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# Early Blight Infection and the Influence of Biocontrol Agents on Three Wild Potato Relatives: Implications for Integrated Pest Management (IPM) in Potato

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## Abstract

Integrated pest management (IPM) is an important tool for sustainable crop production. IPM includes a diversity of methods, e.g., the use of biological control agents (BCAs) for disease control or growth promotion. While there is an increasing interest in the use of BCAs, less is known about their environmental costs and benefits on wild species, such as wild crop relatives. For example, a BCA may have the positive effect of controlling disease in wild relatives, but could also have the negative effect of growth promotion on wild relatives that act as weeds. In this study, we investigated if three wild potato relatives—the perennial climber *Solanum dulcamara*, and the annual weeds *S. nigrum* and *S. physalifolium*—could be infected by *Alternaria solani*, the causal agent of early potato blight in Sweden, and studied how two BCAs, *Pythium oligandrum* (a laboratory strain) and *Bacillus amyloliquefaciens* (Serenade), affected the disease and growth promotion in a series of greenhouse and field experiments. Our studies confirmed the semantic knowledge that *A. solani* can infect all three wild species, in particular the two annual species often growing as weeds in potato fields. We also found a disease-controlling effect of *B. amyloliquefaciens*, but not *P. oligandrum*, in the greenhouse. Some growth effects were found for both BCAs, but whether these were positive or negative varied with trait, plant species, and genotype. In conclusion, BCAs can confer both environmental costs and benefits on the three wild relatives of potato investigated in the current study, which should be taken into consideration for development of sustainable potato cultivation.

**Keywords** *Alternaria solani* · *Bacillus amyloliquefaciens* · Disease epidemiology · Growth promotion · *Pythium oligandrum* · Wild *Solanum* species

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## Introduction

In recent decades, it has become clear that our cultivation systems need to develop more sustainably, in particular to reduce the frequent use of agrochemicals (Aktar et al. 2009; Indira Devi et al. 2022). One solution is to implement integrated pest management (IPM), which is mandatory within the EU (Directive 2009/128/EC, EC 2009). In IPM programmes, a combination of alternative management methods is used first, and then pesticides as the last resort to combat pests and diseases (Barzman et al. 2015). Alternative pest management methods range from preventive methods, e.g., mechanical and cultural control, to ecologically based methods, e.g., using resistant cultivars, enhanced biological diversity, low-risk plant protection products, and biological control agents (BCAs) (Stenberg 2017). BCAs are commonly defined as living organisms with a direct or indirect effect on the pest (Stenberg et al. 2021). BCAs may also have the added function of biostimulation such as growth promotion by improving the uptake of nutrients in the plant or by regulation via phytohormones (Calvo et al. 2014; El-Saadony et al. 2022). While there is an increasing interest in the use of IPM, current problems involve e.g., a lack of knowledge about the efficacy of alternative methods, how, and when they should be combined or applied, estimations of economic thresholds in various cultivation systems and a lack of knowledge about the general link between IPM, management, business, and sustainability (Matyjaszczyk 2018; Dara 2019; Karlsson Green et al. 2020; Deguine et al. 2021; Lankinen et al. 2024).

While alternative pest management methods in general do not pose the same risks for humans and non-target organisms as synthetic pesticides, evaluations of these methods, such as the use of BCAs, should not only study their efficacy but also consider environmental impacts (Simberloff and Stiling 1996; Collinge et al. 2022; Hashemi et al. 2022). For example, environmental risks of a BCA could be that it may spread, persist for a long time, become a pathogen, produce toxins or antibiotics, have a negative side effect on non-target species or that the pest will evolve to tolerate the BCA (Keswani et al. 2019; Ke et al. 2021; Bardin and Siegwart 2022; Collinge et al. 2022). Another potential risk, which has received less attention, is the influence of alternative methods on wild plants growing in or next to the agricultural field (e.g. weeds). Such risks are considered when testing weed biocontrol (Hinz et al. 2019), but are seldom taken into consideration for pest biocontrol. In the case that a BCA also has a growth-promoting effect, this may lead to an increased weed problem if applied on weeds present in an agricultural field (Rabiey et al. 2017; Ray et al. 2018). On the other hand, a BCA may also control pests and diseases on wild crop relatives, which may reduce their spread and severity.

Potato is the fourth most important crop worldwide (Lovat et al. 2016), but because it suffers from many diseases including potato late blight, it is also one of the most sprayed (Hashemi et al. 2022). For example, in Sweden in 2013 potato was grown on 0.9% of the cultivated land, but used 21% of the total fungicides (Eriksson et al. 2016). Despite a reduction in the use of fungicides in potato in Sweden from 2.51 to 1.95 kg per hectare of active substance between 2017 to 2021, the percent of the total fungicides used in potato was still high (26% in 2021)

(SCB 2022). While potato late blight is one of the most serious diseases, in recent years early blight has increased in Europe with identified yield losses of up to 50% (Leiminger and Hausladen 2012; Odilbekov et al. 2014). This increase is believed to be partly caused by a change in the climate and partly caused by rapid resistance development to fungicides by *Alternaria solani*, the causal agent of early blight (Kapsa 2008; Landschoot et al. 2017; Einspanier et al. 2022; Mostafanezhad et al. 2022). The resistance development to fungicides in *A. solani* is particularly worrying, as this may lead to a lack of any available fungicide for treatment of severe early blight. Adopting IPM in potato cultivation is therefore crucial. Currently, using BCAs as part of an IPM strategy in potato to control early blight or for growth promotion is gaining increasing interest despite challenges in large-scale fields (Andersen 2023; Stridh et al. 2022). For example, BCAs approved to be used against early blight within the EU include the oomycete *Pythium oligandrum* (commercial product Polygandron) and the bacterium *Bacillus amyloliquefaciens* (formerly *subtilis*) (commercial product Serenade) (EC 2024). Both these BCAs have been shown to have growth-promoting effects in potato (Syed and Prasad Tollamadugu 2019; Andersen et al. 2024). The growth-promoting effect of *P. oligandrum* in potato is genotype specific (Andersen et al. 2024). However, we still lack knowledge about the environmental risks associated with the use of these BCAs in potato cultivation in relation to wild relatives.

In the current study, we focus on the environmental risks of using BCAs in relation to potential effects on wild hosts of *A. solani* in Sweden, including three wild *Solanum* species—the annual weeds *S. nigrum* and *S. physalifolium* (Taab 2021) and the perennial climber *S. dulcamara*. The annual weeds are commonly found in potato fields. The perennial species can grow close to agricultural fields, e.g., in wetlands or small streams. These species are known hosts of *Phytophthora infestans*, the causal agent of late blight (Abreha et al. 2018). Surprisingly, we are not aware of any studies identifying infection of *A. solani* in these species, even though there appears to be semantic knowledge that this occurs. We studied early blight infection in these three wild potato relatives in the greenhouse and in a small-scale field trial, and investigated the presence of natural early blight infections in the annual wild species growing in potato fields. We also studied the potential benefits and risks of using two BCAs. We focused on the questions:

- Can the three studied wild *Solanum* species host *A. solani*?
- Do BCAs (*P. oligandrum* and *B. amyloliquefaciens*) reduce *A. solani* infection in wild *Solanum* species?
- Do BCAs cause growth promotion in wild *Solanum* species, and do these effects differ between plant genotypes or vary with timing of application?

## Materials and Methods

We performed two greenhouse experiments (experiments 1 and 2) and a small-scale field trial to investigate the interactions between the three wild *Solanum* species, the pathogen *A. solani*, and the potential effects of the biocontrol agents

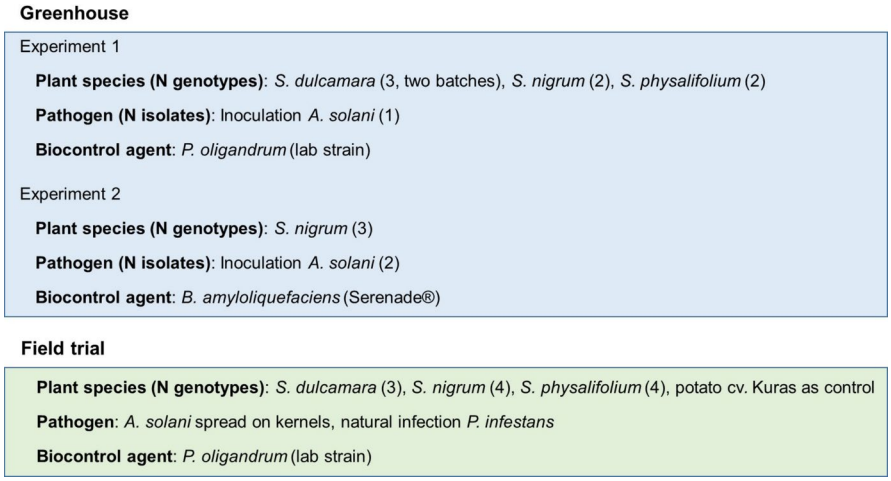


**Fig. 1** Overview of greenhouse experiments and field trial to study the interaction between wild *Solanum* species, the pathogen *A. solani* and biocontrol agents on infection and plant performance. Two batches of *S. dulcamara* were used with an age difference of 2 weeks. *N* = number

*P. oligandrum* and *B. amyloliquefaciens* on disease suppression and on plant performance (Fig. 1).

## Plant Material

We used three *S. dulcamara* genotypes in both the greenhouse and field trials. The genotypes were collected as seeds in two nearby wild populations (one genotype from population Lomma 2 (L2:3.6), a coastal forest in Lomma and two genotypes from population Geneticum (G20:1 and G21:1), an urban parking space outside the Genetics Department at Lund University) (Masini et al. 2019). *S. dulcamara* in the greenhouse (experiment 1) was grown from seeds collected from the three genotypes in a field plot established with micro-propagated clones in 2016 at Campus Alnarp, Swedish University of Agricultural Sciences (SLU) in Lomma, south Sweden (Masini et al. 2019). The field plot of this perennial plant was used in the field trial of the current study.

We used five *S. nigrum* genotypes collected at five sites in Skåne, of which three were used in the greenhouse (greenhouse experiments 1–2) and field: Lillgårda, Löderup farm field (Nr 2 L), Stockholmsgården, Valleberga farm field (Nr 6 T), see Lankinen et al. (2016) for coordinates of close by *S. physalifolium* sites; only experiment 2: Helgegården, Kristianstad field trial (He E, coordinates N56.02404, E14.06913); only field: Simrishamn ruderal land, Alnarp farm field (Abreha et al. 2018)). For *S. physalifolium*, we used four genotypes collected at two ruderal sites in Skåne. Two of these were used in both greenhouse and field trials (Borgeby (Nr 1 B), Spillepengen, Malmö (Nr 5 S)), and another two

genotypes were used only in the field (Spillepengen, Malmö, Abreha et al. 2018, note that the coordinates for Borgeby is given as a *S. nigrum* site). Seeds from all wild species were germinated using the method described in Lankinen et al. (2016).

We used commercial tubers of the starch potato cultivar Kuras (a commonly used cultivar in Sweden) as a control to promote *A. solani* infection in a known host in the field trial. In a parallel study, we tested induction of growth promotion by *P. oligandrum* in these potato plants (Andersen et al. 2024).

### Preparation of *Alternaria solani* Inoculum

We used the *A. solani* strain AS112 (isolated from a potato field in south Sweden and used in previous studies (Odilbekov et al. 2019; Stridh et al. 2022; Andersen 2023) in all experiments. In experiment 2 in the greenhouse, we also used another strain isolated from *S. nigrum* in Helgegården in 2019 (strain ASH4).

#### Experiment 1

Preparation of spore inoculum of *A. solani* for greenhouse experiment 1 was performed following the Shahin and Shepard (1979) protocol. Briefly, ~ 254-$mm^2$ agar blocks from 20% PDA (potato dextrose agar) plate containing dark growth *A. solani* were placed growth-down in new plates with S-medium and incubated in the dark at 18 °C for 5–7 days. The S-medium was composed of 10 g sucrose, 15 g $CaCO_3$ and 500 mL milliQ water, adjusted to 7.4 pH. Once 10 g of Bacto Agar was added, the medium was autoclaved. Spores were collected by flooding the plate with 10 mL autoclaved tap water amended with 0.01% (v/v) Tween-20 and gently scraped the surface of the plate with a sterile spatula. A second S-medium plate with conidia was flooded with the 10-mL suspension from the first plate and the surface was gently scraped with a sterile spatula. The suspension was collected and spores were counted using a Fuchs–Rosenthal counter chamber. The final spore concentration was adjusted to 25,000 spores $mL^{-1}$, supplemented with 0.1% Bacto Agar and MilliQ water with a final volume of 25 mL.

#### Experiment 2 and the Field Trial

Preparation of spore inoculum of *A. solani* for the greenhouse experiment 2 and the field trial was done following Stridh et al. (2022), with minor modification. Briefly, *A. solani* pure cultures were grown on 20% PDA media plates supplemented with 12 g $L^{-1}$ Bacto Agar in the dark for 7 days at 25 °C. The plates were then exposed to UV-c light (254 nm dominant wavelength) for 7 days (5–6 h per day). The plates were flooded with 1 mL of MilliQ water amended with 0.01% (v/v) Tween 20, and the spores were dislodged using a sterile L-shaped cell spreader. The final concentration of the spore suspension was adjusted to $10^4$ spores $mL^{-1}$ using a Fuchs–Rosenthal counter chamber and supplemented with 0.1% Bacto Agar.

## Preparation of Biocontrol Agents

### *Pythium oligandrum*

*Pythium oligandrum* (CBS-strain 530.74) inoculum was prepared as described previously (Stridh et al. 2022; Andersen et al. 2024). Briefly, one agar plug of *P. oligandrum* was inoculated on solid V8 agar plates and allowed to completely overgrow the plates for approximately 5 days at 20℃. Five agar plugs from the solid *P. oligandrum* cultures were inoculated into each of six 1-L bluecap bottles, each containing 300 mL of clarified V8 broth. The bottles were put into a rotary incubator, shaking at 120 rpm at 20℃ for 7 days. To harvest the oospores from the liquid cultures, the mycelium was macerated using a high-speed blender and 200 mL of sterile water was amended. The inoculum was then filtered through cheesecloth and a final concentration of $2.5 \times 10^4$ oospores/mL, resuspended in sterile water, was used in all treatments.

### *Bacillus amyloliquefaciens*

For the biocontrol agent *B. amyloliquefaciens* (formerly *subtilis*), we used Serenade ASO from Bayer Crop Science containing strain QST 713 with a minimum of $1.05 \times 10^{12}$ cfu $L^{-1}$. A solution of Serenade was prepared by diluting 12.5 mL of Serenade in tap water with a final concentration of 0.5% Serenade as previously described (Stridh et al. 2022).

## Greenhouse Experiments

In both experiments, we transferred germinated seeds to soil and cultivated plants in a greenhouse with 16 h light at an approximate temperature of 22 °C. We repotted plants in larger pots as they grew. We used unfertilized potting compost (Krukväxtjord med Lera & Kisel; SW Horto AB) and the final size of the pots was 1.5 L. In both experiments, plants were moved around weekly to avoid border effects. Plants in experiment 1 were watered twice with water supplemented with 15 mL of Osmocote Exact 3–4 months fertilizer beads (containing nitrogen-phosphorus-potassium 16–9-12 + 2MgO + trace elements) as described in Brouwer et al. (2023).

### Greenhouse Experiment 1—Three Wild Species and Biocontrol Agent *P. oligandrum*

In total, 15–24 plants per genotype of the three species were randomly divided into two groups where one was treated with *P. oligandrum* and the other served as control. For *S. dulcamara*, we used two batches of plants, where the second batch was grown from seeds ca. 2 weeks after the first batch (Fig. 1). This allowed us to test how developmental age affected interactions with the biocontrol agent. Plants treated with *P. oligandrum* were sprayed with spores twice with 1 week apart at about the age of 2.5 months (annual plants had started flowering) (second set of *S. dulcamara* 2 months). Both treatments consisted of 10 mL oospore inoculum applied as foliar

spray with a high-pressure handheld sprayer. At the first spraying time, a soil drench using an additional 10 mL of *P. oligandrum* was also conducted in all treated *S. dulcamara* plants. At the second spraying time, a soil drench was applied to all treated plants. Control plants were treated with sterile water of the same volume.

Five days after the second spraying with *P. oligandrum*, we selected 12 plants (6 treated with *P. oligandrum* and 6 control plants) of one genotype from the two annual species and two genotypes from *S. dulcamara*, respectively, for the inoculation experiment with *A. solani*. We inoculated 6 plants per genotype (3 treated with *P. oligandrum* and 3 control plants).

### Greenhouse Experiment 2—*S. nigrum* and Biocontrol Agent *B. amyloliquefaciens*

We selected *S. nigrum* for experiment 2 and added a third genotype, based on the fact that this species showed an interaction between genotype and biocontrol treatment in experiment 1 (see 'Results' section). A total of 18–24 plants per genotype of *S. nigrum* were randomly distributed in four groups: (i) treatment with biocontrol agent *B. amyloliquefaciens*, (ii) treatment with *B. amyloliquefaciens* and infected with *A. solani*, (iii) infected with *A. solani* and (iv) control. For genotypes with fewer than 24 plants, six plants were still included in groups (ii) and (iii) to allow infection by two isolates of *A. solani* (three plants per isolate). Plants were sprayed with *B. amyloliquefaciens* at the age of 2.5 months, and at 9 and 2 days before inoculation with *A. solani*.

### Inoculation and Disease Scoring in the Greenhouse

On each inoculated plant of experiment 1, we placed 2 droplets of 10 µL of *A. solani* on either side of the central vein on seven young, fully expanded leaves. Two additional leaves of the same plant were mock-treated with 0.033% Bacto Agar control. In experiment 2, inoculation was performed by placing 1 droplet of 10 µL of *A. solani* on one side of the central vein on ten young, fully expanded leaves per plant. No leaves were mock-treated in this experiment, as we never detected any response on such leaves in experiment 1 (see 'Results' section). After inoculation, we placed a plastic tent over the plants to maintain high humidity (around 95%) during the first 24 h after inoculation. We then used a misting system within the chamber to stabilise relative humidity at 85%.

We estimated disease development 9, 12 and 20 days after inoculation in experiment 1, and after 7 and 10 days after inoculation in experiment 2 by measuring the lesion area. Lesion area (LA) was measured as an oval area, using the equation $LA = \pi/4 \times D1 \times D2$, where D1 and D2 are two perpendicular diameters.

### Estimates of Plant Performance in the Greenhouse

To investigate the effects of the biocontrol agents on plant performance, we measured plant performance traits before treatment with biocontrol agents and at the end of the experiment (at plant age of approximately 4 months). In experiment 1, early

performance was recorded as plant size. At the end of the experiment, we recorded final plant size, leaf area, number of flowers or berries, dry above ground biomass, and dry root biomass.

Early plant size was measured as total length of all shoots in *S. dulcamara* (batch 1) and as length of the longest shoot (batch 1, 2). Final plant size in *S. dulcamara* (batch 1, 2) was recorded as total length of all shoots. Early and final plant size were measured as plant height in *S. nigrum* and *S. physalifolium*. Leaf area was measured as the length multiplied by the width of three fully expanded leaves per plant. The number of flowers was counted in *S. dulcamara* as an indication of reproductive effort as this outcrossing species did not set seeds in the greenhouse. In the annual, self-compatible species, we counted the number of berries. Plant biomass above ground was separated into green mass and fruit mass for *S. nigrum*, as this species had a substantial number of fruits at the time of harvest. We hereafter refer to 'green biomass' for all above-ground biomass for *S. dulcamara* and *S. physalifolium*, and for *S. nigrum* to the above-ground measure separated from fruit biomass. Roots were rinsed to remove soil. Rinsing resulted in some loss of fine roots. Green biomass, fruit biomass and root biomass were weighed after drying for 24 h at a temperature of 60 °C.

## Field Trial

We conducted the small field trial at Campus Alnarp in June to September 2019 to investigate the interaction between the three wild *Solanum* species, and *A. solani* and the biocontrol agent *P. oligandrum* under field conditions (Fig. 2).

### Establishment of Plants and Management of Field Trial

At establishment of the perennial species *S. dulcamara* in 2016, the three genotypes were randomly mixed and planted in six blocks (12–14 plants per block, arranged in two rows along the length of the plot, i.e., one row in the control area and one row in the *A. solani* area, Fig. 2). In a previous study, which finished a year before the present work commenced, two thirds of the plants were treated with *P. infestans*, but

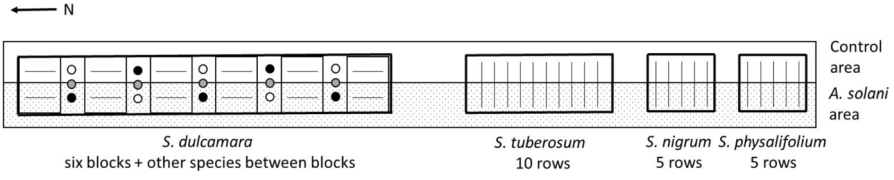


**Fig. 2** Design of small field trial at Campus SLU in Alnarp in 2019 (37 × 2.5 m). The three wild *Solanum* species and a starch potato cultivar (Kuras) were grown in their own individual plots. The *S. dulcamara* plot was established in 2016 and plants were arranged in six blocks. Between these blocks, three plants of the other species (grey circle = potato, white circle = *S. nigrum* and black circle = *S. physalifolium*) were planted to allow more immediate comparison. In half of the field, *A. solani* was spread by dispersing infected kernels to the soil. The other half of the field served as control. In both areas of all species, six selected plants were treated with the biocontrol agent *P. oligandrum*

only showed weak disease symptoms. All above-ground plant material was removed at the end of each season. We could not detect any long-term treatment effects on survival (survival rate in early season 2019; inoculated: 94%, control: 96%, $\chi^2 = 0.001$, $df = 1$, $P > 0.05$) or on performance traits (shoot length, flower and fruit production late season 2018; $P > 0.53$) (Lankinen unpublished data).

The two annual species were planted out as seedlings when they reached ca. 15 cm in height (at approximately 4 weeks old) on the 24th of June. Plants were arranged in 5 rows per species across the control and *A. solani* treatment areas with 7 plants per row (Fig. 2). The four genotypes per species were planted randomly across rows.

Commercial potato tubers, cv. Kuras, were sown on the 7th of May 2019 in 10 rows with 6 plants per row (70 cm between rows, 30–40 cm between plants) (Fig. 2). Additionally, to get an immediate comparison between the four different species, we planted three plants (one per species of *S. nigrum*, *S. physalifolium* and potato) in the five areas between the existing *S. dulcamara* blocks (Fig. 2).

The field was fertilised with 100 kg N/ha at the beginning of July. See Andersen et al. (2024) for measurements of soil properties. Weeding and watering were performed when needed over the growing season.

### Field Trial Treatments

*Alternaria solani* inoculum was spread as infected kernels in half of the field on the 31st of July using the method by Adolf and Hausladen (2015) to have a control area for treatments with the biocontrol agent *P. oligandrum*. However, because of the narrow field, we expected that plants in both areas could be infected. To study the potential effects of *P. oligandrum* on the infection and on plant performance, 12 plants per species were treated with *P. oligandrum* (6 plants in the control area and 6 plants in the area exposed to *A. solani* kernels) five times during the season, starting at the beginning of July and ending at the beginning of September (8th and 25th of July, 6th and 19th of August, 3rd of September). Twelve plants per species served as controls (6 per area). Plants were treated with the *P. oligandrum* oospore inoculum in sterile water or with sterile water (control), both as foliar application and soil drenching as described above and in Andersen et al. (2024). We used a volume of 300 L/ha, corresponding to 200 mL per plant, following the recommendations for application of commercial products of *P. oligandrum.*

### Disease Scoring in the Field Trial

Disease scoring of early blight was conducted three times from the middle of August to the middle of September (21st of August, 6–9th of September, 23–24th of September) using the method of Duarte et al. (2013) developed for potato. We identified infection as the percentage of green leaf area covered by typical dark early blight spots per individual plant. We also noted defoliation as the percentage of leaves that were dead or defoliated. Because infection levels were low < 5%, we used the percentages per individual plant at the last estimation date for statistics rather than calculating the relative area under the disease progression curve (rAUDPC).

Additionally, a spontaneous late blight infection (caused by naturally occurring *P. infestans*) affected *S. physalifolium* around the middle of August. Only minor signs of late blight symptoms were seen on the other species, which are known to be more resistant. We scored late blight infection in *S. physalifolium* at one occasion (21st of August) as a percentage of infected leaves.

### Estimates of Plant Performance in the Field Trial

Plant performance traits were measured three times between July and the middle of September (8th of July, 2–7th of August, 11–19th of September), including plant size, and number of flowers and berries. In *S. dulcamara*, plant size was estimated as total length of all shoots, and in *S. nigrum* and *S. physalifolium* as plant height. In *S. dulcamara*, the number of flowers and berries was counted, while in the other species we counted the number of inflorescences containing flowers or berries. In *S. physalifolium*, we noted survival and measured regrowth of the plant after the spontaneous *P. infestans* infection (24th of September), as a percentage of plant size at the time of infection. All above-ground material was also collected on the 14th of November. Plant biomass above ground was separated into green mass and fruit mass for *S. dulcamara*, but not for the other species as fruits had been lost. The material was weighed after drying for 24 h at a temperature of 60 °C.

### Confirmation of *A. solani* Infection in the Wild *Solanum* Species

Lesions of infected leaves of the three wild species were collected from the small field trial and grown on water agar and kept in an UV incubator for 2 days with a temperature of 18 °C and then kept near the window for a week. Plates were then inspected under the microscope to confirm the presence of *A. solani*. As a complement, we included leaves of *S. nigrum* with typical *A. solani* lesions collected from naturally infected potato field trials at Helgegården in 2019 (*S. nigrum*, at the same site where we collected *S. nigrum* seeds and field inoculum; see above).

### Production of Single-celled Isolates

Single-cell isolates of putative *A. solani* from *S. dulcamara* (Alnarp) and *S. nigrum* (Helgegården) leaves were confirmed to be *A. solani* by genetic markers (PCR). To produce single-cell isolates, a lesion was cut from the leaf, washed in bleach and MilliQ water, then cultured on water agar for at least 2 weeks in the UV incubator at 18 °C with 30% intensity UV-c light for 9 h per day. After 2 weeks, the spores were transferred to new plates, this time 20% PDA and incubated for 10 days in the UV chamber. Spores were then detached from the mycelium with 40 µL of MilliQ water. The droplet was moved on a new 20% PDA plate and spread with an L-shaped spreader. The plate was incubated at room temperature for 3 h to allow spore germination. Finally, a sterile scalpel was used to cut out a piece of the PDA containing one single spore and moved to a new 20% PDA

plate. The plate was then incubated in the UV chamber for the culture to grow for 14 days at room temperature. One agar plug of 1 $cm^2$ was cut out from the edge of a growing culture and transferred into a bottle of potato dextrose broth medium. The bottles were incubated in the dark on a laboratory shaker at room temperature for 7 days. The mycelium was then separated from the agar plugs using forceps. Mycelial samples were frozen in liquid nitrogen and ground to a find powder using a mortar and a pestle.

### Molecular Confirmation of *A. solani*

DNA extraction was carried out using the DNA-Plant mini kit from Qiagen, following the manufacturer's protocol. We confirmed the quality of the extracted DNA using Nano-drop ND1000 and diluted it to a concentration of 100 ng/µL. As a positive control for the presence of *A. solani* in the PCR assay, DNA from the reference strain AS 112 (Odilbekov et al. 2014) was utilised. Negative controls consisted of distilled water as template.

PCR was performed using two sets of *A. solani*–specific primers: AS1 (5′-GCT CCCACTCCTTCCGCGC-3′) and AS2 (5′-GGAGGTGGAGTTACCGACAA-3′) from Kumar et al. (2013), or forward primer Asol 129 (319) (ATGCGGGTGAAT ACGGTTAA) and reverse primer 143 (CTCTACTTTGTTTATGTTATTTAACCA AGAATG), as published in Edin et al. (2019). PCR reactions were carried out in 25-µL reactions, using 50 ng/µL DNA as the template. The PCR conditions followed the protocols described in Kumar et al. (2013) for the AS1 and AS2 primers and Edin et al. (2019) for the Asol 139 (319) and 143 reverse primer. Subsequently, the PCR product was separated on a 1% agarose gel (confirmational pictures can be found in Online Resource 1).

### Data Analysis of Greenhouse Experiments and Field Trial

Data was analysed in SPSS, version 29 (IBM SPSS Statistics for Windows 2022), using a series of ANOVAs. Type III sum of squares was used in the ANOVAs. Continuous covariates were standardised to a mean of zero and a standard deviation of 1.

### Greenhouse Experiments

In experiment 1, we tested lesion area in a model involving the factors *Solanum* species, *S. dulcamara* genotype nested within species, treatment with *P. oligandrum* and the interaction between species and treatment. In experiment 2, we tested lesion area in a model with the factors *S. nigrum* genotype, *A. solani* isolate, treatment with *B. amyloliquefaciens* and all two- and three-way interactions.

To investigate plant performance following biocontrol treatment in experiments 1–2, we used models with plant genotype, treatment and their interaction. We included early plant size as a covariate when it was significant (for *S. dulcamara*) and the factor growth chamber nested under genotype (for *S. nigrum* and *S. physalifolium*) as plants of some genotypes were split between greenhouse chambers. To

analyse whether biocontrol treatment affected plant allocation above versus below ground, we added the covariate dry root weight to the models with the dependent variable dry green mass and all interactions with genotype and treatment. Significant interactions between dry root weight and treatment would indicate an altered allocation in treated plants.

In experiment 1, we tested how plant performance was influenced by age at the biocontrol treatment in two batches of *S. dulcamara* plants with a model involving plant genotype, treatment, plant batch and all two- and three-way interactions. We also included the covariate early plant size when significant.

Dependent variables were transformed when needed to obtain normal distribution of residuals in the models (experiment 1; log-transformed: *S. dulcamara*: early plant size, plant size at harvest, *S. dulcamara* two batches: early plant size, plant size at harvest, dry green mass and dry root mass, *S. nigrum*: leaf area, dry root mass, *S. physalifolium*: dry green mass and dry root mass; experiment 2: power-transformed: plant size, log-transformed: dry root mass).

### Field Trial

To investigate how the percentage infected leaf area by *A. solani* and wilting (both arcsine-transformed) differed among wild species and potato, we first tested *S. nigrum* and potato in their two growth places (own plot and between *S. dulcamara* plants). We used a model with growth place, species and their interaction. Because percentage infection did not differ between growth places (ANOVA; growth place: $F_{1,95}=0.581$, $P=0.45$, species: $F_{1,95}=3.14$, $P=0.079$, interaction: $F_{1,95}=2.48$, $P=0.12$), we pooled all samples of these species in tests for differences among species. As wilting was affected by growth place (see 'Results' section), we also performed an additional analyses of the differences among the four species growing in the *S. dulcamara* plot. Genotype differences in percentage infection and wilting within each species were tested with non-parametric Kruskal–Wallis test.

We tested the effect of *P. oligandrum* on percentage infection in the selected 24 plants per species by using a model with species, treatment (control in *A. solani* or control area, *P. oligandrum* in *A. solani* or control area), scoring date and all two- and three-way interactions. For effects on percentage wilting on the last scoring date, we used a model with species, treatment and their interaction.

Additionally, we tested how *S. physalifolium* genotype affected *P. infestans* infection percentage and the percentage regrowth with non-parametric Kruskal–Wallis test. Moreover, the effect of *P. oligandrum* on percentage infection and regrowth was tested with non-parametric Man–Whitney *U*-test.

To study how plant performance was affected by *P. oligandrum* treatment, we used a model with treatment and if significant included the covariate early plant size (*S. dulcamara* and *S. nigrum*). Some traits were log-transformed to obtain normally distributed residuals of the models (*S. dulcamara*: number of flowers, number of berries, dry berry mass; *S. nigrum*: number of inflorescences with flowers and berries, dry green mass).

## Results

### *Alternaria solani* Infection in the Greenhouse

All three wild *Solanum* species inoculated with *A. solani* developed lesions typical for early blight disease in the greenhouse experiments (Fig. 3). In experiment 1, involving all species, mock-treated leaves showed no signs of lesions. Lesions in inoculated plants were possible to measure 9 days post inoculation (dpi). After 12 and 20 days, leaves started to drop, so we concluded that the data was most reliable at 9 dpi. Lesion size at 9 dpi differed among species across *A. solani* treatments (with and without *P. oligandrum* treatment), showing that *S. physalifolium* was more susceptible to infection than *S. dulcamara* and *S. nigrum* (Fig. 3a, Table 1). There was no significant difference between the two tested *S. dulcamara* genotypes.

In experiment 2, lesion area at 7 and 10 dpi in *S. nigrum* showed similar results. Lesion size at 7 dpi was similar across the three *S. nigrum* genotypes and for the two isolates (Fig. 3b, Table 1). However, there was a non-significant trend that the newly collected isolate caused larger lesions ($P = 0.069$) and the variance for this isolate was significantly larger ($F$-test; $F = 0.047$, $P = 0.0001$).

### Effect of Biocontrol Agents on Early Blight Disease in the Greenhouse

In experiment 1, no effect of the biocontrol agent *P. oligandrum* on lesion area was detected in any of the three wild *Solanum* species (Fig. 3a, Table 1). In experiment 2, the biocontrol agent *B. amyloliquefaciens* had a strong negative effect on lesion area in *S. nigrum* (Fig. 3b, Table 1).

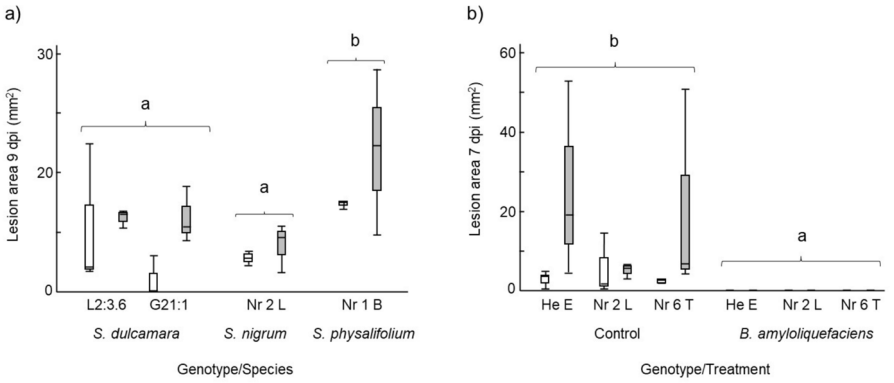


**Fig. 3** Box plots for lesion area following inoculation with *A. solani* in (**a**) experiment 1 and (**b**) experiment 2 in the greenhouse. In experiment 1, the biocontrol agent *P. oligandrum* (grey bars) was compared to control (white bars) across three wild *Solanum* species. In experiment 2, the biocontrol agent *B. amyloliquefaciens* was tested against control on three *S. nigrum* genotypes. In experiment 1, we used *A. solani* isolate AS112. In experiment 2, we used isolates AS112 (white bars) and ASH4 (grey bars). Genotype names of the wild *Solanum* species are specified on the *x*-axes. Different letters indicate a significant ($P < 0.05$) difference. dpi = days post infection

**Table 1** Analyses of variance of lesion area following inoculations by *A. solani* in wild *Solanum* species treated with a biocontrol agent (*P. oligandrum* or *B. amyloliquefaciens*) in the greenhouse in experiments 1–2

| Source of variation | *df* | *F* | *P* |
|---|---|---|---|
| Experiment 1: lesion area ($mm^2$) 9 dpi | | | |
| *Solanum* species | **2** | **5.63** | **0.013** |
| *S. dulcamara* genotype[a] | 1 | 1.14 | 0.30 |
| Treatment (*P. oligandrum*) | 1 | 3.54 | 0.077 |
| Species × treat | 2 | 0.361 | 0.70 |
| Error | 17 | | |
| Experiment 2: lesion area ($mm^2$) 7 dpi | | | |
| *S. nigrum* genotype | 2 | 0.511 | 0.61 |
| *A. solani* isolate | 1 | 3.62 | 0.069 |
| Treatment (Serenade) | **1** | **8.26** | **0.008** |
| Genotype × isolate | 2 | 0.956 | 0.40 |
| Genotype × treat | 2 | 0.508 | 0.61 |
| Isolate × treat | 1 | 3.61 | 0.070 |
| Genotype × isolate × treat | 2 | 0.952 | 0.40 |
| Error | 24 | | |

Bold indicates significant ($P < 0.05$) factors

[a]*S. dulcamara* genotype was nested within species

## Effect of Biocontrol Agents on Plant Performance in the Greenhouse

In experiment 1, the biocontrol agent *P. oligandrum* affected plant performance in all three *Solanum* species, but in slightly different ways (Fig. 4, Table 2). In the three *S. dulcamara* genotypes, treated plants became smaller (Fig. 4a). The effect on dry root weight varied among genotypes (Fig. 4c), as indicated by the significant genotype by treatment interaction. In the two *S. nigrum* genotypes, a significant genotype by treatment interaction was seen for both plant size, estimated as plant height (Fig. 4d) and dry root weight (Fig. 4f). Thus, the response to the treatment varied with genotype. In *S. physalifolium*, the two genotypes responded in a similar way to *P. oligandrum*; they became shorter (Fig. 4j) and produced a higher dry green biomass (Fig. 4k) and a higher dry root biomass (Fig. 4l). Leaf area was not affected by treatment in any species (Table 2). Likewise, no treatment effects were seen for berry production in *S. nigrum* or *S. physalifolium* (Table 2).

In experiment 2, the three *S. nigrum* genotypes treated with the biocontrol agent *B. amyloliquefaciens*, significant genotype by treatment interactions were found for plant size and dry root weight (Fig. 4, Table 3). This pattern was similar to that seen in *S. nigrum* genotypes treated with *P. oligandrum*. Interestingly, the two genotypes used in both experiments (Nr 2 L and Nr 6 T) showed a similar plant size response to both biocontrol agents, but an opposite response regarding dry root mass (Fig. 4). The strong genotype effect detected on dry green mass was similar for the two genotypes across biocontrol agents.

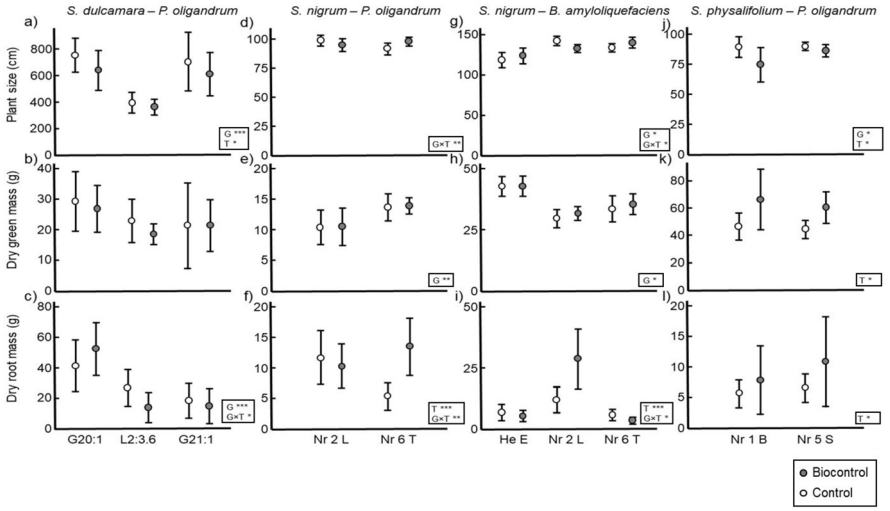


**Fig. 4** Plant performance traits measured in (**a**–**c**) three *S. dulcamara* genotypes, (**d**–**i**) three *S. nigrum* genotypes and (**j**–**l**) two *S. physalifolium* genotypes treated with a biocontrol agent or control in the greenhouse experiments 1–2. Genotype names of the wild *Solanum* species are specified on the *x*-axes. The biocontrol agent *P. oligandrum* was tested on all species, while the biocontrol agent *B. amyloliquefaciens* was tested only on *S. nigrum*. Plant size in *S. dulcamara* was estimated as length of all shoots, and in *S. nigrum* and *S. physalifolium* as plant height. G = effect of genotype, T = effect of treatment, G × T = effect of genotype by treatment interaction, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

## Effect of Biocontrol Agents on Above- versus Below-ground Allocation in the Greenhouse

To test if plants allocated resources differently above- versus below-ground in response to the biocontrol agents, we included dry root mass as a covariate in the models with the dependent variable above green mass, including all interactions. However, we found no significant differences in allocation in either tested species, as indicated by the interaction between treatment and dry root mass, or the interaction between treatment, dry root mass and genotype (*S. dulcamara*: $P > 0.61$, *S. nigrum*: $P > 0.16$, *S. physalifolium*: $P > 0.67$). Thus, there was no evidence for a change in above- versus below-ground allocation in response to treatment with the two tested biocontrol agents. There were, however, positive correlations between dry green mass and dry root mass across treatments (both with and without BCAs) in *S. dulcamara* ($P = 0.005$) and *S. nigrum* (experiment 1: $P = 0.011$, experiment 2: $P = 0.013$), but not in *S. physalifolium* ($P = 0.085$).

## Plant Developmental Stage and Performance in Response to *P. oligandrum* in the Greenhouse

To get an indication of whether plant developmental stage influenced the response to treatment with the biocontrol agent *P. oligandrum*, plant performance traits in *S. dulcamara* were also evaluated in plants that were 2 weeks younger

**Table 2** Analyses of variance of plant performance traits in three wild *Solanum* species treated with the biocontrol agent *P. oligandrum* in the greenhouse experiment 1

| Source of variation | Plant size (cm)[a] | | | Leaf area ($mm^2$)[b] | | | Number of berries | | | Dry berry mass (g) | | | Dry green mass (g)[c] | | | Dry root mass (g)[d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* |
| *S. dulcamara* | | | | | | | | | | | | | | | | | | |
| Genotype | **2** | **36.8** | **< 0.001** | 1 | 3.08 | 0.054 | | | | | | | 2 | 4.41 | 0.17 | **2** | **19.2** | **< 0.001** |
| Treatment | **1** | **7.12** | **0.010** | 1 | 0.784 | 0.38 | | | | | | | 1 | 1.37 | 0.25 | 1 | 0.651 | 0.42 |
| Early plant size[e] | **1** | **20.4** | **< 0.001** | – | – | – | | | | | | | **1** | **5.18** | **0.027** | **1** | **6.37** | **0.015** |
| Genotype × treat | 2 | 0.951 | 0.39 | 2 | 1.19 | 0.31 | | | | | | | 2 | 0.258 | 0.77 | **2** | **3.61** | **0.034** |
| Error | 52 | | | 51 | | | | | | | | | 52 | | | 52 | | |
| *S. nigrum* | | | | | | | | | | | | | | | | | | |
| Genotype | 1 | 0.885 | 0.35 | **1** | **13.6** | **0.001** | **1** | **7.73** | **0.008** | 1 | 0.402 | 0.53 | **1** | **9.77** | **0.003** | 1 | 1.68 | 0.20 |
| Treatment | 1 | 0.50 | 0.48 | 1 | 0.048 | 0.83 | 1 | 0.200 | 0.66 | 1 | 2.22 | 0.14 | 1 | 0.030 | 0.86 | **1** | **4.80** | **< 0.001** |
| Chamber (G)[f] | **1** | **13.8** | **0.001** | 1 | 2.45 | 0.13 | **1** | **6.04** | **0.018** | 1 | 0.489 | 0.49 | 1 | 2.46 | 0.12 | 1 | 0.063 | 0.80 |
| Genotype × treat | **1** | **7.53** | **0.009** | 1 | 0.025 | 0.87 | 1 | 3.01 | 0.090 | 1 | 0.793 | 0.38 | 1 | < 0.001 | 1.0 | **1** | **10.5** | **0.002** |
| Error | 43 | | | 43 | | | 43 | | | 43 | | | 43 | | | 43 | | |
| *S. physalifolium* | | | | | | | | | | | | | | | | | | |
| Genotype | **1** | **7.76** | **0.008** | 1 | 0.014 | 0.91 | 1 | 1.56 | 0.22 | | | | 1 | 0.048 | 0.83 | 1 | 0.286 | 0.60 |
| Treatment | **1** | **5.69** | **0.022** | 1 | 0.026 | 0.87 | 1 | 0.710 | 0.41 | | | | **1** | **11.1** | **0.002** | **1** | **4.44** | **0.042** |
| Chamber (G)[f] | **1** | **15.8** | **< 0.001** | 1 | 1.64 | 0.21 | 1 | 2.84 | 0.10 | | | | 1 | 1.48 | 0.23 | 1 | 4.07 | 0.052 |
| Genotype × treat | 1 | 1.32 | 0.26 | 1 | 1.26 | 0.27 | 1 | 1.29 | 0.26 | | | | 1 | 0.337 | 0.57 | 1 | 1.83 | 0.19 |
| Error | 37 | | | 37 | | | 36 | | | | | | 36 | | | 35 | | |

Bold indicates a significant ($P < 0.05$) difference

[a]Plant size in *S. dulcamara* was estimated as length of all shoots (log-transformed), and in *S. nigrum* and *S. physalifolium* as plant height

[b]Log-transformed in *S. nigrum*

[c]Dry green mass included berries in *S. dulcamara* and in *S. physalifolium* (log-transformed in this species), but not in *S. nigrum* which had produced a very high number of berries

[d]Log-transformed in *S. nigrum* and in *S. physalifolium*

[e]Early plant size was controlled for in *S. dulcamara* models to take into account early size differences in this species

[f]Chamber nested under genotype (G) was included in the models for *S. nigrum* and *S. physalifolium* because some genotypes were split between two different chambers in the greenhouse

**Table 3** Analyses of variance of plant performance traits in *S. nigrum* treated with the biocontrol agent *B. amyloliquefaciens* in the greenhouse experiment 2

| | Plant size (cm)[a] | | | Number of berries | | | Dry berry mass (g) | | | Dry green mass (g) | | | Dry root mass (g)[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* |
| Genotype | **2** | **15.3** | **< 0.001** | **2** | **106** | **< 0.001** | **2** | **16.8** | **< 0.001** | **2** | **20.1** | **< 0.001** | **2** | **22.6** | **< 0.001** |
| Treatment | 1 | 0.052 | 0.82 | 1 | 0.086 | 0.77 | 1 | 1.14 | 0.29 | 1 | 0.030 | 0.86 | 1 | 0.074 | 0.79 |
| Genotype × treat | **1** | **3.95** | **0.025** | 1 | 0.094 | 0.91 | 1 | 1.22 | 0.30 | 1 | 2.46 | 0.12 | **1** | **4.39** | **0.017** |
| Error | 58 | | | 58 | | | 58 | | | 58 | | | 58 | | |

Bold indicates a significant ($P < 0.05$) difference

[a]Estimated as plant height and power-transformed

[b]Log-transformed

than the first replicate of plants and therefore smaller in size (mean ± SD longest shoot: first batch = 101 ± 22 cm; second batch = 36 ± 10 cm). Even though plants in the second replicate (batch 2) were harvested 2 weeks later than the first replicate (batch 1), these plants remained smaller (mean ± SD total shoot length: first replicate = 563 ± 30 cm; second replicate = 238 ± 8 cm, Table 4). The response to *P. oligandrum* was significantly different between replicates (batches) for plant size and dry root biomass at the end of the experiment, as suggested by the significant interactions involving treatment and plant batch (Table 4). Separate analyses including only plants from the second batch could not detect any significant effect of either biocontrol treatment or its interaction with genotype (ANOVA; plant size: genotype: $F_{2,55} = 1.72$, $P = 0.22$, treatment: $F_{1,55} = 0.573$, $P = 0.45$, T × G: $F_{2,55} = 1.11$, $P = 0.34$; dry root weight: genotype: $F_{2,55} = 1.00$, $P = 0.38$, treatment: $F_{1,55} = 0.299$, $P = 0.59$, T × G: $F_{2,55} = 1.52$, $P = 0.23$). No difference in response to the biocontrol treatment was seen for dry green biomass, indicating that this trait was not affected by *P. oligandrum* in either of the plant batches (Tables 2 and 4).

### Disease Progression in the Field Trial and Confirmation of *A. solani*

All three wild *Solanum* species and the potato cultivar Kuras started to show lesions typical of infection by *A. solani* (Fig. 5) from the middle of August, i.e., 3 weeks post inoculation of kernels. The percentage of infected leaves increased over the three scoring events performed during 5 weeks between the middle of August

**Table 4** Analyses of variance of plant performance traits in two *S. dulcamara* plant batches with an age difference of 2 weeks treated at the same time with the biocontrol agent *P. oligandrum* in the greenhouse experiment 1

| | Plant size (cm)[a] | | | Dry green mass (g)[b] | | | Dry root mass (g)[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| Source of variation | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* |
| Genotype | **2** | **10.3** | **< 0.001** | 2 | 0.968 | 0.38 | **2** | **6.76** | **0.002** |
| Treatment | 1 | 0.692 | 0.41 | 1 | 1.40 | 0.24 | 1 | 1.69 | 0.20 |
| Plant batch | **1** | **19.1** | **< 0.001** | **1** | **4.08** | **0.046** | 1 | 1.41 | 0.24 |
| Early plant size[c] | **1** | **5.22** | **0.024** | **1** | **10.6** | **0.002** | **1** | **8.29** | **0.005** |
| Genotype × treat | 2 | 0.116 | 0.89 | 2 | 0.442 | 0.64 | 2 | 0.26 | 0.77 |
| Genotype × plant batch | **2** | **22.1** | **< 0.001** | **2** | **12.7** | **< 0.001** | **2** | **14.3** | **< 0.001** |
| Treat × plant batch | **1** | **4.42** | **0.038** | 1 | 0.243 | 0.63 | 1 | 0.293 | 0.59 |
| Genotype × treat × plant batch | 2 | 1.45 | 0.24 | 2 | 0.949 | 0.39 | **2** | **3.43** | **0.036** |
| Error | 107 | | | 105 | | | 104 | | |

Bold indicates a significant ($P < 0.05$) difference

[a]Estimated as length of all shoots (log-transformed)

[b]Log-transformed

[c]Estimated as longest shoot (log-transformed)


**Fig. 5** Leaves of (**a**) *S. dulcamara*, (**b**) *S. nigrum* and (**c**) *S. physalifolium* with lesions following infection by *A. solani* in a field trial at Campus Alnarp, Sweden

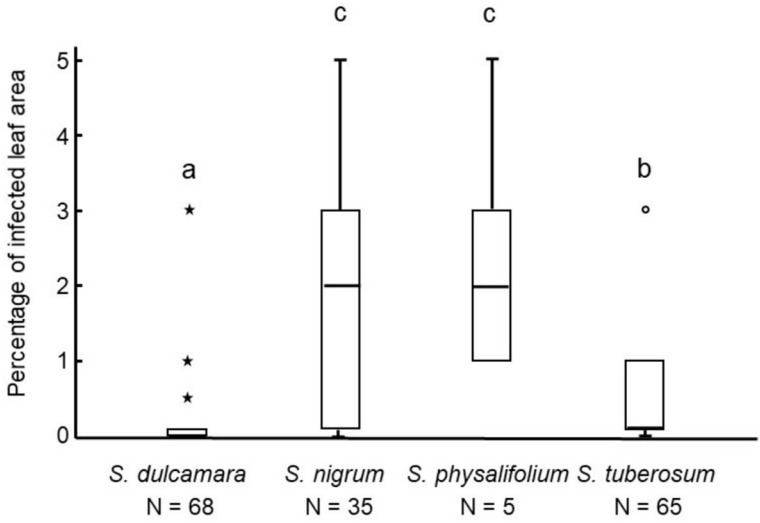


**Fig. 6** Boxplot for percentage of infected leaf area by *A. solani* in three wild *Solanum* species—*S. dulcamara*, *S. nigrum* and *S. physalifolium*—and the potato (*S. tuberosum*) cultivar Kuras in a field trial at Campus Alnarp, Sweden (plants grown in different places were pooled) at three occasions between 21 st of August and 24th of September. Different letters indicate a significant ($P<0.05$) difference. $N$ = number of plants

and the middle of September, but at the latest scoring date the infection was still low (Fig. 6). The *S. physalifolium* plot was affected by a spontaneous *P. infestans* infection at the end of August, causing the plants to drop all of their leaves. Thus, for *S. physalifolium* early blight could only be scored in plants growing between *S. dulcamara* plants ($N=5$). Despite the low infection level, the four species differed in percentage infected leaves ($F_{3,168}=31.9$, $P<0.001$, using pooled values for growth place as this factor was non-significant). *S. physalifolium* and *S. nigrum* were more susceptible to infection compared to *S. dulcamara* (Fig. 6). There were no differences in percentage infection among the three genotypes of *S. dulcamara*

(Kruskal–Wallis; 1.15, $N=68$, $df=2$, $P=0.56$) or the four genotypes of *S. nigrum* (Kruskal–Wallis; 5.47, $N=29$, $df=3$, $P=0.14$).

Percentage wilting of plants at the last scoring event (in mid-September) differed between growth place for *S. nigrum* and potato, but also between species as potato had slightly higher wilting percentage (ANOVA; own plots: *S. nigrum* $0.32 \pm 0.11$ (SE) %, potato $1.3 \pm 0.22$%, *S. dulcamara* plot: *S. nigrum* $0.90 \pm 0.56$%, potato $4.0 \pm 1.64$%; ANOVA; growth place: $F_{1,95}=9.51$, $P=0.003$, species: $F_{1,95}=22.0$, $P<0.001$, interaction: $F_{1,95}=2.33$, $P=0.13$). A separate analysis for all four species in the *S. dulcamara* plot showed a lower wilting percentage in *S. dulcamara* than in the five *S. physalifolium* plants and five potato plants (ANOVA; $F_{3,78}=5.1$, $P=0.003$; *S. dulcamara* $1.2 \pm 0.26$%, *S. physalifolium* $4.2 \pm 1.64$%). This difference was in line with the percentage infected leaves (Fig. 6).

Inspecting the water agar plates from all wild species under the microscope confirmed the presence of typical *A. solani* spores. Moreover, PCRs of isolates from *S. dulcamara* in our small field trial at Campus Alnarp and from naturally infected *S. nigrum* in a larger field trial confirmed the infection of *A. solani* in these two wild species (see Online Resource 1).

### Effect of *P. oligandrum* on Disease and Plant Performance in the Field Trial

We were unable to detect any significant effect of treatment with *P. oligandrum* on the percentage of infection by *A. solani* or wilting involving the 24 selected plants of *S. dulcamara*, *S. nigrum* or potato per species grown in the *A. solani* or control area across the three scoring dates (ANOVA; infection: species: $F_{2,180}=16.7$, $P<0.001$, treatment: $F_{3,180}=1.57$, $P=0.20$, date: $F_{2,180}=64.7$, $P<0.001$, species × date: $F_{4,180}=8.55$, $P<0.001$, other two-way interactions and three-way interaction: $P=0.64$–$0.78$; wilting: species: $F_{2,60}=13.5$, $P<0.001$, treatment: $F_{3,60}=1.60$, $P=0.20$, interaction: $F_{6,60}=1.73$, $P=0.13$).

*Solanum physalifolium* scored for infection by *P. infestans* on the 21st of August showed that $64 \pm 15$ (SD) % of leaves were infected (ranging between 50 and 100% per individual plant). The two plants with 100% infected leaves died, while plants with < 100% infected leaves all survived and started regrowing new leaves. Plant genotype did not influence percentage infection (Kruskal–Wallis; 3.16, $N=34$, $df=3$, $P=0.37$) or the percentage of regrowth in late September (0–75%, Kruskal–Wallis; 6.63, $N=32$, $df=3$, $P=0.093$). Treatment with *P. oligandrum* did not influence infection by *P. infestans* in the selected plants (Mann–Whitney *U*-test; 93, $N=23$, $P=0.089$) or regrowth in the surviving 22 of the selected plants (Mann–Whitney *U*-test; 63, $N=22$, $P=0.87$).

No plant performance traits were affected by *P. oligandrum* treatment in the *A. solani*–treated or control area, for either of the three wild species (Table 5). For both *S. dulcamara* and *S. nigrum*, early plant size had a big impact on later performance.

## Discussion

In this study, we showed that three wild *Solanum* species growing either as weeds in potato fields or close by potato fields can host the potato pathogen *Alternaria solani*. We also tested how two BCAs used in potato to control *A. solani* affect these wild plants. We found some indication of disease control of one of the BCAs—*B. amyloliquefaciens* in the commercial product Serenade. Both BCAs had effects on plant growth, but the effect varied with investigated trait, species, and genotype within species. When assessing environmental risks of BCAs, it may therefore be of interest to also consider their effects on wild relatives present in the field.

### Wild *Solanum* Species as Alternative Hosts of *A. solani*

Disease epidemiology in crops is not only affected by the crop per se but can also be influenced by presence of alternative hosts (Kumar et al. 2021; Susi 2024). For this reason, it is important to investigate if crop-wild relatives can act as alternative hosts of crop pathogens. Potato has three wild relatives in Sweden—the perennial climber *S. dulcamara* and the two annual weeds *S. nigrum* and *S. physalifolium*. Previous studies showed varying susceptibility to late blight in these species (Grönberg et al. 2012; Abreha et al. 2018)—and that *S. dulcamara* can act as an overwintering host (in the rhizosphere) (Vetukuri et al. 2020). Surprisingly, we were unable to find studies that had tested if these three species could act as alternative hosts also to *A. solani*, but there appears to be some sematic knowledge. For example, the two annual weeds often grow in potato fields and lesions similar to those resulting from early blight are often noted by farmers. In our study, we inoculated these three species with *A. solani* both in the greenhouse and in a small-scale field trial. We found that *A. solani* was able to infect all three species. The greenhouse results suggested that *S. physalifolium* was more susceptible than the other species. Furthermore, results from the field trials indicated that the two annual species showed higher levels of infection than *S. dulcamara*, indicating a different pattern compared to infection by *P. infestans* where *S. physalifolium* is highly susceptible and *S. nigrum* is mostly resistant (Abreha et al. 2018). The higher levels of infection in the greenhouse compared to in the field for *S. dulcamara* was seen previously for *P. infestans* (Masini et al. 2019), and is probably related to the production of thicker and smaller leaves in the field. It should be noted that our field trial was small and the degree of infection by *A. solani* quite low. Moreover, a spontaneous *P. infestans* infection in *S. physalifolium* caused these plants to drop their leaves and therefore reduced the number of plants we could score for early blight.

Confirmation of *A. solani* was also carried out by inspection of the spores under the microscope for all three species and through PCR from samples from our small field trial for *S. dulcamara* and for *S. nigrum* from a potato field trial with natural infection. These data further support the hypothesis that the wild species can

**Table 5** Analyses of variance of plant performance traits in three wild *Solanum* species treated with the biocontrol agent *P. oligandrum* in a field trial at Campus Alnarp, Sweden

| Source of variation | Plant size (cm)[a] August | | | Number of flowers[b]/ flower + berries[c] August | | | Plant size (cm)[a] September | | | Number of berries[d] September | | | Dry green mass (g)[e] November | | | Dry berry mass (g)[d] November | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* |
| *S. dulcamara* | | | | | | | | | | | | | | | | | | |
| Treatment | 1 | 2.24 | 0.15 | 1 | 0.020 | 0.89 | 1 | 0.214 | 0.65 | 1 | 0.214 | 0.65 | 1 | 0.184 | 0.67 | 1 | 2.68 | 0.12 |
| Plant size[f] July | **1** | **22.8** | **<0.001** | 1 | 19.2 | **<0.001** | **1** | **9.86** | **0.005** | **1** | **9.86** | **0.005** | – | – | – | **1** | **10.7** | **0.006** |
| Error | 16 | | | 16 | | | 20 | | | 20 | | | 20 | | | 14 | | |
| *S. nigrum* | | | | | | | | | | | | | | | | | | |
| Treatment | 1 | 0.50 | 0.48 | 1 | 0.048 | 0.83 | 1 | 0.369 | 0.55 | | | | 1 | 0.068 | 0.80 | | | |
| Plant size[f] July | 1 | 26.3 | **<0.001** | – | – | – | **1** | **10.2** | **0.005** | | | | – | – | – | | | |
| Error | 19 | | | 19 | | | 19 | | | | | | 20 | | | | | |
| *S. physalifolium* | | | | | | | | | | | | | | | | | | |
| Treatment | 1 | 0.191 | 0.66 | | | | 1 | 0.006 | 0.94 | | | | 1 | 0.003 | 0.96 | | | |
| Error | 21 | | | | | | 20 | | | | | | 20 | | | | | |

Bold indicates a significant ($P < 0.05$) difference

[a]Plant size in *S. dulcamara* was estimated as length of all shoots, and in *S. nigrum* and *S. physalifolium* as plant height

[b]Number of flowers in *S. dulcamara* (log-transformed)

[c]Number of inflorescences with flowers and berries in *S. nigrum* (log-transformed)

[d]Log-transformed

[e]Log-transformed in *S. nigrum*

[f]Early plant size was controlled for in *S. dulcamara* and *S. nigrum* models to take into account early size differences in these species

be alternative hosts for *A. solani*. From other observations of potato field trials and commercial potato fields with naturally occurring *A. solani* infection in south Sweden, we have noted that *S. nigrum* and *S. physalifolium* growing next to the field often show early blight disease symptoms, which may indicate an influence on disease epidemiology (Lankinen et al. unpublished observations). However, we have also seen that disease symptoms in the wild species usually appear later than in potato. Because early blight disease is positively correlated to plant age (Odilbekov et al. 2020), it is possible that the later life-cycle of these wild species (from July to October), compared to potato, reduces their potential influence on the epidemiology of early blight. Interestingly, the isolate collected from *S. nigrum* in potato field trials showed a trend towards higher pathogenicity than a commonly used laboratory strain. The trend of higher pathogenicity in the isolate collected from the wild species may be caused by the more recent collection of this isolate compared to the laboratory strain, reflecting the well-known degeneration of pathogenicity in laboratory strains of plant pathogens in general (Danner et al. 2023). In future studies, it would be of interest to understand better if the wild *Solanum* species influence *A. solani* evolution, and if this impacts pathogenicity on potato, as has been indicated for *P. infestans* (Grönberg et al. 2012).

### Control of Early Blight by BCAs in Wild *Solanum* Species

While environmental risks must be taken into consideration in the approval process of BCAs (Simberloff and Stiling 1996; Collinge et al. 2022), less is known about potential positive effects, e.g., if the BCA can control disease also in wild relatives and thereby reduce the presence of the pathogen. In the current study, we investigated if the oomycete BCA *Pythium oligandrum* (using a laboratory strain) and the bacterial BCA *Bacillus amyloliquefaciens* (formerly *B. subtilis*) (using the commercial product Serenade) could control early blight in the three wild *Solanum* species. Previous studies in potato showed that both *P. oligandrum* (the commercial product Polygandron and our laboratory strain) and *B. amyloliquefaciens* (Serenade) controlled early blight in the greenhouse, and that *B. amyloliquefaciens* was more effective than *P. oligandrum* (Stridh et al. 2022). In line with these studies, we found that *B. amyloliquefaciens* was effective at controlling early blight when tested in *S. nigrum* in the greenhouse. In contrast, *P. oligandrum* had no disease-controlling effect in either of the tested three species in the greenhouse. It is possible that the lack of effect of *P. oligandrum* was because this was a small experiment in combination with an expected smaller effect size for this BCA compared to Serenade. It is also possible that *P. oligandrum* is unable to control early blight in these wild species.

In our field trial, we investigated the biocontrol effect of *P. oligandrum.* In line with the results of the greenhouse study, we were unable to detect a disease controlling effect in either *S. dulcamara*, *S. nigrum* or the starch potato cultivar Kuras. This result was not surprising given that in potato, BCAs are less effective in the field compared to in the greenhouse (Stridh et al. 2022) or show a transient effect (Andersen 2023). It is also possible that the low infection pressure made our studies less reliable or made

the infection less easy to detect. In *S. physalifolium*, we were not able to investigate a biocontrol effect on *A. solani* because of the spontaneous *P. infestans* infection. However, *P. oligandrum* had no disease-controlling effect on the *P. infestans* infection or on the capacity of these plants to regrow. Interestingly, even though plants lost most of their leaves, 22 out of 24 plants survived the *P. infestans* infection and started to regrow. Abscission of leaves either as a response to damage or as a defence response has been observed in many species (Kong and Yang 2023). In future studies, knowledge about the disease-controlling capacity of a BCA not only on a given crop but also on nearby alternative hosts may be of interest to quantify for a better understanding of the environmental impact of BCAs and also for a more comprehensive understanding of how important it is to control these weeds in potato fields.

### Growth Promotion in Wild *Solanum* Species following BCA Treatment

The added benefit of growth promotion of BCAs is receiving increasing interest (El-Saadony et al. 2022), but less is known about environmental effects on wild plants. In this study, we evaluated a potential growth-promoting effect on the three wild relatives of potato of the BCAs *P. oligandrum* and *B. amyloliquefaciens* (Serenade), known to be growth promoting in potato (Syed and Prasad Tollamadugu 2019; Andersen et al. 2024). In the greenhouse, we found that growth was affected following treatment with *P. oligandrum*, but the outcome varied across investigated traits, species and genotypes within species. In general, root mass was more often positively affected, while the response in above-ground biomass or plant size was more variable. *S. physalifolium* responded positively to *P. oligandrum* treatment in terms of both root mass and above-ground mass, but plant size (measured as height) was slightly reduced. *Solanum dulcamara* and *S. nigrum* showed variation among genotypes in the root mass response. In *S. dulcamara*, there was a slight negative effect on plant size (measured as shoot length), while in *S. nigrum* the response in plant size (measured as height) again differed among genotypes. When we tested the effect of *B. amyloliquefaciens* (Serenade) in *S. nigrum*, the outcome was similar to the effect of *P. oligandrum.* However, the genotype response was not consistent across BCAs tested. The variation in response among genotypes is in line with studies showing that growth is promoted only in some potato cultivars following treatment with *P. oligandrum* (Andersen et al. 2024). Moreover, one of the few other studies that investigated how a BCA influences growth promotion in wild species found that the endomycorrhizal fungus *Serendipita vermifera* had a variable effect on three nearby weed species occurring with the crop switchgrass (Ray et al. 2018). Our greenhouse results suggest that at least for *S. physalifolium* and potentially also for *S. nigrum*, exposure in a potato field may enhance the weed problem, as increased root mass is likely to increase water and nutrient update, and therefore performance of these weeds. It is, however, uncertain if these effects are dependent on the plant development stage that is exposed to the BCA, as our greenhouse results indicated for *S. dulcamara*, or if the same results will be seen under field conditions. We were unable to detect any effects of *P. oligandrum* treatment in the field trial, but here only plant height was measured, which showed an unclear or

negative effect in the greenhouse. Even though this was a small field trial, we could detect a positive effect on plant height of the potato plants grown in the same field trial (Andersen et al. 2024). From these greenhouse results, we conclude that growth promotion can happen in wild *Solanum* relatives of potato exposed to BCAs, but the effect can vary and it is therefore not so easy to predict unless investigated. Future studies should evaluate growth promotion effects of BCAs on wild plants under field conditions and at time points that reflect their use in agriculture. Such data on wild crop relatives may also be useful for finding genetic differences in the response to BCAs that could contribute to plant breeding for genotypes that respond well to BCAs (Schmidt et al. 2020).

## Conclusions

In this study, we showed that *A. solani* can infect the three wild *Solanum* species that occur in Sweden, supporting the hypothesis that they can be alternative hosts of this pathogen. To our knowledge, this is the first study to report infection of *A. solani* in these wild species, which is important for the prediction of early blight disease epidemiology in the future. We also found that to some extent, the BCA *B. amyloliquefaciens*, but not the BCA *P. oligandrum*, can control early blight in wild *Solanum* species. Moreover, these BCAs affected growth of the wild species, but the effects were not always positive. We conclude that the investigated BCAs can result in both positive and negative environmental effects when affecting these wild species within or near to potato fields. The variability in the responses suggests that these effects may be difficult to predict beforehand and therefore it may be beneficial to take the effects of BCAs on wild species into consideration for successful use of them in sustainable agriculture.

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**Author Contribution** ÅL initiated and coordinated the study. ÅL, CBA, HM, EL and LGB contributed to the study conception and design. Material preparation and data collection was performed by ÅL, CBA, HM, FQ, CDP, VH and LJS. Data analysis was performed by ÅL. The first draft of the manuscript was written by ÅL with help from CBA and CDP. ÅL, HM, CDP, VH, EL and LGB contributed to manuscript editing and reviewing. All authors read and approved the final manuscript.

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**Data Availability** Data are available from the Swedish National Data Service (SND) Repository: https://doi.org/10.5878/t350-qh54 (Lankinen et al. 2025).

## Declarations

**Conflict of interest** The authors declare no competing interests.



## References

Abreha KB, Lankinen Å, Masini L, Hydbom S, Andreasson E (2018) Late blight resistance screening of major wild Swedish Solanum species: *S. dulcamara, S. nigrum* and *S. physalifolium*. Phytopathol 108:847–857. https://doi.org/10.1094/PHYTO-10-17-0355-R

Adolf B, Hausladen H (2015) Protocol for the artificial inoculation with *A. solani* in field trials (with infected kernels). Euroblight Protocols for Alternaria. https://agro.au.dk/forskning/internationale-platforme/euroblight/alternaria/protocols. Accessed 6 Nov 2024

Aktar W, Sengupta D, Chowdhury, (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol. 2:1–12. https://doi.org/10.2478/v10102-009-0001-7

Andersen CB, Aleklett K, Digdarshika G, Lankinen Å, Grenville-Briggs L (2024) *Pythium oligandrum* induces growth promotion in starch potato without significantly altering the rhizosphere microbiome. Appl Soil Ecol 199:105423. https://doi.org/10.1016/j.apsoil.2024.105423

Andersen C (2023) Friend or foe? Biocontrol interactions of *Pythium oligandrum* within the potato cropping system. Dissertation, Swedish University of Agricultural Sciences

Bardin M, Siegwart M (2022) Can pests develop resistance to biocontrol products? In: Fauvergue X, Rusch A, Barret M, Bardin M, Jacquin-Joly E, Malausa T, Lannou C (eds) Extended biocontrol. Springer, Netherlands, pp 267–272. https://doi.org/10.1007/978-94-024-2150-7_23

Barzman M, Bàrberi P, Birch ANE, Boonekamp P, Dachbrodt-Saaydeh S, Graf B, Hommel B, Jensen JE, Kiss J, Kudsk P, Lamichhane JR, Messéan A, Moonen A-C, Ratnadass A, Ricci P, Sarah J-L, Sattin M (2015) Eight principles of integrated pest management. Agron Sustain Dev 35:1199–1215. https://doi.org/10.1007/s13593-015-0327-9

Brouwer SM, Wolters PJ, Andreasson E, Liljeroth E, Vleeshouwers VGAA, Grenville-Briggs LJ (2023) Double trouble: co-infection of potato with the causal agents of late and early blight. Plant Pathol 72:1365–1379. https://doi.org/10.1111/ppa.13759

Calvo P, Nelson L, Kloepper JW (2014) Agricultural uses of plant biostimulants. Plant Soil 383:3–41. https://doi.org/10.1007/s11104-014-2131-8

Collinge DB, Jensen DF, Rabiey M, Sarrocco S, Shaw MW, Shaw RH (2022) Biological control of plant diseases – what has been achieved and what is the direction? Plant Pathol 71:1024–1047. https://doi.org/10.1111/ppa.13555

Danner C, Mach RL, Mach-Aigner AR (2023) The phenomenon of strain degeneration in biotechnologically relevant fungi. Appl Microbiol Biotechnol 107:4745–4758. https://doi.org/10.1007/s00253-023-12615-z

Dara SK (2019) The new integrated pest management paradigm for the modern age. J Integr Pest Manag 10:1. https://doi.org/10.1093/jipm/pmz010

Deguine J-P, Aubertot J-N, Flor RJ, Lescourret F, Wyckhuys KAG, Ratnadass A (2021) Integrated pest management: good intentions, hard realities. A Review Agron Sustain Dev 41:38. https://doi.org/10.1007/s13593-021-00689-w

Duarte HSS, Zambolim L, Capucho AS, Júnior AFN, Rosado AWC, Cardoso CR, Paul PA, Mizubuti ESG (2013) Development and validation of a set of standard area diagrams to estimate severity of potato early blight. Eur J Plant Pathol 137:249–257. https://doi.org/10.1007/s10658-013-0234-3

EC (2009) Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for community action to achieve the sustainable use of pesticides. http://data.europa.eu/eli/dir/2009/128/2019-07-26. Accessed 10 Jan 2023

EC (2024) EU pesticide database for active substances. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances. Accessed 8 Apr 2024

Edin E, Liljeroth E, Andersson B (2019) Long term field sampling in Sweden reveals a shift in occurrence of cytochrome b genotype and amino acid substitution F129L in *Alternaria solani*, together with a high incidence of the G143A substitution in *Alternaria alternata*. Eur J Plant Pathol 155:627–641. https://doi.org/10.1007/s10658-019-01798-9

Einspanier S, Susanto T, Metz N, Wolters PJ, Vleeshouwers VGAA, Lankinen Å, Liljeroth E, Landschoot S, Ivanović Ž, Hückelhoven R, Hausladen H, Stam R (2022) Whole-genome sequencing elucidates the species-wide diversity and evolution of fungicide resistance in the early blight pathogen Alternaria solani. Evol Appl 15:1605–1620. https://doi.org/10.1111/eva.13350

El-Saadony MT, Saad AM, Soliman SM, Salem HM, Ahmed AI, Mahmood M, El-Tahan AM, Ebrahim AAM, Abd El-Mageed TA, Negm SH, Selim S, Babalghith AO, Elrys AS, El-Tarabily KA, AbuQamar SF (2022) Plant growth-promoting microorganisms as biocontrol agents of plant diseases: mechanisms, challenges and future perspectives. Front Plant Sci 13:923880. https://doi.org/10.3389/fpls.2022.923880

Eriksson D, Carlson-Nilsson U, Ortíz R, Andreasson E (2016) Overview and breeding strategies of table potato production in Sweden and the Fennoscandian region. Potato Res 59:279–294. https://doi.org/10.1007/s11540-016-9328-6

Grönberg L, Andersson B, Yuen JE (2012) Can weed hosts increase aggressiveness of *Phytophthora infestans* on potato? Phytopathol 102:429–433. https://doi.org/10.1094/PHYTO-12-11-0326

Hashemi M, Tabet D, Sandroni M, Benavent-Celma C, Seematti J, Andersen CB, Grenville-Briggs LJ (2022) The hunt for sustainable biocontrol of oomycete plant pathogens, a case study of *Phytophthora infestans*. Fungal Biol Rev 40:53–69. https://doi.org/10.1016/j.fbr.2021.11.003

Hinz HL, Winston RL, Schwarzländer M (2019) How safe is weed biological control? A global review of direct nontarget attack. Q Rev Biol 94:1. https://doi.org/10.1086/702340

IBM SPSS Statistics for Windows (2022) SPSS (version 29.0). IBM Corp, Armonk, New York, USA

Indira Devi P, Manjula M, Bhavani RV (2022) Agrochemicals, environment, and human health. Annu Rev Environ Resour 47:399–421. https://doi.org/10.1146/annurev-environ-120920

Kapsa JS (2008) Important threats in potato production and integrated pathogen/pest management. Potato Res 51:385–401. https://doi.org/10.1007/s11540-008-9114-1

Karlsson Green K, Stenberg JA, Lankinen Å (2020) Making sense of integrated pest management (IPM) in the light of evolution. Evol Appl 13:1791–1805. https://doi.org/10.1111/eva.13067

Ke J, Wang B, Yoshikuni Y (2021) Synthetic biology of plant-associated microbiomes in sustainable agriculture. Trends Biotechnol 39:244–261. https://doi.org/10.1016/j.tibtech.2020.07.008

Keswani C, Prakash O, Bharti N, Vílchez JI, Sansinenea E, Lally RD, Borriss R, Singh SP, Gupta VK, Fraceto LF, de Lima R, Singh HB (2019) Re-addressing the biosafety issues of plant growth promoting rhizobacteria. Sci Total Environ 690:841–852. https://doi.org/10.1016/j.scitotenv.2019.07.046

Kong F, Yang L (2023) Pathogen-triggered changes in plant development: virulence strategies or host defense mechanism? Front Microbiol 14:1122947. https://doi.org/10.3389/fmicb.2023.1122947

Kumar S, Singh R, Kashyap PL, Srivastava AK (2013) Rapid detection and quantification of *Alternaria solani* in tomato. Sci Hortic 151:184–189. https://doi.org/10.1016/j.scienta.2012.12.026

Kumar S, Bhowmick MK, Ray P (2021) Weeds as alternate and alternative hosts of crop pests. Indian Journal of Weed Science 53:14–29. https://doi.org/10.5958/0974-8164.2021.00002.2

Landschoot S, Carrette J, Vandecasteele M, De Baets B, Höfte M, Audenaert K, Haesaert G (2017) Boscalid-resistance in *Alternaria alternata* and *Alternaria solani* populations: an emerging problem in Europe. Crop Prot 92:49–59. https://doi.org/10.1016/j.cropro.2016.10.011

Lankinen Å, Abreha KB, Alexandersson E, Andersson S, Andreasson E (2016) Nongenetic inheritance of induced resistance in a wild annual plant. Phytopathol 106:877–883. https://doi.org/10.1094/PHYTO-10-15-0278-R

Lankinen Å, Witzell J, Aleklett K, Furenhed S, Karlsson Green K, Latz M, Liljeroth E, Larsson R, Löfkvist K, Meijer J, Menkis A, Ninkovic V, Olson Å, Grenville-Briggs L (2024) Challenges and opportunities for increasing the use of low-risk plant protection products in sustainable production. A Review Agron Sustain Dev 44:21. https://doi.org/10.1007/s13593-024-00957-5

Leiminger JH, Hausladen H (2012) Early blight control in potato using disease-orientated threshold values. Plant Dis 96:124–130. https://doi.org/10.1094/PDIS-05-11-0431

Lovat C, Nassar AMK, Kubow S, Li XQ, Donnelly DJ (2016) Metabolic biosynthesis of potato (*Solanum tuberosum* L.) antioxidants and implications for human health. Crit Rev Food Sci Nutr 56:2278–2303. https://doi.org/10.1080/10408398.2013.830208

Masini L, Grenville-Briggs LJ, Andreasson E, Råberg L, Lankinen Å (2019) Tolerance and overcompensation to infection by *Phytophthora infestans* in the wild perennial climber *Solanum dulcamara*. Ecol Evol 9:4557–4567. https://doi.org/10.1002/ece3.5057

Matyjaszczyk E (2018) "Biorationals" in integrated pest management strategies. J Plant Dis Prot 125:523–527. https://doi.org/10.1007/s41348-018-0180-6

Mostafanezhad H, Edin E, Grenville-Briggs LJ, Lankinen Å, Liljeroth E (2022) Rapid emergence of boscalid resistance in Swedish populations of *Alternaria solani* revealed by a combination of field and laboratory experiments. Eur J Plant Pathol 162:289–303. https://doi.org/10.1007/s10658-021-02403-8

Odilbekov F, Carlson-Nilsson U, Liljeroth E (2014) Phenotyping early blight resistance in potato cultivars and breeding clones. Euphytica 197:87–97. https://doi.org/10.1007/s10681-013-1054-4

Odilbekov F, Edin E, Mostafanezhad H, Coolman H, Grenville-Briggs LJ, Liljeroth E (2019) Within-season changes in Alternaria solani populations in potato in response to fungicide application strategies. Eur J Plant Pathol 155:953–965. https://doi.org/10.1007/s10658-019-01826-8

Odilbekov F, Selga C, Ortiz R, Chawade A, Liljeroth E (2020) QTL mapping for resistance to early blight in a tetraploid potato population. Agronomy 10:728. https://doi.org/10.3390/agronomy10050728

Rabiey M, Ullah I, Shaw LJ, Shaw MW (2017) Potential ecological effects of *Piriformospora indica*, a possible biocontrol agent, in UK agricultural systems. Biol Control 104:1–9. https://doi.org/10.1016/j.biocontrol.2016.10.005

Ray P, Guo Y, Kolape J, Craven KD (2018) Non-targeted colonization by the endomycorrhizal fungus, *Serendipita vermifera*, in three weeds typically co-occurring with switchgrass. Front Plant Sci 8:2236. https://doi.org/10.3389/fpls.2017.02236

SCB (2022) Pesticides in agriculture and horticulture 2021. Use on crops. Statistiska meddelanden MI 31 SM 2202, Statistiska Centralbyrån, Sweden. https://www.scb.se/contentassets/6e042f0902bb449fb15edc4c1eb8e22c/mi0502_2021i20_br_mi31br2202.pdf. Accessed 2 Dec 2024

Schmidt J, Dotson BR, Schmiderer L, van Tour A, Kumar B, Marttila S, Fredlund KM, Widell S, Rasmusson AG (2020) Substrate and plant genotype strongly influence the growth and gene expression response to *Trichoderma afroharzianum* T22 in sugar beet. Plants 9:1–14. https://doi.org/10.3390/plants9081005

Shahin EA, Shepard JF (1979) An efficient technique for inducing profuse sporulation of *Alternaria* species. Phytopathol 69:618–620

Simberloff D, Stiling P (1996) How risky is biological control? Ecol 77:1965–1974. https://doi.org/10.2307/2265693

Stenberg JA (2017) A conceptual framework for integrated pest management. Trends Plant Sci 22:759–769. https://doi.org/10.1016/j.tplants.2017.06.010

Stenberg JA, Sundh I, Becher PG, Björkman C, Dubey M, Egan PA, Friberg H, Gil JF, Jensen DF, Jonsson M, Karlsson M, Khalil S, Ninkovic V, Rehermann G, Vetukuri RR, Viketoft M (2021) When is it biological control? A framework of definitions, mechanisms, and classifications. J Pest Sci 94:665–676. https://doi.org/10.1007/s10340-021-01354-7

Stridh LJ, Mostafanezhad H, Andersen CB, Odilbekov F, Grenville-Briggs L, Lankinen Å, Liljeroth E (2022) Reduced efficacy of biocontrol agents and plant resistance inducers against potato early blight from greenhouse to field. J Plant Dis Prot 129:923–938. https://doi.org/10.1007/s41348-022-00633-4

Susi H (2024) Alternative host shapes transmission and life-history trait correlations in a multi-host plant pathogen. Evol Appl 17:e13672. https://doi.org/10.1111/eva.13672

Syed S, Prasad Tollamadugu NVKV (2019) Role of plant growth-promoting microorganisms as a tool for environmental sustainability. In: Buddolla V (ed) Recent developments in applied microbiology and biochemistry. Elsevier, pp. 209–222. https://doi.org/10.1016/B978-0-12-816328-3.00016-7

Taab A (2021) *Solanum nigrum* and *Solanum physalifolium*. In: Bhagirath Singh Chauhan (ed) Biology and management of problematic crop weed species, 1st edn. Elsevier pp 357–373. https://doi.org/10.1016/B978-0-12-822917-0.00005-7

Vetukuri RR, Masini L, McDougal R, Panda P, de Zinger L, Brus-Szkalej M, Lankinen Å, Grenville-Briggs LJ (2020) The presence of *Phytophthora infestans* in the rhizosphere of a wild *Solanum* species may contribute to off-season survival and pathogenicity. Appl Soil Ecol 148:103475. https://doi.org/10.1016/j.apsoil.2019.103475

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