applied. Maybe the treatments must start earlier if a microorganism should have time to establish on the canopy for example. In 2020, when we observed a weak but significant effect of Serenade® in the large field trial the first application was done much earlier than in 2019 where no effect was observed. In a recently published article, da Silva et al. (2021) showed a significant disease reduction in potato early blight of around 90% after treatment with *Clonostachys* in greenhouse like our results with Serenade®. They further suggest that this BCA could be used in field before planting to reduce the soil inoculum, and not as a direct treatment during the season.

The variation of efficacy of disease suppression between field and greenhouse assays might also be related to differences in the microbiome of the plant. In a greenhouse experiment UV-light, soil, humidity and irrigation will be very different from a field. Studies have shown that the bacterial community in potatoes are recruited from the soil (Buchholz et al., 2019). Microbial agents may be harmed by the continuous UV-light present in the field, and this may also be part of the explanation for the better effect in the greenhouse. A better strategy might be to introduce the biocontrol agent in the soil before planting for possible reduction of soil-borne inoculum of the pathogen. Abiotic factors such as environmental conditions (Rasche et al., 2006) or different soil types (İnceoğlu et al., 2012) are known to influence the structural and functional diversity of for example the bacterial microbiota of potato plants. Similar trends have been seen for fungi. Hou et al. (2020) reported that the change of the microbiome in potato plants was most significant at seedling stage and that potato root exudates contributed to the growth of the rhizobiome. Zimudzi et al. (2018) reported that the rhizospheric fungal microbiome of potatoes were different between the two seasons and in the different plant growth stages within a given season, indicating the significance of the rhizosphere in shaping microbial communities. Hence it matters greatly, in which environment, and existing interactive microbial community, the biocontrol agent will be amended, and thereby to what extent it will have capabilities of disease control.

Application method

The application methods were different in the greenhouse, the small and the large field trial. In the greenhouse and the small trials, a hand sprayer was used which will have a lower pressure, larger droplets, and a higher coverage of lower foliage than the tractor sprayer used for the large trial. In the small hand-sprayed trials, we also made effort to try to hit all the leaves. The absence of effect of BCAs in the large tractor-sprayed trials could be due to that the agents did not reach the lower leaves resulting in a high initial infection rate. Early blight infection usually starts in the lower aging leaves of the plants. This might explain some of the divergence of the results. If alternatives to fungicides are to be used in conventional farming, the products need to fit the already practiced routines and equipment or that application technologies are developed to better fit BCAs and other alternative agents.

We used doses of the products as recommended by the suppliers. It is possible that higher doses are required to obtain significant effects in the field. In 2020, we used double dose of Serenade® compared to 2019 and the application was done earlier. This might explain why the rAUDPC was significantly reduced in 2020 but not in 2019, but it might also be explained by a different disease pressure.

The BCAs and PRIs we investigated had no or very limited effect on early blight in the field. However, still a small effect could be of importance if there were a combinatory, or even better a synergistic effect, when used in combination or together with reduced amounts of chemical fungicides. In the greenhouse, we observed weak additive effects when two or more agents were combined. In the field trials, no such effect was observed when combining the alternative agents in small trials. In the big field trials, on one occasion Actisil® combined with half dose fungicide resulted in the same level of control as full-dose fungicide. However, it was not repeatable.

Conclusions

In summary, it can be concluded that there is a need for more field-based research on the use of alternative treatments against early blight in potato. The plant biological interactions need to be further evaluated. There seems to be a gap in the understanding of how and when alternative treatments should be applied with tractor sprayers to sustain the effect of the products. It might be of importance to cover all the foliage of the crop, which a flat fan nozzle cannot conduct. Another possible solution might be to formulate the products in a way that gives the BCAs better opportunities to colonize the foliage. Serenade® and Actisil® showed a small potential in reducing the infection of early blight in the field, in some of the years, but no tuber yield increase was observed. If BCAs and PRIs are going to be used against early blight in potato the efficacy of them must be much higher. Maybe that can be improved by optimizing dose rates, application timing and application technology or by development of more efficient agents and formulations. Their use also must be put in perspective involving other IPM measures like more resistant cultivars, optimized nutrition, crop rotations, optimized timing of fungicide treatments by using decision support systems, etc. Breeding for resistance is important and there may be possibilities to also breed for improved response to BCAs and PRIs in the future.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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