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Faculty of Landscape Architecture, Horticulture and Crop Production Science

Alternative strategies to treat potato early blight

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Cover: Field plot untreated against early blight in a starch potato field.
(photo: Gabriella Malm)

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Errata for Alternative strategies to treat potato early blight

by Linnea Stridh

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Paper III	Location:	Figure 3B, label x-axis
	Is now:	rAUDPC untreated/rAUC treated "relative susceptibility"
	Should be:	rAUDPC untreated/rAUC treated "relative susceptibility" in relation to cv. Kuras (Kuras = 1)

Alternative strategies to treat potato early blight

Abstract

Potato, *Solanum tuberosum*, is a staple crop grown worldwide. Like all other plants that are cultivated in the world's vast agricultural system, potatoes are constantly under attack by plant pathogens. Early blight is a potato disease caused by a fungal pathogen called *Alternaria solani*. In Sweden this pathogen is particularly problematic in the starch potato industry causing premature defoliation and reduced starch yield. The most common current treatment is application of fungicides. The focus of the research presented in this thesis has been to test and evaluate alternative ways to combat this pathogen in an applied Swedish field environment. A three-year observational study was conducted, as were multiple field trials, to achieve a broader understanding of how to manage early blight. The results of the observational study led us to design further field trials to test the importance of potassium. We found interesting differences in disease severity among the farms. The field trials consisted of evaluating cultivar tolerance, biological control measures such as the use of biocontrol agents (BCAs) and plant resistance inducers (PRIs), and the role of plant nutrients. The most important finding in this thesis is that the best treatment strategy is highly farm specific, and it is crucial to customize the treatments at a field level. The soil composition is the single largest factor that impacts the rate of infection. A sandier soil is much more likely to suffer from early blight induced yield loss and the recommended treatments should be based on the sand content of the soil in the specific field. Further results conclude that the potassium content in the soil and leaf plays a role in disease rate since a depletion caused heavier infection. The BCAs and PRIs evaluated showed potential for future alternative strategies but none of the evaluated substances proved to be efficient under field conditions. Lastly, it was observed that there are differences among starch potato cultivars currently grown, that affect the disease rate of early blight.

Keywords: Early blight, alternative strategies, IPM, *Alternaria solani*, Integrated Disease Management

Alternativa strategier för behandling av torrfläcksjuka i potatis

Sammanfattning

Potatis, *Solanum tuberosum*, är en stapelgröda som produceras i hela världen. Liksom alla andra grödor som odlas i världens omfattande agrara system, så är potatisen under ständigt hot från växtpatogener. Torrfläcksjuka, eller Alternaria, är en potatissjukdom orsakad av svamppatogenen *Alternaria solani*. I Sverige är denna sjukdom särskilt problematisk inom potatisstärkelseproduktionen där den orsakar en tidigare nedvissning, vilket reducerar stärkelseskörden. Den idag vanligaste behandlingen är applicering av fungicider. Forskningsfokus i denna avhandling har varit att testa och utvärdera alternativa sätt att bekämpa denna växtpatogen i en tillämpad svensk fältmiljö. En treårig observationsstudie utfördes, tillika flera fältförsök, med målet att ge en bredare förståelse för hur man bäst behandlar Alternaria. Resultatet från observationsstudien ledde till utformningen av ytterligare fältförsök för att utvärdera betydelsen av kalium. Vi fann även intressanta fältskillnader i sjukdomstrycket. Fältförsöken omfattade utvärdering av sortskillnader i sjukdomsmottaglighet, användandet av biologisk bekämpning, samt vilken roll som växtnäringsämnen spelar. Den viktigaste slutsatsen i denna avhandling är att den bästa bekämpningsstrategin är platsspecifik, och det är avgörande att anpassa bekämpningsstrategin på fältnivå. Jordens sammansättning är den enskilt viktigaste faktorn som avgör sjukdomstrycket. Skördeförluster orsakade av Alternaria uppträder med mycket större sannolikhet på jordar med hög sandhalt och den rekommenderade bekämpningen bör baseras på sand- och grovmohalten i det specifika fältet. Vidare visar resultaten att kaliumhalten i jorden och blasten inverkar på infektionstrycket då kaliumbrist gav större sjukdomsangrepp. De utvärderade biologiska substanserna visar på en potentiell framtida användning men ingen av de studerade preparaten hade i nuläget tillräckligt hög verkan i fältmiljö. Slutligen observerades sortskillnader i sjukdomsmottaglighet bland aktuella stärkelsesorter.

Nyckelord: Torrfläcksjuka, alternativa strategier, IPM, Alternaria solani, Integrerat växtskydd

Dedication

To the four men who made this thesis possible,
to my father who gave me the incentive,
to Michael Pollan who gave me the interest,
to Harald Berg who got me to initiate,
to Magne who made the investment.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Stridh, L. J[a].**, Malm, G., Lankinen, Å. and Liljeroth, E. (2023) Field and management factors can reduce potato early blight severity – An observational study on farms combined with field trials in southern Sweden. *Potato Research*. DOI: 10.21203/rs.3.rs-3187902/v1.

II. **Stridh, L. J*.**, Mostafanezhad, H*., Andersen, C. B., Odilbekov, F., Grenville-Briggs, L., Lankinen, Å. and Liljeroth, E. (2022) Reduced efficacy of biocontrol agents and plant resistance inducers against potato early blight from greenhouse to field. *Journal of Plant Diseases and Protection*, 129:923-938.

III. **Stridh, L. J[a].**, Alexandersson, E., Liljeroth E. and Sivarajan, R. S. Cultivar differences in susceptibility to early blight in starch potato is not only linked to maturity type. (Manuscript)

Papers I-II are reproduced with the permission of the publishers.

*Equally contributing authors
[a] Corresponding author

Linnea J. Stridh also contributed to the studies presented in the following articles that are not included in this thesis.

I. Olayide, P., Large, A., **Stridh, L. J.**, Rabbi, I., Baldermann, S., Stavolone, L. and Alexandersson, E. (2020) Gene Expression and Metabolite Profiling of Thirteen Nigerian Cassava Landraces to Elucidate Starch and Carotenoid Composition. *Agronomy 10*, 3: 424.

II. Vilvert, E., **Stridh, L. J.**, Andersson, B., Olson, Å., Aldén, L. & Berlin, A. (2022) Evidence based disease control methods in potato production: a systematic map protocol. *Environ Evid*, 11:6.

III. Andersen, C. B., Lassen, S. B., Brus-Szkalej, M., **Stridh, L. S.**, Lankinen, Å. and Grenville-Briggs, L. Resilience of the potato rhizosphere microbiome during both early blight disease and application of the biocontrol agent *Pythium oligandrum*. (Manuscript)

1. Introduction

The planet earth is supplying 8 billion people with food every day (UN, 2022). This is only possible due to the fast development and success of agricultural sciences. Even though there are issues with the dispersion of the available food to all parts of the planet, the fact is that there is plenty of food produced for all the people around the globe, according to the Food and Agriculture Organization of the United Nations (2009a). One aspect of the development of agriculture is the capacity to combat plant diseases. This thesis will be focusing on one such disease, in one particularly important food crop: early blight in potato.

Early blight is a fungal disease caused by the soil born pathogen *Alternaria solani*. The pathogen mainly destroys the foliage of the potato plant, causing the tubers to stop developing (Abuley et al., 2019). There are several ways to halt this pathogen from decreasing the yield, the most common one today being the application of fungicides. However, as most people nowadays are aware of, fungicide usage comes along with risks that would be preferable to avoid. Integrated Pest Management (IPM) is a concept summarizing all measures that are used to create a more environmentally friendly and less pesticide dependent agricultural system (Barzman et al., 2015). This thesis will go through ways to tackle early blight on potatoes in the most efficient and sustainable ways possible following IPM strategies.

First and foremost, it is fundamental to understand why the disease hits hard in certain fields. Therefore, an observational study of Swedish starch potato farms was performed and the results from this study were used to design field trials. Additionally, the use of biological control agents (BCAs), and plant resistance inducers (PRIs), to tackle the disease were evaluated as were cultivar resistance and linkage to nutrient deficiencies.

2. Background

2.1 Potato and early blight

2.1.1 Importance of the potato crop

Potato (*Solanum tuberosum L.*) is a nutritious, tasty, and reliable staple crop grown all over the world. It delivers high yields in different climate zones, from temperate, subtropical to tropical zones. Grown over all inhabited continents, potato is one of the top crops of this agriculturally dominated planet, with over one billion daily consumers and an estimated annual global production of 360 million tonnes (Caliskan et al., 2023). Potato was first introduced in Sweden as a food crop in the beginning of 18^{th} century, but it was not until the 19^{th} century that the knowledge and production of potato was more widely spread in Sweden (Lundequist, 1828). Today, Sweden cultivates around 24 000 ha of potato every year with yields around 37 tonnes/ha for table potato and 42 tonnes/ha for starch potato (Jordbruksverket, 2022). Mentioning yield numbers for potato makes it necessary to compare yield averages for other staple crops. In Sweden yield averages for winter wheat are 7 tonnes/ha and 5 tonnes/ha for spring wheat (Jordbruksverket, 2022). Sweden does not produce rice, but the global yield average for rice lies just below 5 tonnes/ha (FAO, 2021). Thus, it is safe to state that even when taking water content into consideration, potato is still a high yielding crop.

Potato is a starch rich crop. In dry matter, the starch content is 60-80% of the potato tuber, mostly in the form of amylopectin but also as amylose and resistant starch (Robertson et al., 2018). Potato starch production in Sweden began in the 1750s, where Countess Eva De la Gardie played an important role in increasing the demand for potato starch to be used as body powder and in the production of alcohol (De la Gardie, 1748). From the end of the 1700s until the beginning of the 1900s, there were many small potato starch factories and starch potato farmers in southern Sweden. In 1927 they came together and formed a cooperative to increase the efficacy in their business, and Lyckeby Sveriges Stärkelseproducenter, förening, was founded. Lyckeby SSF is located outside Kristianstad, Sweden. Today, Lyckeby SSF is the only potato starch company in Sweden and all starch potatoes grown in Sweden are delivered to, and processed by, Lyckeby SSF. Around 400 potato growers contribute to an annual yield of around 325 000 tonnes (Gianuzzi, 2022). Today, potato starch is not used for alcohol or body powder, except for a tiny fraction, but instead it is mostly used in the food and technical industries as a component to make glue, or coatings, and to give food certain textural properties. Potato starch has a neutral taste, high viscosity, transparency, and a relatively low gelatinization point, all of which are relevant to the industry (Grommers & van der Krogt, 2009).

There are particular potato cultivars high in starch and yield, that are fit for starch production. Today the land usage for starch potato is around 7500 ha in Sweden (Sveriges Stärkelseproducenter, 2022), meaning that the land use for starch potato is currently a little higher than the fresh table potato area (unprocessed potato that goes directly to consumption). Starch potatoes are quite different from table potatoes from an agronomical point of view. They have a longer season, deliver higher yields and are less sensitive to drought and pests. Further, since the tubers are processed, the farmer does not need to be as careful with green tubers, size distribution, lumps, discoloration, and mechanical damage (personal communication, Gabriella Malm, Lyckeby SSF).

Figure 1. Germinating *Alternaria solani* conidia in microscope. Photo: Sophie M Brouwer

Figure 2. Lesions on potato leaves caused by *Alternaria solani*. Photo: Linnea Stridh

2.1.2 Early blight biology

As for all cultivated crops, many different plant pathogens cause problems in the fields. This thesis will focus on the fungal pathogen *Alternaria solani*, the causal agent of early blight disease in potato. There are different *Alternaria* species attacking potato as well, but in the Swedish starch potato industry, *A. solani* seems to be the most aggressive and yield reducing species (Odilbekov et al., 2019).

Alternaria solani was first described in 1882 by Ellis and Martin but was then originally named *Macrosporium solani*. Jones (1893) suggested the name early blight to separate the disease from late blight, since early blight was more common in early maturing cultivars of potato (Galloway, 1891). *A. solani* conidia can be easily recognized under the microscope (Figure 1), with quite large (200 µm), dark coloured (melanized), segmented, bottle shaped spores (Gannibal et al., 2014). The *Alternaria* conidia are easily detached and transported by wind, dust, or water splashes (Jambhulkar et al., 2016). During the infection process, *Alternaria*-spores penetrate the potato leaves causing necrotic lesions with concentric rings (Figure 2). The lesions cause premature defoliation of the plant and the tuber growth is reduced. *Alternaria* spp. are mostly saprophytic and necrotrophic, hence they do not normally infect living tissue, but dying or senescing leaves are vulnerable (Thomma, 2003). *Alternaria* spores are also *de facto* one of the most common causes of hay fever (Rabe, 2020). Conidia start to produce germ tubes under free moisture at a wide temperature range of 8-32°C. The spores or mycelia overwinter in the soil or in infected plant debris, and when a host is present, the germinating spores penetrate susceptible tissue to start an infection (Agrios, 2005; Rotem, 1994). *A. solani* has an asexual life cycle but still has a high genetic, morphological, and pathogenetic diversity (Van der Waals, 2004).

2.1.3 Yield loss and economic importance

Alternaria solani causes early blight in many Solanaceous crops such as potato, tomato, and eggplant (Rotem, 1981). The potato tuber yield is directly negatively correlated with the rate of early blight disease (Yellareddygari et al., 2015) primarily due to the defoliation (Herriott et al., 1990). Yield losses as high as 50 % have been reported, especially in late maturing cultivars (Leiminger & Hausladen, 2014). Furthermore, considering the polycyclic nature of the pathogen, a three- or four-times fungicide treatment strategy could be seen as an economic insurance to keep the crop protected. However, this thesis and the included articles show that some farms will not be as affected by the disease and therefore it would be more economically beneficial to reduce or even skip early blight treatments.

2.1.4 Control methods

Chemical control

Chemical control with the use of synthetic fungicides is today the most common way to battle potato early blight (Campo Arana et al., 2007; Odilbekov et al., 2016; Jordbruksverket, 2023; Stridh et al., 2023). Currently the available fungicides are highly efficient (Stridh et al., 2022), but this can change rapidly, as it has before (Mostafanezhad et al., 2021), with the rise of fungicide resistance. It is questionable to rely solely on chemical control as a control method for pests, since a rapid loss of efficacy in the pesticide can lead to a devastating crop failure (Yellareddygari et al., 2019). Additionaly using substances harmful to the soil and environment is problematic, not to mention the high costs associated with such a strategy (Siddique et al., 2016; Kemmit, 2002). There are multiple ways to decrease the dependence on chemical control e.g., as part of Integrated Pest Management (IPM) and these will be listed below.

Biocontrol agents and Plant Resistance Inducers

Biological control of pests can be defined as the use of another living organism to control a pest (Stenberg et al., 2021), this method has been used for a long time in agriculture (Smith, 1919). The mode of action of these substances varies with the product but includes outcompeting the pathogen, infecting, feeding on or producing toxins to kill the pathogen. An alternative treatment, or “bioprotection”, can also work as a plant resistance inducer (PRI) by activating plant innate immunity or producing plant hormones that further protect the plant from pathogen attack (Stenberg et al., 2021; Andersen, 2023). In **Paper II**, four different alternative substances were evaluated in greenhouse and field trials for their effects on the rate of disease. As demonstrated in previous studies (Sharma et al., 2019) the efficacies of these control methods are low, and there is a need to develop biological substances that are more efficient in the field environment or re-evaluate the way that they are stored and applied to the crop.

Decision Support Systems

A Decision Support System, DSS, is a tool based on computer modelling that helps the farmer or advisor to take an active decision whether and when to spray against a disease or not, and what dose and fungicide to use. There is a widely used DSS for potato late blight called Skimmelstyring (Hansen & Abuley, 2019). Around 80 % of Lyckeby's potato growers are using this highly efficient model today (personal communication, Gabriella Malm, Lyckeby SSF). For potato early blight there are different DSS being developed, one of them is called TOMCAST (Abuley & Nielsen, 2017). This model was used in several field trials within this thesis. The model collects weather data in the form of temperature and relative humidity from weather stations and calculates a disease risk value from these parameters (Figure 3).

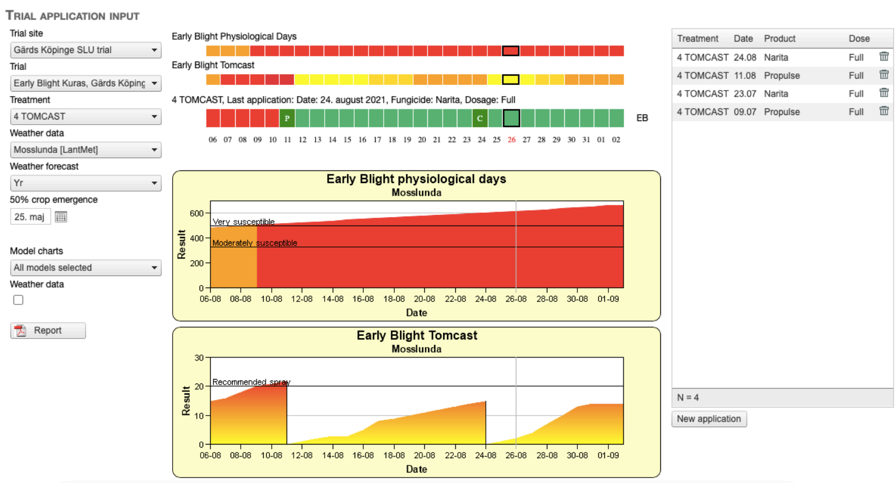

Figure 3. TOMCAST model as a farmer or advisor would see the DSS on a computer or a smart phone (Potato Late Blight Toolbox, 2023).

Cultivar susceptibility

The cornerstone of Integrated Pest Management, IPM, is to grow cultivars that perform the best with as little pesticide usage as possible. This is the case also for control of *Alternaria* diseases (Odilbekov, 2015). Identification of genetic traits and resistance genes is a primary step in obtaining resistant cultivars. There are currently no early blight resistant potato cultivars and the susceptibility in the cultivars available is not so well mapped out either

(Mathelemuse et al., 2022). No resistance genes have been identified yet but putative QTLs for early blight resistance have been demonstrated (Odilbekov et al., 2020; Xue et al., 2022). The resistance seems to be linked to the maturity of the potato and later maturing cultivars have shown lower infection rates (Abuley et al., 2017; Duarte et al., 2014; Rodriguez et al., 2006). However, in our trials (2017-2019) we saw that there might be exceptions to this rule. This gave us an indication that there are genetic traits separated from the maturity traits of importance for early blight resistance. This has also has been observed before (Zhang, 2004; Boiteux et al., 1995). Identification of these genes would facilitate the development of more resistant cultivars.

Field management

There are multiple parameters of field management that could possibly influence the rate of infection. The crop rotation and crops included in the rotation, tillage, seed tuber quality, irrigation, fertilization, sanitation of equipment and choice of cultivars are all management factors that could potentially affect disease development (**Paper I**; **Paper III**). Since these factors could be influenced by the farmer, they are of importance to study and understand. Crop rotation includes the crops that are grown on the same field in the seasons following each other. Crop rotation is very important for several different plant diseases, since it can have devastating effects on the yield with a too short rotation (**Paper I**; Walters, 2013).

2.2 Using the principles of Integrated Pest Management

Integrated Pest Management, IPM, plays a critical role in achieving a pesticide reduction without a yield penalty. IPM can be explained as a combination of measures that can be used to create a more sustainable and less pesticide dependent agriculture (Stenberg, 2017). IPM is stipulated by an EU directive (2009/128/EG), meaning all EU countries are required to work with these strategies. Barzman et al. (2015) summarized the general principles of IPM into eight categories. The categories are:

1. Prevention and suppression,
2. Monitoring,
3. Decision making,
4. Non-chemical control methods,

5. Pesticide selection,
6. Reduced pesticide use,
7. Anti-resistance strategies and
8. Evaluation.

This thesis evaluates various IPM strategies to mitigate potato early blight. These strategies encompass cultivar tolerance (Category 1), decision support systems (Categories 2, 3, 5, 6, 7), biocontrol agents (Category 4), plant resistance inducers (Category 4), nutritional status (Category 1), field management (Categories 1, 3), and a comprehensive understanding of the disease (Categories 7, 8). The primary goal is to minimize reliance on fungicides, by refining the toolbox for the agronomic advisor and farmer through improved knowledge.

In addition to these eight categories, a visual representation of IPM principles, as depicted in a pyramid (SmartProtect, 2023; Figure 4), highlights the importance of preventive measures at the base layers. Emphasis is placed on agronomic practices, decision support, and various controls (mechanical, physical, natural, and biological) at the foundation. Pesticides, representing chemical control, are positioned as a last resort at the top of the pyramid. This strategic approach aids in the development of sustainable pest management plans.

It is crucial to distinguish IPM strategies from organic agriculture. Unlike in organic farming, where chemical control methods and artificial fertilizers are prohibited, IPM acknowledges their use as necessary options for protecting crops and preserving yield.

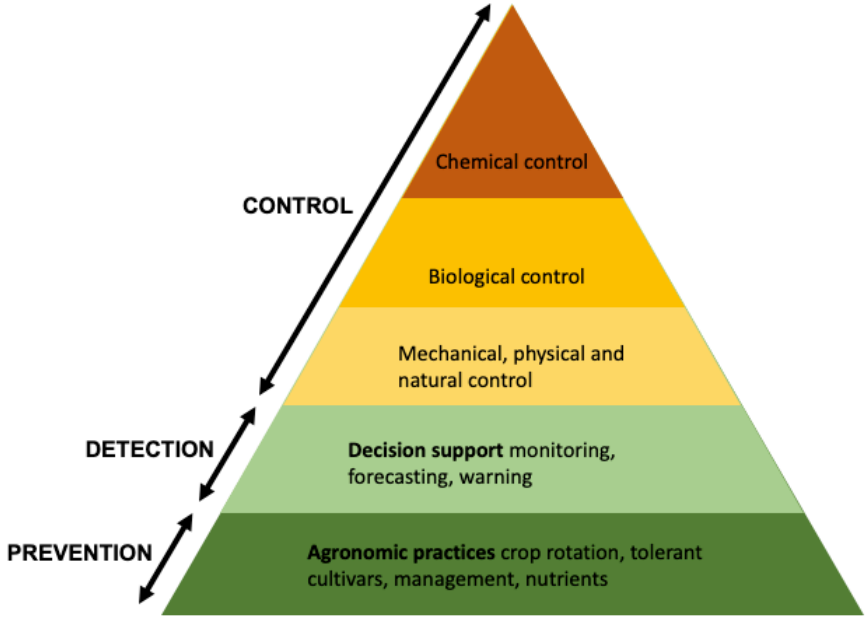

Figure 4. IPM pyramid used to help portray and plan a sustainable pest management strategy. Emphasis lies in working preventatively at the bottom of the pyramid, and to use the top layer control methods as a last resort (SmartProtect, 2023).

2.3 International and national goals for pesticide use

The European Commission has set ambitious goals to reduce the use of synthetic pesticides in the European agricultural system to 50 % by the year 2030. Hence our food system needs to adapt fast to minimize yield reductions. The European Union has directives (the main one being 2009/128/EG) concerning the sustainable use of pesticides. These directives are complimentary to other rules and directives and the purpose of the directives is to minimize the requirement for pesticides in the EU. In addition to the EU directives, Sweden has a national plan of action for pesticides (LI2023/02045) the current version of which, was agreed on at a government meeting March 16th, 2023. The Swedish board of agriculture (Jordbruksverket) is the authority responsible for leading, coordinating and evaluating the national action plan. The action plan was evaluated during 2021 and the conclusions were that the Swedish usage of pesticides was reduced drastically in the 1980s and 1990s but since then it has been stable. However, usage is measured as hectare doses but the overall risks for health and environment have been further going down since the 1990s, meaning that the pesticides that are in use today are less dangerous than those used previously. The action plan highlights the importance of using Integrated Pest Management (IPM) and alternative strategies for crop protection that are effective and safe for the environment (Swedish government, 2023).

3. Thesis objectives

The objectives of this thesis are to achieve a profound understanding of the current situation surrounding early blight in Swedish starch potato production, to develop and evaluate alternative treatment strategies against this disease, and lastly to communicate the results to the farming community. The underlying hypothesis posits that the severity of the disease is intricately tied to the unique characteristics of individual farms, necessitating tailored treatment approaches. The aim is to unravel the factors contributing to the varied impact of early blight infections on different farms (**Paper I**) and to assess the effectiveness of Integrated Pest Management (IPM) methods in combating this disease. Additionally, the thesis seeks to explore alternatives to pesticides (**Paper II**) and evaluate the susceptibility levels of commonly grown starch potato cultivars (**Paper III**).

4. Methods

4.1 Observational study

Over three seasons, 2019, 2020 and 2021, an observational study was performed in the Swedish starch potato production area. Fifty-two unique field plots were observed over the three years (**Paper I**). The specific farms were identified with the help of Lyckeby's agronomists to balance the conditions, e. g. crop rotation history, soil type and geographical location. The farmers left an untreated 24m x 24m plot in their potato field and from this plot information was collected. The early blight disease was scored twice per season, a management form was filled out by the farmer, and leaf and soil analyses were performed. From these input parameters statistical analysis was performed to search for correlations with the early blight disease severity.

4.2 Field trials

Field trials were used in all three studies (**Papers I-III**). All field trials were performed in the Southern Sweden starch potato production area with a complete randomized block design. The Rural Economy and Agricultural Society (Hushållningssällskapet, Kristianstad) managed all field trials.

4.2.1 Potassium field trials

As a follow up study to the observational study, a potassium field trial was performed for two seasons, 2021 and 2022 (**Paper I**; Figure 5). The trials were placed in fields with low levels of available potassium in the soil to easier achieve a depletion. A ladder of three potassium fertilization levels was evaluated in both treated and untreated plots.

4.2.2 Biocontrol agents and plant resistance inducers

Biocontrol agents based on *Bacillus subtilis* (Serenade®), and *Pythium oligandrum* (Polygandron®) and Plant resistant inducers/fertilizers containing silicon, HortiStar and Actisil, were evaluated in a greenhouse to field study based on efficacy in earlier studies (Kurzawińska & Mazur, 2009; Abbasi & Weselowski, 2014; Egel el al., 2019; Gulzar et al., 2021). The study consisted of three different setups: greenhouse trials, small scale hand sprayed field trials and large-scale standard field trials (**paper II**).

Figure 5. Potassium field trial at Åsums Boställe photographed with a drone 12th of September 2022. The field trial showed that the lower the potassium fertilization was, the more severe the early blight disease became. Photo: Kristoffer Gustavsson

4.2.3 Cultivar field trials

In **Paper III** differences in susceptibility between starch cultivars were evaluated. The two cultivars Kuras and Avenue were compared in a set of field trials first during 2017-2019. The following years, 2020-2022, there was an additional field trial series evaluating the susceptibility of eight different starch potato cultivars (Figure 6).

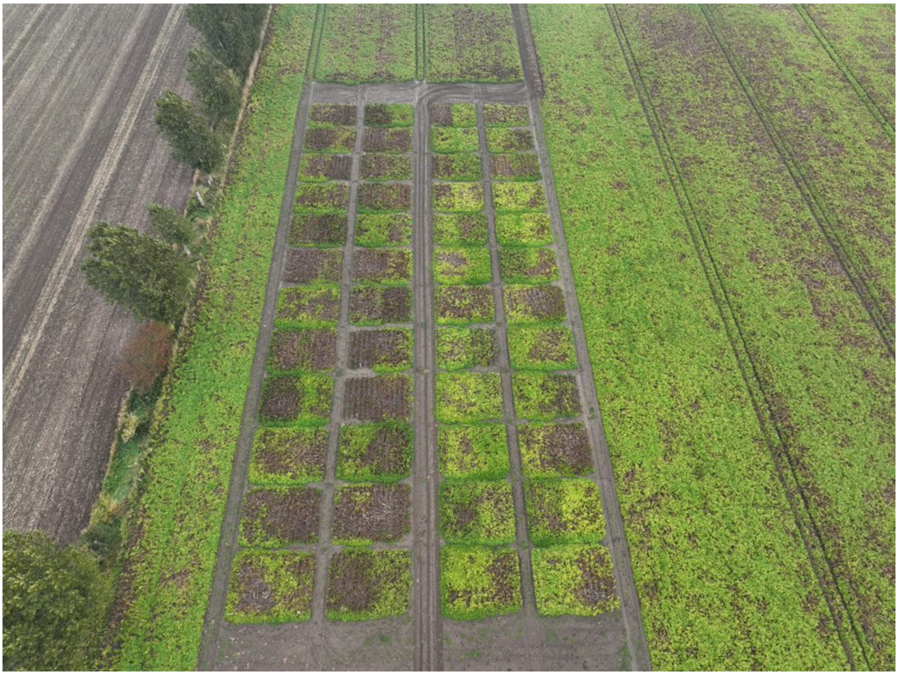

Figure 6. Cultivar field trial at Vittskövle photographed 26th of September 2022. The left part of the trial is untreated against early blight. The senescence seen is caused by both early blight and natural defoliation. Photo: Kristoffer Gustavsson

4.2.4 Decision Support Systems – TOMCAST

The TOMCAST model, as outlined by Abuley and Nielsen in 2017, was integrated into field trials spanning from 2018 to 2021. The TOMCAST treatments were integrated in the same trials as reported in **Paper II** but not reported there. Thus, the management of the field trials was as described in **Paper II**. During the initial years, the dose and timing of applications were decided through direct communication with the model developer, Isaac Abuley (personal communications). Subsequently, an online tool (Figure 3) was introduced to automate these processes. In the first two years (2018-2019), two versions of the TOMCAST model were evaluated (TOMCAST Maturity and TOMCAST DSV), providing identical treatment dates but differing in dosage (Table 1). Specifically, the maturity model recommends a lower dose early in the season, based on the plant's maturity-related resistance. In 2020, we shifted focus solely to treatment dates and timing of treatments (TOMCAST DSV), omitting reduced doses (TOMCAST

Maturity). The first online version of the Decision Support System (DSS) tool was released to selected users for research purposes in 2020 (Figure 3), streamlining the decision-making process. Notably, the initial treatment date holds paramount importance as it is a key output from the decision support system. This significance arises from the unpredictable onset of infection due to weather conditions and maturity dependence. Subsequent treatments follow a 14-day interval, acknowledging that once the infection is well established in the field, its progression is likely to remain unhindered.

Table 1. Overview of the field evaluations with Decision Support Systems.

Year	**How decision was taken**	**DSS Model**	**Date of the first treatment**	**Treatments* and trial sites**
2018	Email correspondence	TOMCAST DSV	31/7	Nymö: Na/P+Na/Na/P (2nd treatment was incorrectly sprayed with both substances) Helgegården: Na/P/Na
2018	Email correspondence	TOMCAST Maturity	31/7	Nymö: discarded, Helgegården: Na 75%/P/Na
2019	Email correspondence	TOMCAST DSV	1/7 Nymö, 9/7 Helgegården	Na/P/Na/P
2019	Email correspondence	TOMCAST Maturity	1/8 Nymö, 7/8 Helgegården	Na 50%/P/Na
2020	Online tool	TOMCAST DSV	23/7 Helgegården, 28/7 Nymö	Na/P/Na
2021	Online tool	TOMCAST DSV	9/7 Nymö, 9/7 Gärds Köpinge	Nymö: P/Na/P/Na Gärds Köpinge: P/Na/P

*Na = Narita, P = Propulse, alternated every 14th day, full dose if nothing else is stated.

4.3 Disease assessment

The level of disease and defoliation in field trials, was assessed weekly, following the methodology outlined by Duarte et al. (2013). Disease development and defoliation rates, represented by the relative area under disease progress curve (rAUDPC) and relative area under defoliation curve (rAUC), respectively, were calculated according to the method described by Shaner & Finney (1977). The disease score quantifies the percentage of green leaf area covered by early blight lesions, while the defoliation score indicates the percentage of the entire plant that is dead or necrotized. All infections during both the field trials and the observational study occurred naturally.

4.4 Laboratory analysis

The presence of *A. solani* spores was confirmed in all field trials with microscopic examination of the characteristic conidia (Figure 1) and by PCR (Landschoot et al., 2017).

4.5 Statistical analysis

Except for the calculation of standard deviations, disease rate (rAUDPC) and defoliation rate (rAUC) values (Shaner & Finney, 1977), which was done in MS Excel, all statistical analysis was done in R-studio (version 1.1.456– 2009–2018 RStudio, Inc). Detailed descriptions are presented in the materials and methods sections in **Papers I-III**.

5. Results and Discussion

5.1 Observational study

The 52 untreated farm field plots gave novel insights into how early blight disease progresses, and thus, how to prioritize different control measures against the disease (**Paper I**). The front cover of this thesis shows a drone photo of one of the particularly heavy infected field plots from this study. The aim of this study was to investigate which environmental and management parameters affect the severity of early blight. A summary of the results are highlighted below.

5.1.1 Soil quality

The relationship between soil sand and clay content in the observational study and the rate of early blight disease was highly significant (**Paper I**; Figure 7) as has been reported previously (Shtienberg, 2014). Even though the infection pressure varies between seasons, with exceptions for very light soil with low infection, it is clear that starch potato fields with a lower sand content than 80% have a much lower risk of being affected by early blight. From this analysis, the risk of early blight infection can be categorized as in Table 2.

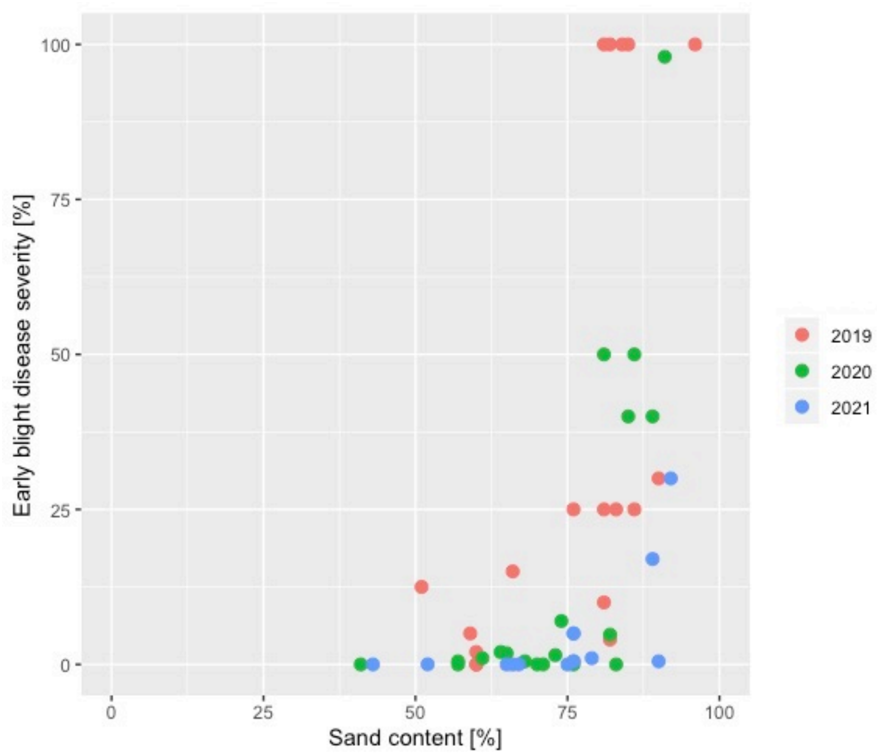

Figure 7. In this plot (**Paper I**), every data point indicates a unique untreated field plot. The scored early blight disease severity (x-axis) is strongly correlating with the soil sand content (y-axis). Seasons are presented in colour.

Table 2. General advice for early blight fungicide treatment strategies in starch potato.

Risk of early blight infection	Sand content [% dw]	Clay content [%]
Low risk	< 70	> 10
Intermediate risk	70-80	7.5-10
High risk	> 80	< 7.5

From the soil analysis there was another parameter that correlated significantly with the disease rate. The soil phosphorous (P-Al) content and its relationship with early blight severity is portrayed in Figure 8. This graph also shows the soil sand content using a colour scale, to elucidate the genuine associations between the variables in this context. Also notable is the apparent connection between the available phosphorous in the soil and the amount of infection, however, the soil phosphorous is also linked with the sand content. The intricate interrelationships among numerous parameters within field environments contribute to the inherent complexity and scientific intrigue of this study. One possible explanation for this relationship is that historically, and today, farms with lighter soils have also had animals to add income to the lower yield that lighter fields deliver (personal communication, Gabriella Malm, Lyckeby SSF). If a farm keeps animals, it is more likely that the fertilization of the field will be done with manure. Manure as a fertilization form will bring up certain nutrients, e. g. phosphorous, to a higher level than the needs of the crop (Qin et al., 2020). Thus, what we see in this relationship, is probably still mainly just explained by the sand content.

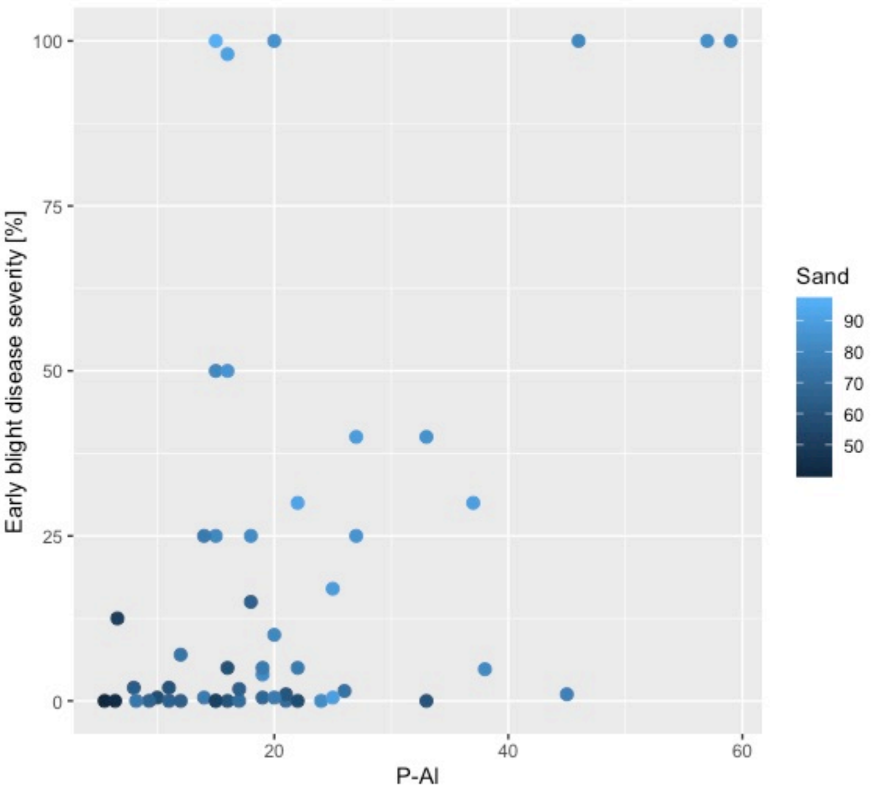

Figure 8. Relationship between the soil phosphorous content (P-Al), measured at harvest time, and early blight disease severity with the soil sand content shown in colour. Each datapoint represents a unique field plot.

5.1.2 Nutritional status

In the observational study, a leaf nutrient analysis was performed and the results were correlated with the disease severity scoring values. The complete correlation plot can be found in **Paper I**. The strongest correlation with disease was found for the potassium content (Figure 9), but correlations with Boron (positive), Copper (negative) and Magnesium (positive) were also found. A leaf potassium content below 2%, measured in mid-august, seemed to impose a higher risk of severe infection. The preliminary results after the first year (2019) of the observational study led us to design field trials to evaluate the effect of potassium fertilization levels on disease severity (Section 5.3; **Paper I**).

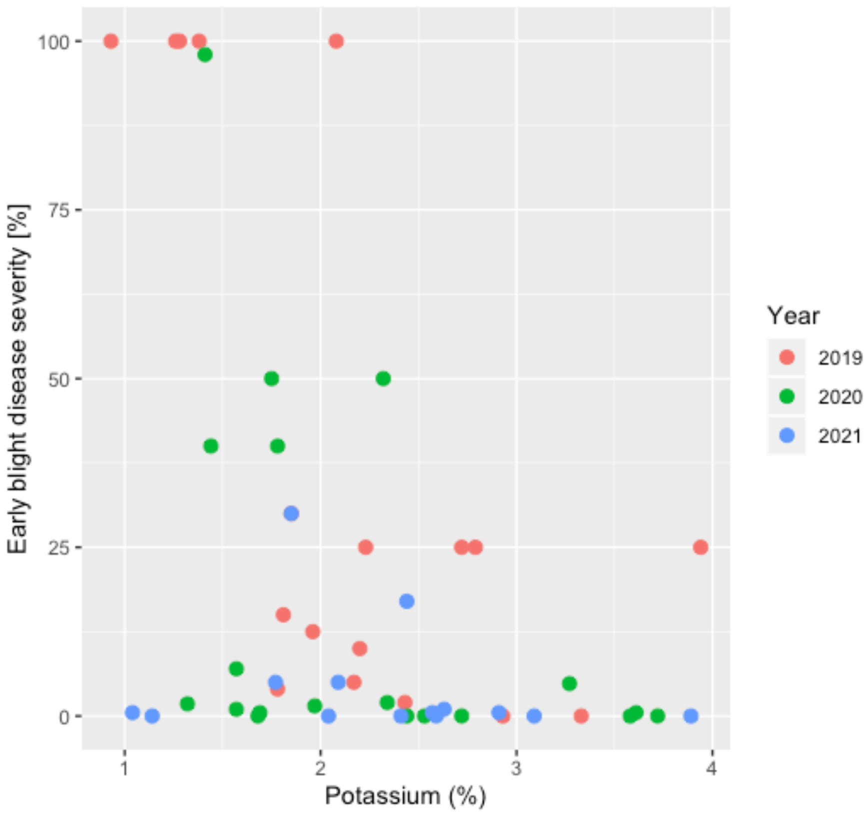

Figure 9. Leaf potassium content from leaves collected mid-August correlates strongly with early blight disease severity. Each data point represents a unique field plot.

5.1.3 Management strategies

Crop rotation is an important parameter that effects the rate of early blight infection since the pathogen is soil borne (Delgado-Baquerizo et al., 2020). However, we could not find any significant decrease of infection at a lower crop rotation than seven years, which makes the discussion about crop rotation questionable since exceeding a seven-year crop rotation is not economically justifiable for the farmer. If you have made investments for farming potatoes, a 3–4-year crop rotation is needed (personal communication, Kristoffer Gustavsson, Lyckeby SSF). Since a 6–7-year crop rotation with potatoes is uncommon due to economic constraints, it was hard to find fields and map out where exactly the soil borne inoculum declines in a notable way. The few fields incorporated in the study with a crop rotation exceeding seven years, had a very low disease severity.

Below is an observation made by a farmer (Figure 10). This is a field of starch potatoes, and the senescence seen is mainly due to early blight. The dotted red line separates the field into two parts, where the disease severity is clearly much higher in the right side of the photo. The only difference in management from the left and right side of the line, is that the right side had potatoes once in the crop rotation three years prior to the season where the photo was taken, and the left side had not had potatoes in the rotation for more than thirty years. The cause of the much more extensive defoliation to the right was early blight. The main wind direction is coming from the west, which is from the right in the picture, meaning that spores would be assisted in spreading from the right to the left in the photo (Bashan et al., 1991). In this specific case, the wind dispersal seems to be of less importance compared to the soil inoculum since there is not notably more infection closer to the red line. It appears that a single season of potato cultivation within a three year period can elevate the soil-borne inoculum.

Figure 10. Drone photo of a field with starch potato cultivar Kuras taken at harvest 7th of October 2020. The left side of the dotted red line has not had potatoes in 30 years, the right side of the dotted red line had potatoes once in 2017. The main wind direction is in this location from the west, which is from right to left in the photo. Photo: Linnea Stridh

5.2 Potassium field trials

In figure 11, a photo of a potato leaf with lesions caused by both potassium deficiency and early blight can be seen. This photo portrays how hard it can be for a farmer or advisor (or researcher) to distinguish lesions caused by disease versus those caused by nutrient deficiencies. The relationship between the leaf potassium content and early blight disease severity shown in the observational study led to the planning and execution of potassium field trials for two seasons. A ladder with three different levels of potassium fertilization was designed (**Paper I**). In the first season, 2021, the potassium deficiency was not reached as planned and therefore the results from the first season were not conclusive. Therefore, the potassium fertilization levels were lowered for the following season, 2022, leading to higher deficiencies in potassium and to a significant correlation with the rate of disease (Figure 12). The standard recommended level of potassium fertilizer was sufficient to reduce the rate of infection compared to lower fertilization levels even in these light soils. But since the observational study showed many plots with a potassium level low enough to influence the disease severity, it can be hypothesized that farmers might not follow the recommended potassium fertilization in some cases. It appears that there is a need to highlight the importance proper potassium fertilization in fields with a high risk of early blight infection, to potato growers. **Paper I** summarizes the findings.

Figure 11. Potato leaves (cv Kuras) with early blight lesions (top arrow) and potassium deficiency (bottom arrow). Photo taken mid-September 2022 in the potassium trial at Åsums Boställe. Photo: Linnea Stridh

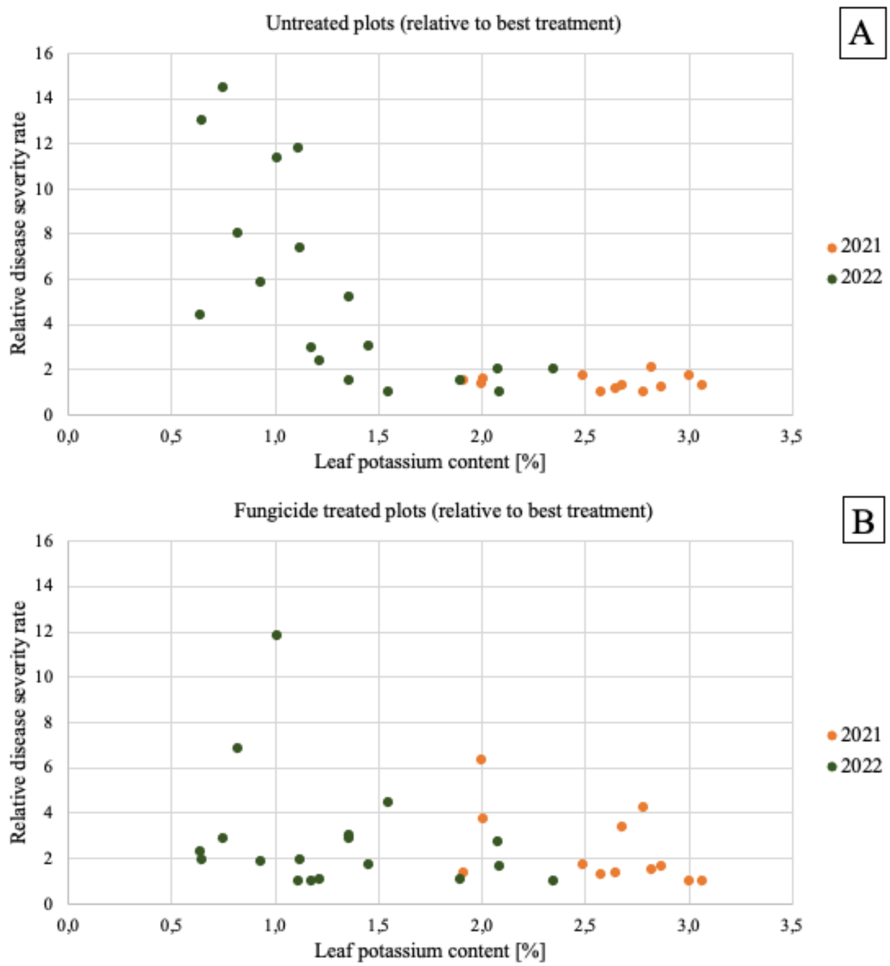

Figure 12. Correlation between leaf potassium content and relative disease severity rate for untreated (A) and treated (B) plots, relative to the best treatment (the plot with the lowest disease severity). The potassium content is lower in 2022 than in 2021 due to the change of trial setup with decreased potassium fertilization to create a heavier depletion. The correlation is strongest in the untreated plots.

5.3 Alternative strategies with BCAs and PRIs

In **Paper II** treatments with four alternatives to fungicides were evaluated, i.e. the biocontrol agents, (BCAs) *Pythium oligandrum* and *Bacillus subtilis* and the Plant Resistance Inducers (PRIs) Actisil and HortiStar. The study compared different scales and settings to treat potato against early blight. The substances evaluated showed the greatest treatment effect in the smallest setting, the greenhouse, but when the trials were scaled up the efficiency was lost (Figure 13). When the products were tested in large-scale field trials and applied with a tractor sprayer, the effect almost disappeared and there was no effect on the yield whatsoever. This led to a discussion on why the efficiency is lost, landing in a need for further research concerning application methods and potential interference of natural microorganisms and environmental factors under field conditions.

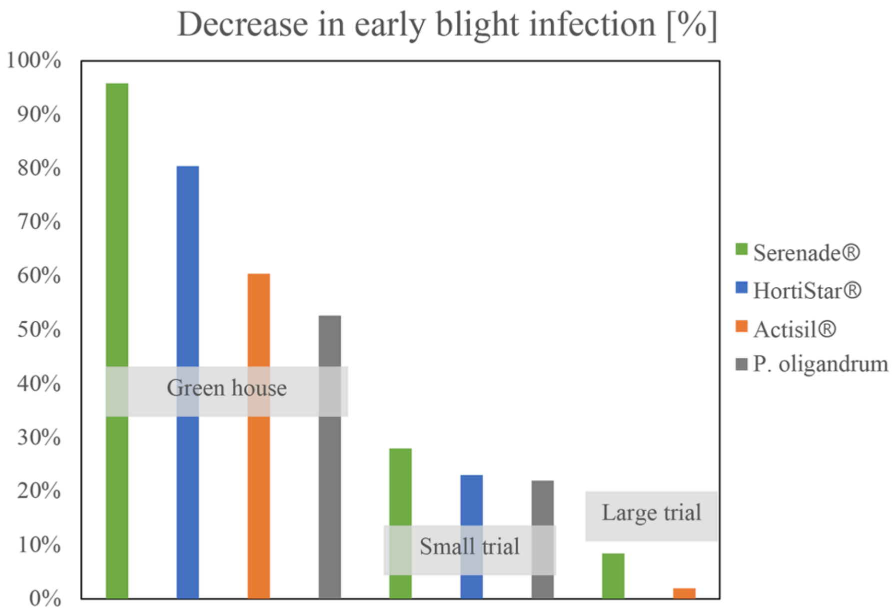

Figure 13. Comparison between average reduction in early blight infection between the different trial setups for the four different agents. To compare the results from the different trial settings, the percentage of infection reduction from the treatments compared to control is used. For the greenhouse trial, this means reduction in the size of the lesion, and for the field trial the numbers come from the visual scoring.

5.4 Cultivar trials

Two separate series of cultivar evaluations were completed. From the two site field trial series from 2018 to 2020, cultivars Avenue and Kuras were compared. Additionally, there was an eight-cultivar trial planned and executed in collaboration with Lyckeby SSF. Lyckeby SSF have annual cultivar trials to evaluate the cultivars available for the farmers to buy as seed tubers and to take in new cultivars that might be of interest for future seed production. During the three seasons 2020, 2021 and 2022, those trials were extended at one of the two sites with additional blocks that were left untreated against early blight. The results are presented in **Paper III**. We classified the eight evaluated cultivars into two classes, depending on their relative susceptibility and the yield loss when left untreated (Figure 14).

I. Less susceptible: Avenue, Saprodi, Sprinter and Ydun
II. More susceptible: Dartiest, Allstar, Kuras and Seresta

Since early blight is a maturity dependent disease, and the cultivars included in the trials differed in maturity rate (meaning some cultivars matured later and some earlier in the season), a new parameter, “relative susceptibility” comparing the degree of resistance between cultivars of different maturity rate and calculated using the formula below was suggested.

$$\text{"relative susceptibility"} = \frac{rAUDPC\ untreated}{rAUC\ treated}$$

The relative susceptibility value correlated better with yield loss, than just using the rate of infection (rAUDPC untreated), leading us to the conclusion that this is a more accurate way to compare susceptibility in cultivars with different maturity rates.

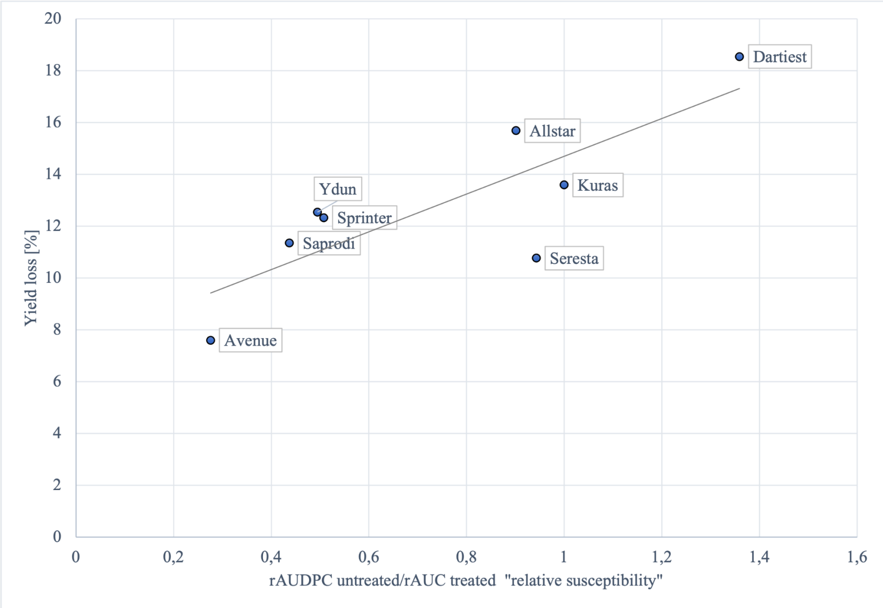

Figure 14. Significant relationship between relative susceptibility of eight different starch potato cultivars and yield loss when left untreated against early blight. The data points are mean values from the three years in trial. $F = 11.6$, $R^2 = 0.660$, $p = 0.0143$.

5.5 Decision support systems

In the pursuit of refining disease management, a Decision Support System (DSS) was implemented to enhance the precision of disease control, with a focus on the integration of epidemiological considerations. The primary objective is to optimize the use of pesticides by reducing doses and administering treatments at the most effective time points. However, our trials utilizing the DSS did not yield a substantial reduction in fungicide application. This contrasts with reductions observed in the case of late blight and DSSs developed for that disease (Wiik et al., 2017). This disparity might be attributed to inherent differences in the epidemiology of the two diseases.

Potato early blight, known for its dependence on senescing tissue, tends to manifest consistently around late August each year in Sweden (**Paper II**; Figure 15). The severity of the disease is notably also dependent on weather conditions, primarily humidity and temperature (Abuley et al., 2023). Hence, there appears to be potential to develop a DSS capable of helping farmers to optimize doses and application timing. Figure 15 depicts the disease severity at the trial site Nymö over multiple seasons, suggesting a relatively consistent soil composition across fields used in rotation. Seasonal variations in weather likely contribute to fluctuations in the date of the onset of infection. The figure indicates a span of approximately twenty days during which there can be a rapid increase in disease severity, emphasizing the value of accurately predicting the onset of infection, so that control measures can be applied before the disease becomes too severe. An accurate DSS could potentially result in the conservation of one or even two (out of approximately four) fungicide applications.

In Figure 16, the disease severity for the Nymö site in 2021 is presented, While the TOMCAST DSV (Disease Severity Value) DSS treatment dates closely aligned with standard treatments that farmers typically use without a DSS (arrows in Figure 16), no significant difference in disease severity was observed. Although the standard treatment demonstrated lower infection rates than the TOMCAST plots, these differences were not statistically significant.

Conclusively, the implementation of the DSS demonstrated improvement in user-friendliness and precision over the trial years, incorporating more input data and design enhancements (user interface). However, the treatment dates generated by the DSS remained consistent with standard recommendations for all the evaluated seasons. The recommended standard treatment for early blight in Sweden appeared to align with the DSS suggestions, and under the climatic conditions observed during the trial seasons, the DSS did not exhibit superiority over the existing practice. It may be possible to further reduce doses with the help of this model, but we decided to focus on the timing of treatments. Additionally, it would be desired for future development of the model to take cultivar specific susceptibility and soil type into consideration.

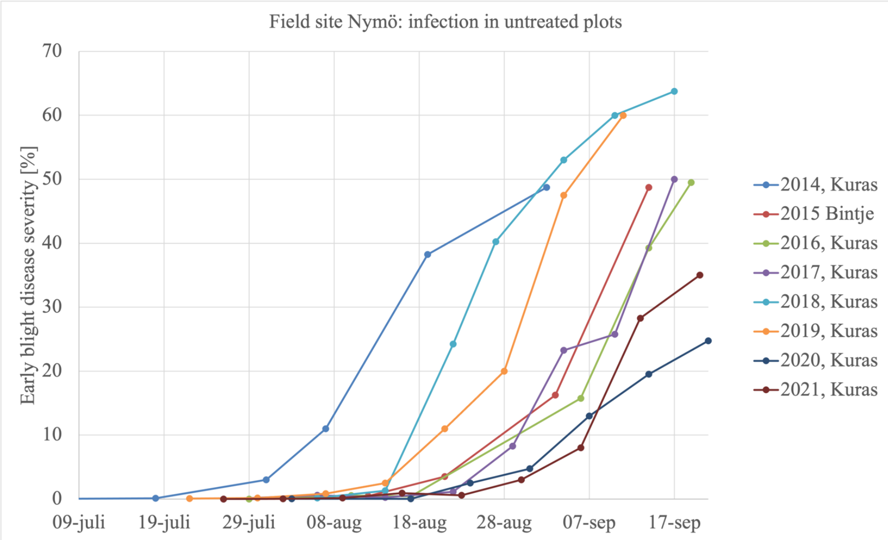

Figure 15. Diagram showing the scored early blight disease development over eight seasons in the same geographical area. The seasonal differences are significant and it would therefore be helpful with a model predicting the disease outbreak.

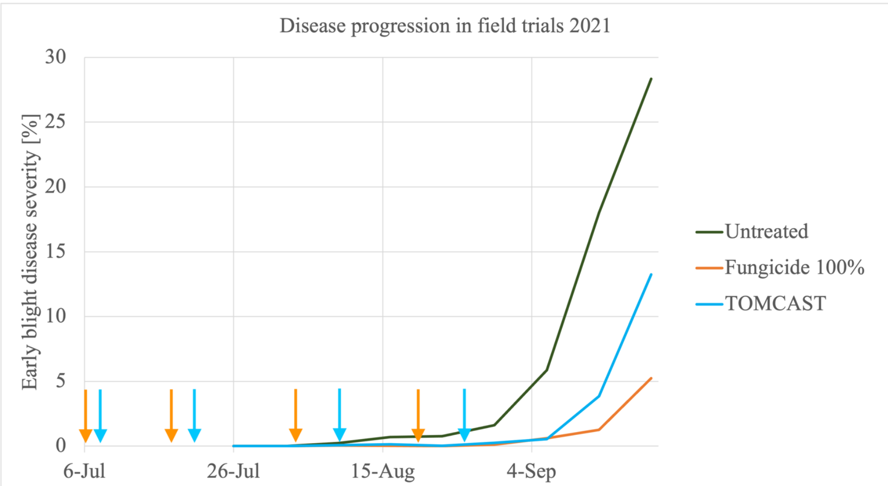

Figure 16. Scored early blight disease severity from the trial site Nymö in 2021. Mean values from untreated, treated and TOMCAST treated. To illustrate the treatments applied (Table 1) arrows are added for the TOMCAST treatments (blue) and standard treatments (orange).

5.6 How can these results help optimize Integrated Pest Management for early blight?

Proposing a strategic approach, I contend that by utilizing efficient substances at optimal doses, accounting for factors such as crop rotation history, cultivar, soil composition, and adhering to potassium fertilization recommendations, a substantial 50% reduction in fungicide usage for early blight can be achieved. This not only serves to minimize the environmental impact of disease control strategies but also to mitigate the risk of fungicide resistance development within early blight populations.

The findings of this research can be distilled into a priority order of parameters influencing early blight severity: soil sand content, season, cultivar susceptibility, and potassium fertilization. Consequently, the following recommendations are proposed: customize treatments based on soil sand content, invest in the development of Decision Support Systems (DSS) predicting the onset of infection, conduct a thorough exploration of cultivar differences for those in use, and meticulously adhere to potassium fertilization recommendations, particularly in high-risk fields.

One of my overarching goals throughout this thesis has been to enhance the accessibility of research findings, particularly for those involved in pesticide application, ensuring they are equipped with the latest information to minimize usage. Recognizing the pivotal role of the research community, there lies a responsibility to convey experimental results expediently and comprehensibly to the farming community. As researchers, we are compelled to engage in diverse communication avenues—lectures, articles, farmers' magazines, interviews, and any other means deemed effective—to disseminate our findings effectively. It is also important that the research itself is directed at issues recognized by the farmers. This thesis suggests the importance of two-way communication between farmers and researchers.

6. Conclusions

The interplay between early blight severity and soil sand content introduces a distinct threshold. Below 70% sand content, the option of leaving the field untreated becomes a strategic consideration, challenging traditional treatment paradigms. This revelation prompts a re-evaluation of intervention strategies, emphasizing the importance of incorporating nuanced considerations of soil characteristics into our approach.

Another facet of the discussion unfolds in the realm of nutrient management practices. A compelling correlation between low potassium fertilization and heightened disease severity, independent of soil sand content, was found. This discovery invites a deeper exploration of the complex interplay between nutrient management practices and disease dynamics. It opens avenues for innovative mitigation strategies, that extend beyond traditional treatments, and underscores the need for a holistic understanding of the potato agroecosystem.

The conventional wisdom surrounding the benefits of prolonged crop rotation in battling early blight is brought into question. The observed lack of notable and economically significant benefits challenges our assumptions about the role of crop rotation as a remedy for early blight management. This calls for a critical re-evaluation and exploration of alternative strategies or complementary practices that may offer more effective solutions.

In exploring the domain of early blight management, a critical analysis of various factors reveals intriguing patterns and challenges our prevailing assumptions. The scalability of Biological Control Agents (BCAs) and Plant Resistance Inducers (PRIs) emerges as a central theme, with their efficacy diminishing as trial scales up. This observation urges a deeper investigation into the nuanced dynamics that influence the performance of these control methods on a larger operational scale, emphasizing the need for sophistication in our understanding.

Lastly, utilizing the differences in cultivar-specific susceptibility to early blight are important from an IPM perspective. Notably, these differences in susceptibility do not always hinge on maturity rates. The approach introduced, categorizing cultivar susceptibility into "relative susceptibility" while considering maturity rates, holds promise in facilitating comparisons between cultivars of varying maturity rate. In essence, early blight management proves to be a multifaceted challenge, requiring a comprehensive and adaptive approach to address its complex dynamics.

7. Future perspectives

One of the most important findings in this thesis is the fact that the soil sand content has a large effect on the rate of early blight infection. However, the reason behind this is still unknown. We have two major hypotheses as to why this effect is observed. A sandier soil will more likely cause nutrient and water depletion in the plants, which will further cause a stress that gives the infection a better grip of the plant. The other hypothesis is that the microorganism communities are different in the soils, depending on the sand content, and that would give the *A. solani* spores a higher survival rate in a sandy soil possible due to the competition with other soil living microorganisms. These hypotheses would need to be further evaluated in a more controlled environment.

The comprehensive investigation involved the systematic collection of fungal spore samples from both treated and untreated plots over the course of several years, aimed at elucidating the dynamics of fungicide resistance development. The substances difenoconazole and fluopyram underwent evaluation in agar plate studies, yet the culmination of this research remains pending, marking an ongoing avenue for future exploration. The imperative to monitor fungicide resistance development, encompassing both emerging and established compounds, stems from the vital objective of ensuring the judicious use of fungicides in agricultural fields. This diligence is crucial to proactively address the potential inefficacy of substances, aligning with the broader ethos of sustainable and effective disease management practices in the agricultural system.

A significant portion of our research efforts were dedicated to the evaluation of Decision Support Systems to optimize the timing of fungicide applications. The models TOMCAST and TOMCAST maturity were represented in field trials over several years. However, the observed absence of advantages in comparison to traditional application timing underscores the needs for future refinement of these models. Future research should focus on enhancing their precision to achieve potential reductions in fungicide usage, thereby addressing existing limitations and optimizing their efficacy within practical agricultural contexts.

From the observational study there was an indication of a relationship between soil phosphorous and early blight severity. I partially explained this finding related to the soil sand content and historical use of manure at a farm level. These findings would preferably need further experimental evaluations similar to our potassium field trial.

In conclusion, the imperative lies in the seamless integration of Integrated Pest Management (IPM) principles with the invaluable insights derived from the farmers' perspective, forming an essential synergy for the advancement of plant pathogen management strategies.

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Popular science summary

Potato is a high-yielding, nutritious, trustworthy, multipurpose crop. It is also the world's third most important food crop, following wheat and rice (FAO, 2014). Just like all living things, potatoes are constantly attacked by other organisms. The nemesis of potato is late blight disease, but several other diseases need to be controlled for the crop to deliver its potential yield. This thesis focuses on potato early blight, caused by the fungal pathogen *Alternaria solani*. Central to our exploration is the utilization of alternative strategies, particularly integrated pest management (IPM). The reliance on fungicides raises concerns due to their expense and potential harm to users and the environment. Moreover, overuse creates resistance within pathogen populations, risking dramatic crop failure.

Emphasizing the importance of field trials and collaboration with farmers, this research underscores the need for practical, cost-effective solutions. Evaluating plant protection strategies requires effectiveness in real-world environments without imposing additional burdens on users. The findings unveil multiple strategies to reduce fungicide treatments without compromising disease control. Field trials, greenhouse experiments, and observational studies revealed novel insights. A correlation between soil sand content and disease severity was discovered, paving the way for farm-specific treatment recommendations. Monitoring nutrient status affirmed a link between low potassium and disease severity, emphasizing the role of optimal fertilization in disease reduction.

Crop rotation, a traditional disease control method, proved elusive in terms of economic benefits, challenging existing studies. Biological control agents and plant resistance inducers showed promise in smaller trials, but their efficacy waned in large-scale farm trials, leaving the explanation difficult to pin down. Disease-resistant cultivars were evaluated using a novel approach that considered cultivar differences in maturity rates. Variations in susceptibility for early blight were not solely linked to maturity, hinting at possible separate resistance genes in common starch cultivars. The distinct susceptibility levels among cultivars suggest a potential for customized treatment strategies that could significantly reduce fungicide use in more tolerant varieties.

In conclusion, this research underscores the complexity of disease management in potato cultivation and the potential of IPM strategies. The significance of understanding soil conditions, nutrient status, and cultivar-specific responses offers a holistic approach to sustainable and effective potato farming.

Populärvetenskaplig sammanfattning

Potatis är en högavkastande, näringsrik, pålitlig och mångfacetterad gröda. Potatis är också den tredje viktigaste livsmedelsgrödan i världen, efter vete och ris (FAO, 2014). Precis som alla levande varelser attackeras potatisen ständigt av andra organismer. Potatisens nemesis är sjukdomen bladmögel, men flera andra sjukdomar måste också behandlas för att nå full skördepotential. Den här avhandlingen fokuserar på torrfläcksjuka, Alternaria, i potatis, en svampsjukdom orsakad av patogenen *Alternaria solani*. Användningen av alternativa strategier, då särskilt integrerat växtskydd (IPM), är avhandlingens huvudfokus. Samtidens beroende av fungicider är inte helt optimal, dels på grund av kostnaden, men förstås också med tanke på den potentiella skaderisken för användare och miljö. Dessutom leder överanvändning till resistens, vilket i slutändan kan leda till missväxt.

Genom att betona vikten av fältförsök och samarbete med lantbrukare understryker denna forskning behovet av praktiska och kostnadseffektiva lösningar. Utvärdering av växtskyddsstrategier kräver resultat i verkliga miljöer utan att påföra användarna ytterligare bördor. Resultaten presenterar flera strategier för att minska fungicidanvändningen utan att kompromissa med sjukdomsincidens. Resultat från fältförsök, växthusexperiment och observationsstudier ger tillsammans nya insikter. Jag fann en stark korrelation mellan jordens sand- och grovmohalt och graden av sjukdomsangrepp, vilket kan bana vägen för gårdsspecifika behandlings-rekommendationer. Uppföljning av plantans näringsstatus visade på en koppling mellan lågt kaliuminnehåll och sjukdomsangrepp, vilket betonar betydelsen av presisionsgödsling för att minska risken för sjukdomsangrepp.

Växtföljd, en traditionell metod för att undvika växtsjukdomar, visade sig vara svår att rättfärdiga i ekonomiska termer och utmanade befintliga studier. Biologisk bekämpning visade lovande resultat i mindre försök, men effekten avtog när försöken skalades upp, och i stora fältförsök hade dessa medel nästan ingen effekt alls. Förklaringar till detta var svåra att fastställa.

Ett viktigt fokusområde inom IPM är sjukdomsresistenta sorter. Ett antal sorter utvärderades med en ny metod som beaktade skillnader i hur tidigt eller sent sorten mognar naturligt. Variation i mottaglighet för Alternaria var inte enbart kopplade till tidig mognad, vilket antyder att det finns resistensgener i kommersiella stärkelsesorter. De olika nivåerna av mottaglighet bland sorterna ger en möjlighet till anpassade behandlingsstrategier som skulle kunna minska fungicidanvändningen i mer resistenta sorter.

Denna forskning betonar komplexiteten av växtskydd i potatisodling och potentialen hos olika IPM-strategier. En ökad förståelse för hur jordförhållanden, näringsstatus och sortskillnader påverkar sjukdomar ger underlag för ett holistiskt perspektiv för en hållbar potatisodling.

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I

Potato Research
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Field and Management Factors Can Reduce Potato Early Blight Severity: an Observational Study on Farms Combined with Field Trials in Southern Sweden

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Abstract

Alternaria solani is causing early blight and thereby yield reduction in the potato production. The pathogen is today mainly controlled by fungicide applications. The severity of early blight can vary largely among fields. The aim of this study was to gain understanding of what field and management parameters are the most important for early blight severity to create more farm-specific fungicide treatment recommendations. Over three seasons, 2019–2021, 52 field plots were observed at farms in southern Sweden. In each field a 24 m × 24 m plot was left untreated against early blight. However, late blight fungicides were applied. The disease severity was scored twice in the untreated plot and information about various soil/plant parameters and farmer's management was collected from each field. In addition to the observational study, field trials were performed in 2021 and 2022, evaluating the effect of potassium fertiliser levels on severeness of infection. We found that the soil composition was of significant importance for the severity of infection, in particular the sand, clay, and potassium content. The early blight severity was directly positively correlating with a high sand content. Low levels of leaf potassium increased the severity of early blight infection, and this observation was confirmed in field trials where different levels of potassium fertiliser were applied. Further no reduction in disease severity was observed with a four-year crop rotation. With knowledge about field and management factors that influence disease, field-specific recommendations can be developed supporting an integrated pest management strategy for early blight to reduce and optimise the fungicide usage.

Keywords *Alternaria solani* · IPM · Participatory research · Potassium · Sand content · Soil

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Introduction

The soil-borne fungus *Alternaria solani* is causing early blight disease in potatoes. In Sweden the pathogen is mainly infecting the foliage causing earlier defoliation leading to a lower yield (Andersson and Wiik 2008; Edin et al. 2019). The main method to decrease the yield loss from early blight today is fungicide usage with multiple sprays per season (Horsfield et al. 2010). The development of integrated pest management, IPM, is aiming to optimise holistic methods in disease control strategies with minimised use of chemical pesticides to tackle plant diseases (Barrera 2020). IPM is not a strict principle that applies to all situations uniformly, but rather a philosophy of guidance to use the most suitable and sustainable tool appropriate for the situation (Dara 2019). IPM could be considered for early blight in potatoes as well (Jindo et al. 2021). However, IPM is not being used to a great extent for early blight management in Sweden today, meaning fungicides are probably overused. The EU green deal (Guyomard et al. 2020) is aiming to reduce the use of pesticides by 50%, and to achieve that for potato early blight, studies of factors linked to IPM including the importance of soil, plant and management factors for early blight disease development are of great importance. Late blight treatment is the main reason for the high amounts of pesticides being used in potato production (Haverkort et al. 2008), but also early blight is currently demanding many treatments to be controlled. There are multiple prognosis systems for potato early blight being developed; most of them are only based on weather conditions and plant age (Meno et al. 2022a, b), but it would be beneficial if field soil specific parameters would also be taken into consideration in these models to create more accurate simulations.

Mineral nutrients are important for plant resistance to pathogens even if there are contradicting reports on the effect of nutrients on plant disease (Dordas 2008) that need to be further elucidated. The effect may depend on the type of pathogen, since obligate pathogens may increase disease severity at high nitrogen levels, while disease caused by facultative pathogens usually decrease at high N levels (Dordas 2008). Since nutrients are important for both plants and microorganisms, many interactions between plant and soil factors may occur and the effect of a specific nutrient can vary in different environments (Dordas 2008; Huber et al. 2012; Tripathi et al. 2022). Low nitrogen levels are reported to increase severity of early blight disease in potato (Jindo et al. 2021; Abuley et al. 2019). However, the effect of other nutrients on potato early blight does not seem to be well investigated.

Starch potatoes in Sweden are usually late-maturing and harvested later in the season with a longer growth period than table potatoes and are therefore more prone to early blight infection. Starch potato farmers in southern Sweden started to notice an increase in early blight infection around the season of 2017. This was probably due to an increased incidence of fungicide resistance to boscalid, at that time the most used fungicide available, and azoxystrobin (Mostafanezhad et al. 2021; Odilbekov et al. 2019). This gave rise to awareness of early blight in Sweden and was partly the reason for the initiation of this study.

This investigation was designed as an observational study of commercial farms representing the starch potato production area in Sweden in 2019–2021. The hypothesis of this study, based on the large variations in disease observed by farmers and advisors, was that the severity of infection depends on specific field, soil, plant, and crop management factors. The reason for the large field variation in early blight severity in Sweden is mostly unknown, and we aimed to unravel factors that are most important for the disease development.

To experimentally confirm an observation in the farmer study, field trials were performed in 2021 and 2022. Ladders of three different levels of potassium fertilisation were applied for both fungicide-treated and untreated plots to study the effect on early blight disease.

The main question for the study was are there any soil, plant or crop management parameters that we can observe or control in order to customise and calibrate the disease control management towards more field-specific IPM strategies?

Materials and Methods

Observational Study

Over three seasons, 2019, 2020 and 2021, a total of 52 unique fields were included in this observational study (Fig. 1). The fields were situated in southern Sweden where starch potato is grown for “Sveriges Stärkelseproducenter Förening” (SSF) (Fig. 1). The fields were chosen to represent the different conditions in the area (e.g., crop rotation, soil type, farmer- and advisor perceived infection pressure) of potato starch primary production. It was also of importance to select farms where the

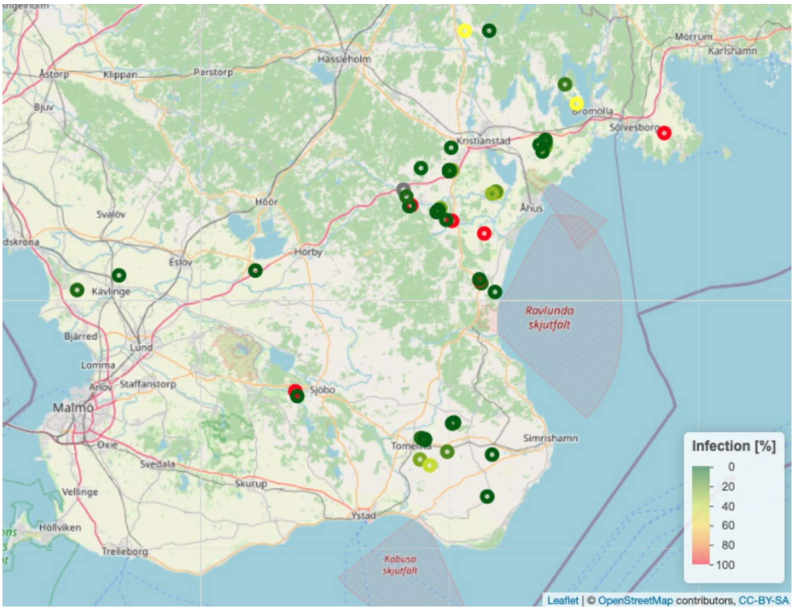

Fig. 1 Map presenting the geographical location in southern Sweden of all untreated farm plots for all three years (2019–2021) with a colour scale indicating the severeness of early blight infection (%)

farmers were interested in participating in the study and ready to collaborate, since it required them to sacrifice time to manage an untreated plot and fill out a survey. Table 1 gives an overview on the data that were collected from each field: soil analysis, leaf analysis and the specific management strategies at each farm.

Field Plots

The 24 m × 24 m (24 m is the width of a standard tractor sprayer) plot untreated against early blight was located inside the field to avoid edge effects. All other

Table 1 Overview of descriptive statistics for measured parameters in farmer field plots from the observational study 2019–2021

Parameter	Max	Min	Mean	CV [%]
Soil analyses				
pH	8.1	4.8	6.6	13
P-AL (mg P/100 g soil)	59	5.5	21	56
K-AL (mg K/100 g soil)	33	2.6	10	61
Mg–AL (mg Mg/100 g soil)	27	4.2	10	50
K/Mg	4.0	0.1	1.2	63
Ca-AL (mg Ca/100 g soil)	1900	54	330	130
Humus content (%)	11	0.9	3.4	52
Clay content (%)	25	2	8.3	54
Sand content (%)	96	41	74	17
Leaf analyses				
Calcium [%]	5.0	1.3	2.6	28
Magnesium [%]	1.9	0.5	0.8	31
Manganese [ppm]	630	27	330	58
Boron [ppm]	170	27	45	4.1
Copper [ppm]	20	2.9	8.1	54
Molybdenum [ppm]	4.5	0.1	1.0	81
Iron [ppm]	560	87	190	50
Zinc [ppm]	33	8.4	16	27
Sulphur [%]	0.8	0.3	0.5	22
Phosphorus [%]	0.4	0.2	0.3	18
Potassium [%]	3.9	0.9	2.3	34
Nitrogen [%]	6.1	3.4	4.4	13
Management				
Planting date	15-May	4-Apr	27-Apr	
50% emergence date	9-Jun	25-Apr	25-May	
Irrigation total (mm)	380	0	81	95
Seed tuber	N/A			
Cultivar	N/A			
Crop rotation year ($n-1$) = years without potato	10	3	5.6	44

management of the fields was left untouched and followed the general management strategy by each farmer.

Soil Analysis

To get an indication of the soil type and fertility (Table 1), soil samples were taken at the beginning of September by using a soil drill at a depth of 5–25 cm inside of the furrow where the tubers grow. Around twenty subsamples from the soil drill were taken from different spots in the field plots in a “W” pattern with an estimated total volume of one litre and then pooled and mixed in a plastic bag. The soil in the sealed plastic bag was stored at room temperature, in darkness until analysis the next day. The soil was delivered to Eurofins in Vä, Kristianstad Sweden, and analysed in their laboratory (Eurofins 2021). The soil parameters analysed were pH, P-Al, K-Al, Mg-Al, Ca-Al, humus content, clay content and sand content (Table 1). Accreditation and methods for soil analysis can be found in the Supplementary materials Table 1.

Leaf Analysis

Leaf samples representing plant nutrient content of the field plots (Table 1) were collected in the middle of August. The collection technique followed the instructions from Yara Megalab™ for whole potato leaf analysis (Yara 2022). The fourth fully developed leaf was picked. In total, twenty leaves from each untreated plot were picked. The leaf samples were put in paper envelopes and mailed the same day as the collection to Yara Analytical Services in Pocklington, UK, that carried out the analyses. The leaf analysis parameters analysed were Ca, Mg, Mn, B, Cu, Mb, Fe, Zn, S, P, K and N (Table 1).

Disease Assessment

The levels of disease and defoliation were evaluated twice during the season, in the middle of August and at the beginning of September. The early blight severity was visually scored according to Duarte et al. (2013). The scoring numbers (0–100) are defined as the percentage of the green foliage covered by dark early blight spots. The level of defoliation was scored as a percentage of the green biomass that had turned brown or fallen off from the plant. Both the disease levels in the untreated plot and in the surrounding field were evaluated, and so was the level of defoliation. This was performed to see if the fungicide management strategy used by the farmer had any effect on the disease. The surrounding fields were fungicide treated at most farms and never showed high levels of infection. For the “Results” section in this paper, the disease severity data (percent infection) from the untreated plots from the second scoring, at the beginning of September, was used. The infection in mid-August had not yet reached a point where larger differences could be seen among the fields (Supplementary materials Table 2).

Farm Management Form

After each field season, the farmers were asked to fill out a form containing information about their management. The following information was collected for each field: planting date, date at which 50% of crop emergence occurred, cultivar, seed tuber certification and/or treatment, irrigation type and amount, the number of potato free years and details about their historical crop rotation the last five years, soil management, fertilising schedule and amounts, yield and finally their own reflections of their disease levels.

Weather Data

Values of average daily temperature and precipitation were obtained from Lantmet, SMHI, 2019–2022, and are presented in the "Season" section. The weather station in Nymö-Fjälkinge was used for the comparison since it was operating during all seasons involved and represented the area of the observational study well.

Potassium Field Trials

Preliminary data from the first two seasons of the farmer study showed that low potassium levels in leaves correlated with high early blight infection. Therefore, to confirm these results, potassium field trials were performed in 2021 and 2022. The trials were located at two separate sites per season, 2021: Nymö (N 56.024848, W 14.335998) and Gärds Köpinge (55.947479, 14.182357), 2022: Åsums boställe (55.958602,14.151002) and Lyngby gård (55.886097, 14.143463). The sites were chosen based on their low levels of potassium available in the soil to be able to create deficiencies at low potassium application rates. The trial was designed with four completely randomised blocks. Each plot consisted of 18 m^2 of plants, except for the trial at Åsums boställe, where the plots had a size of 15.75 m^2 due to lack of space. Each plot contained five rows where the three middle rows were evaluated for disease progression, defoliation, leaf potassium concentration and tuber- and starch yield. Three different levels of potassium fertilisation were applied in both untreated and fungicide-treated plots (Table 3). Fungicides were applied following a full dose recommendation with four treatments and two-week interval with start in mid-July. The fungicide products Narita (0.4 L/ha, active component: difenoconazole 250 g/L) and Propulse (0.45 L/ha, active components: fluopyram 125 g/L, prothioconazole 125 g/L) were alternated. Standard late blight (Revus/RanmanTop alternated every week starting in mid-June) and insecticide treatments were conducted. A tractor sprayer (Lechler IDKT Purple 0,25) with a flat fan nozzle with medium droplet size was used with 300 L water/ha at 3 bar. The starch cultivar Kuras was used in all field trials and seed tubers were obtained from Lyckeby SSF. The trials were, except for potassium, fertilised and managed following standard recommendations by the Swedish Rural Economy and Agricultural Societies in the starch potato growing area in southern Sweden. The planting row distance was 75 cm with 38 cm in between the plants for all trials. All infection that occurred in the trials was natural.

Potassium Fertilisation

The experimental fields were fertilised before planting with a later (end of June) additional N fertilisation and weekly Mn treatments. For 2021, 137 kg/ha N, 63 kg/ha P and 50 kg/ha Mg and for 2022 200 kg/ha N, 63 kg/ha P and 50 kg/ha Mg were added in total, in the form of Axan Ns 27–4, MAP NP 12–23 and Kiserit. The potassium fertiliser (potassium sulphate) was applied by hand individually for each plot to be able to adjust the levels. In the first year, 2021, a potassium ladder was designed according to the soil analysis results with three steps (Table 3). The middle level, K2, was following the recommended fertilisation for the field, and the low level, K1, contained 100 kg K/ha less than K2, and the high level, K3, contained 100 kg K/ha more. Since the potassium leaf analysis for 2021 did not show the depletion that was aimed for (see "Results"), the ladders were changed for the 2022 season. For 2022, the ladder was designed so that the low level, K1, had no potassium fertilisation at all and the highest level, K3, was following the recommended level and the middle step, K2, was in between zero and the recommended value (Table 3).

Potassium Leaf Analysis

The leaf analysis was performed on September 6th in 2021 and on August 25th for 2022, following the same method as for the leaf analysis in the observational study, the "Leaf Analysis" section. It was planned for a later analysis in 2022 as well but due to early defoliation caused by potassium deficiency the later analysis was not possible. Ten leaves from each block were picked. Blocks 1 and 2 and 3 and 4 were pooled, respectively, to 20 leaves each giving two replicates for each treatment in 2021. In 2022, twenty leaves were collected from each of blocks 1, 2 and 3 giving three replicates. Results are presented in Table 3.

Disease Assessment

The level of early blight disease and defoliation was visually scored weekly as in the "Disease Assessment" section. The disease scoring data was used to calculate the relative area under disease progress curve, rAUDPC (Shaner & Finney 1977). The defoliation data was used to calculate the area under the defoliation curve, rAUC, in a similar way. The visual scoring was carried out weekly during the season, but since the disease in the different fields progressed very differently both in the two years and at the different sites, the exact dates for the calculations differed over the seasons and fields. For the 2021 trials, the calculations were done from 9th of August to 13th of September for both rAUDPC and rAUC at both sites. For 2022 at the Lyngby gård trial site rAUDPC was calculated from 18th of July to 22nd of August and rAUC from 8th of August to 5th of September. For the second trial site in 2022, Åsums boställe, both rAUDPC and rAUC were calculated from 9th of August to 12th of September. Thus, Lyngby gård had to be evaluated during a different period than the other sites, due to a very early onset of infection and an earlier defoliation. Disease assessment data is presented in Tables 4 and 5.

Yield and Starch Measurements

The yield from each plot was measured at harvest and the starch content of the tubers was calculated following the standard method set by the International Starch Institute Denmark (1986). The yield and starch data are presented in Tables 4 and 5.

Statistical Analysis

For data analysis and creation of most graphs, the language R was used with R studio as an interface (Version 1.1.456© 2009–2018 RStudio, Inc.). Some graphs were made with MS Excel. To investigate how the rate of infection (as a percentage) was influenced by the measured soil, leaf and farm management parameters in the observational study of the farms, first Spearman correlations were performed between infection rate and all parameters for (i) soil measures, (ii) leaf measures and (iii) farm management parameters. All field plots were considered as independent samples. Second, to test whether selected parameters that correlated with infection rate in the initial tests also had an effect when year of study was taken into account or occasionally the combined effect of two parameters was taken into account, analyses of variances (Anovas) were performed. These models included the fixed factors selected parameter(s), year and their interactions. Type II sums of squares were used and normality of residuals were tested with the Shapiro–Wilk normality test. Correlation plots were created using the corrplot (Wei and Simko 2021) package, cor.mtest. The ggplot2 (Wickham 2016) package was used for the plots. The leaflet package (© 2014–2016 RStudio, Inc.) was used to create the map.

To test whether potassium fertilisation level affected the dependent variables: infection rate (rAUDPC), defoliation rate (rAUC), yield, starch content and starch yield in the field trials, a series of analyses of variances (Anovas) were performed for each of the dependent variables. rAUDPC and rAUC were log-transformed. Data from each of the two years were analysed in separate models because of the difference in design between the years. The models included the fixed factor treatment (potassium treatment K1–3 for each of fungicide-treated and control plots), block nested within field site and the interaction between treatment and field site. Type II sums of squares were used. Post hoc tests were performed to test treatment differences using estimated marginal means with Tukey's method. Normality of residuals was tested with the Shapiro–Wilk normality test. The packages used for statistical analyses were car (Fox and Weisberg 2019), emmeans (Lenth 2022) and stats (R core team 2022).

Results

Observational Study

An overview of the investigated parameters in the observational study is presented in Table 1. Several measured parameters showed very high variation among the investigated farmer's fields indicated by large differences between min and max values and high CV values. For example, the soil parameters K-AL, K/Mg, Ca-AL, and the leaf parameter copper all had a CV higher than 60%. However, several other parameters showed much lower variation. The soil parameters pH and sand content together with the leaf parameters boron, phosphorous and nitrogen content all had CV values lower than 20%.

Soil and Leaf Analyses

Soil Analysis

From the initial correlation analysis, the soil type, i.e., sand and clay content, showed a strong correlation (Spearman) with each other and with the early blight infection ($p < 0.001$, Fig. 2a). Testing the effect of sand content when taking year into account confirmed that a high sand content seemed to imply a higher risk of early blight infection (Anova; sand content: $F = 22.9$, Df = 1, $p < 0.0001$; year: $F = 6.29$, Df = 2, $p = 0.0039$; interaction: $F = 2.88$, Df = 2, $p = 0.067$, Fig. 3). Sand content was divided into low (< 80%) and high (> 80%) based on general agronomic practice in the local area. Infection rates above 20% were only found on soils with a sand content above 75% (Fig. 3). However, in some cases soils with high sand content also had low

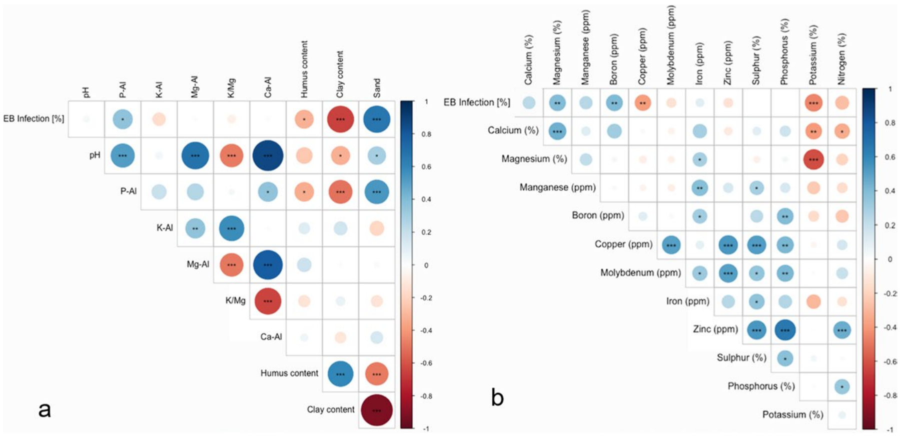

Fig. 2 Correlation plots (Spearman) for the soil (**a**) and the leaf (**b**) parameter analyses together with the scored early blight infection (in percentage) in the untreated plots. The correlations are described with a colour scale (where red is a negative correlation, and blue is positive) and with a circle (bigger circle shows a higher correlation). Also asterisks are indicating the significance in the correlations

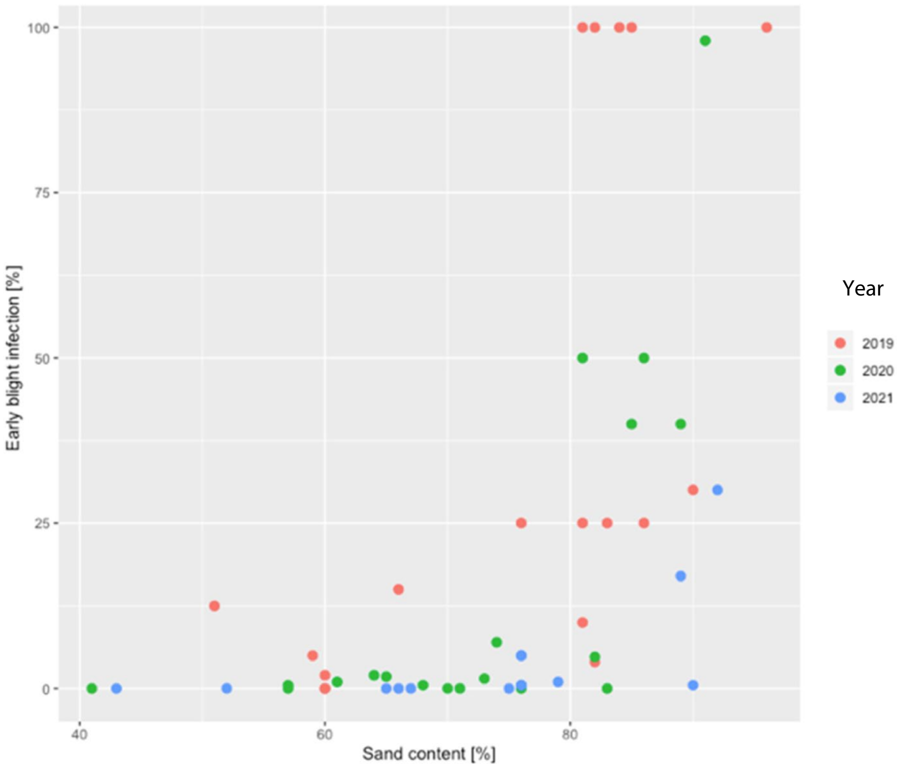

Fig. 3 Infection of early blight in relation to sand content together with season showed in colour

infection rate due to other factors like year (Fig. 3). Tukey's post hoc test confirmed that the average infection rates were lower in 2021 compared to 2019 ($p = 0.0077$, Fig. 3).

A significant positive correlation between P-AL and infection rate was found in the initial analysis (Fig. 2a) and in the Anova also including the effect of year (Anova; P-AL: $F = 10.7$, Df = 1, $p = 0.0020$; year: $F = 3.72$, Df = 2, $p = 0.032$; interaction: $F = 2.14$, Df = 2, $p = 0.13$). However, high P-AL values were mainly found in soils with high sand content (Figs. 2a and 4, Table 2).

Leaf Analysis

Overall, the initial leaf analysis indicated a highly significant negative correlation between leaf potassium content and the severity of early blight infection (Fig. 2b, Table 2). However, that seemed to be mainly the case in sandy soils where a negative correlation was observed all three years (Fig. 5). Testing the combined effects of potassium content of sand content as a categorical factor either above or below 80% across years showed a significant interaction between leaf potassium content, sand content category and year (Table 2). There is also a three-way

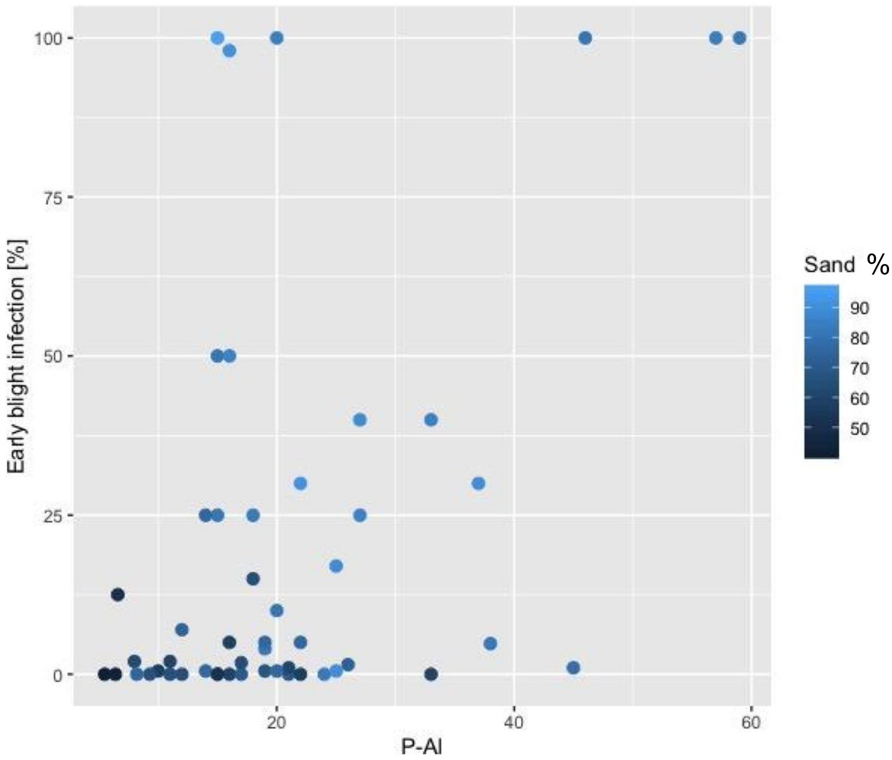

Fig. 4 Relationship between early blight infection rate and soil phosphorus level, P-AL, with sand content (%) shown in colour scale

interaction to be found (Table 2), showing that in years with higher infection pressure the effect of potassium content on infection matters on heavier soils as well. There is a marginally non-significant correlation to be found between leaf potassium content and soil sand content (Df = 1, $F = 3.99$, $p = 0.0529$), and this correlation would much likely be stronger if the fields were not soil type precisely fertilised directly to not cause depletion in potato.

There was a significant negative correlation between the leaf copper content and infection rate in the initial analysis (Figs. 2b and 6). However, when taking year into account the effect of copper was marginally non-significant (Anova: leaf Cu content effect on infection: $F = 3.8$, Df = 1, $p = 0.056$; year: $F = 5.8$, Df = 2, $p = 0.0059$; interaction: $F = 0.22$, Df = 2, $p = 0.80$). Copper content was also related to the soil sand content (Fig. 6), where lighter soils tended to be lower in Cu (Anova: leaf Cu content effect on sand content: $F = 39.5$, Df = 1, $p < 0.0001$). Leaf phosphorus and nitrogen content showed no significant correlation with the infection rate and is therefore not analysed in separate models involving year. Positive correlations between the leaf analysis and infection rate were found for boron and magnesium (Fig. 2b), suggesting that these micronutrients may also have some

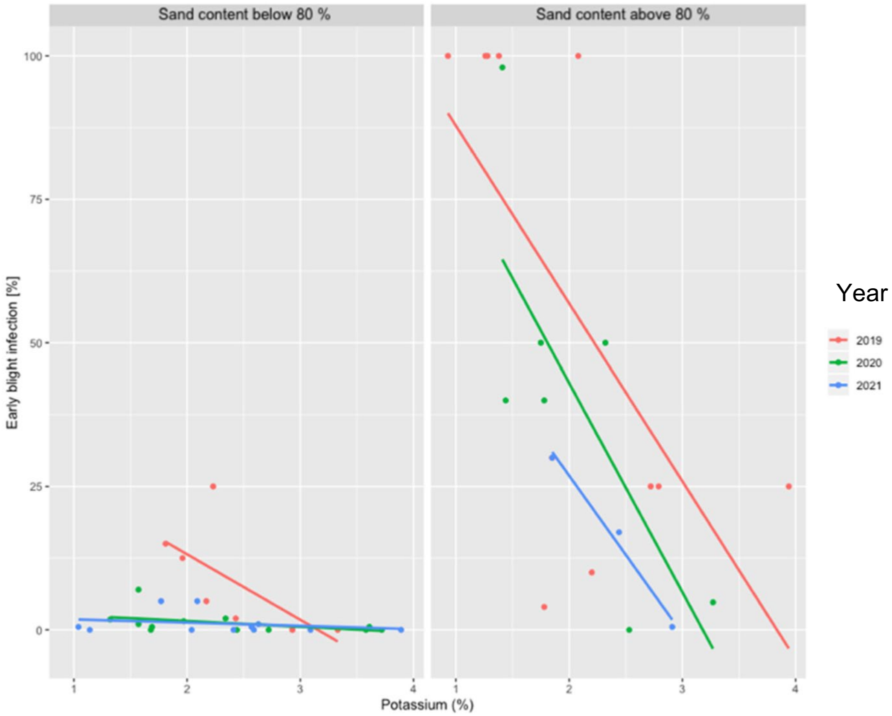

Fig. 5 The relationship between early blight infection rate and leaf potassium content in soils with sand content above and below 80%

Table 2 Anovas for early blight infection rate in potato fields 2019–2021 as an effect of selected soil and leaf parameters and their interactions with year and each other

Source of variation	Df	*F* value	*p* value
Leaf potassium and sand content above 80%			
Potassium (%)	1	18.2	0.00012
Year	2	6.84	0.0028
Sand > 80%	1	59.1	2.5*e* – 09
Potassium × year	2	0.164	0.85
Potassium × sand > 80%	1	1.02	0.32
Year × sand > 80%	2	0.483	0.62
Potassium × year × sand > 80%	2	7.05	0.0024
Soil P-Al and sand content			
P-Al	1	0.183	0.67
Year	2	11.9	9.1e – 05
Sand	1	36.7	4.3e – 07
P-Al × year	2	0.877	0.42
P-Al × sand	1	6.71	0.013
Year × sand	2	1.39	0.26
P-Al × year × sand	2	0.0585	0.94

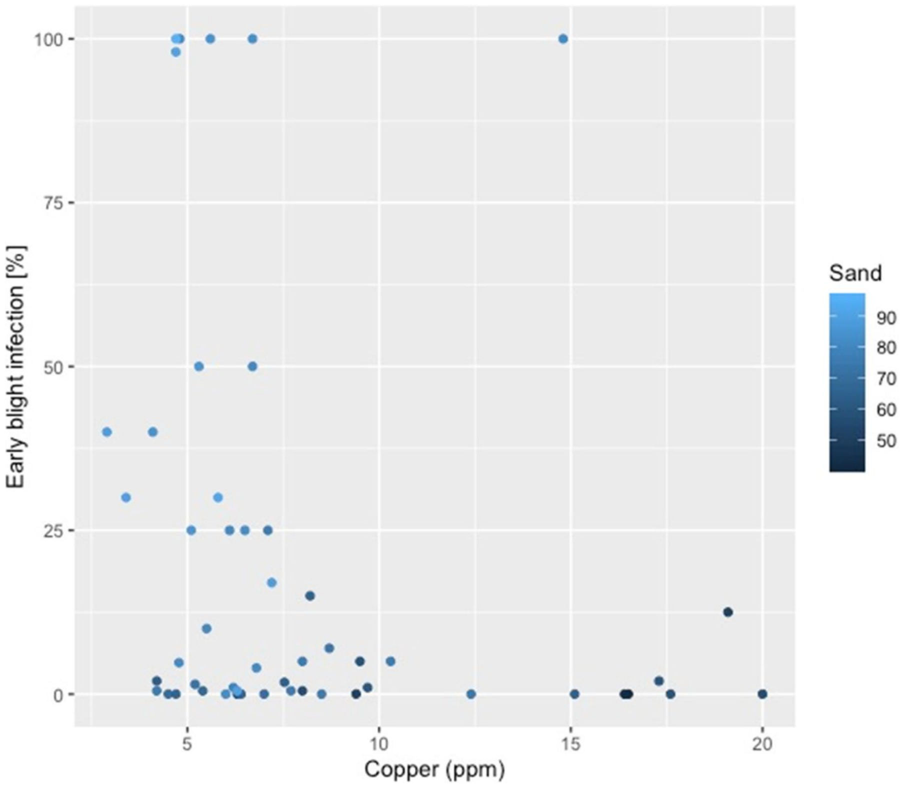

Fig. 6 Relation between copper content in leaves and early blight infection rate, sand content in different colour

importance. However, the effect on infection may also be indirect as magnesium content in the leaf had a strong negative correlation with potassium (Fig. 2b).

Management

Cultivar Resistance The form the farmers filled in gave information on what starch potato cultivar that was used in the plots, but since most farmers were growing the same cultivar, Kuras (67%), and the distribution of other cultivars were not even (Dartiest 10%, Saprodi 8%, Avenue 6%, Maxim 4%, Stratos 2%, Maxim 2%, N/A 2%), this study did not give enough input data on cultivar resistance to disease in order to draw any conclusions.

Season For early blight, as for many other plant diseases, the seasonal disease pressure may largely vary. The average infection rate was lower in 2021 than in 2019 and 2020 (Figs. 3 and 5). The daily average temperature and the precipitation for the years of the observational study and the field trials are seen in Fig. 7. Weather data

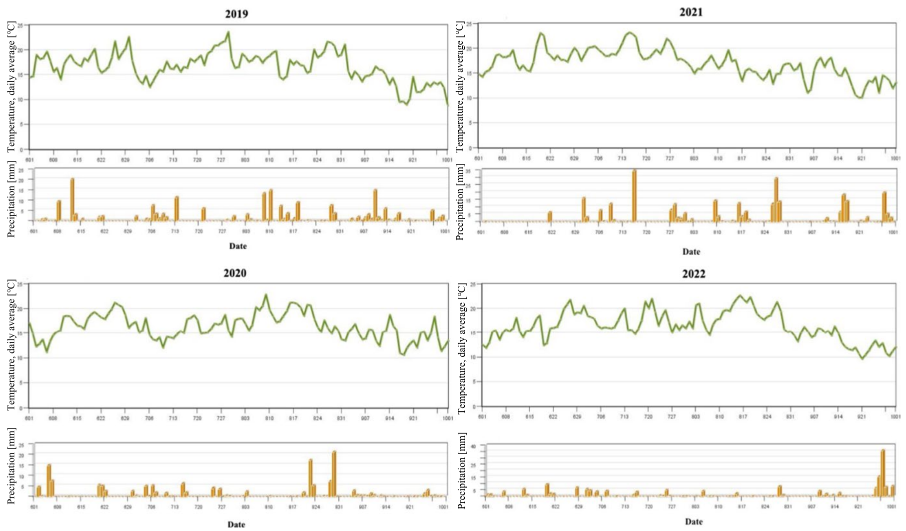

Fig. 7 Weather data for 2019–2022 with daily temperature average and precipitation obtained from Lantmet (2023) from the weather station in Nymö-Fjälkinge

from the years of the observational study 2019–2021 and for the potassium field trials 2021–2022 is presented.

Crop Rotation Visual inspection of the effect of crop rotation on infection rate in relation to the two most significant parameters influencing infection (sand content and leaf potassium content) suggested that longer crop rotations (> 8 years) never gave rise to very high early blight infection rates (Fig. 8a, b). The other factors, soil composition and potassium, seemed to be of bigger importance though. There is a lack of data points from farms with a crop rotation of 6–8 years, which makes it difficult to test the effect of crop rotation statistically. However, the results suggest that there is a weak significant correlation between short crop rotation and high infection rate (Anova: crop rotation effect on infection: $F = 6.30$, $\mathrm{Df} = 1$, $p = 0.0158$).

Planting Date and Emergence Date There was no significant correlation between the planting date or emergence date and severity of early blight infection.

Furrowing Since *A. solani* is a soil pathogen and mostly spreads directly from the soil to the lower foliage on the potato plant, management strategies such as late furrowing could potentially help spread the spores to the foliage. However, there was no correlation between late furrowing and early blight infection.

Seed Tuber The farmers gave information about seed tuber treatment and certification level of tubers planted in the fields. None of these seed tuber parameters seemed to correlate with the early blight infection rate.

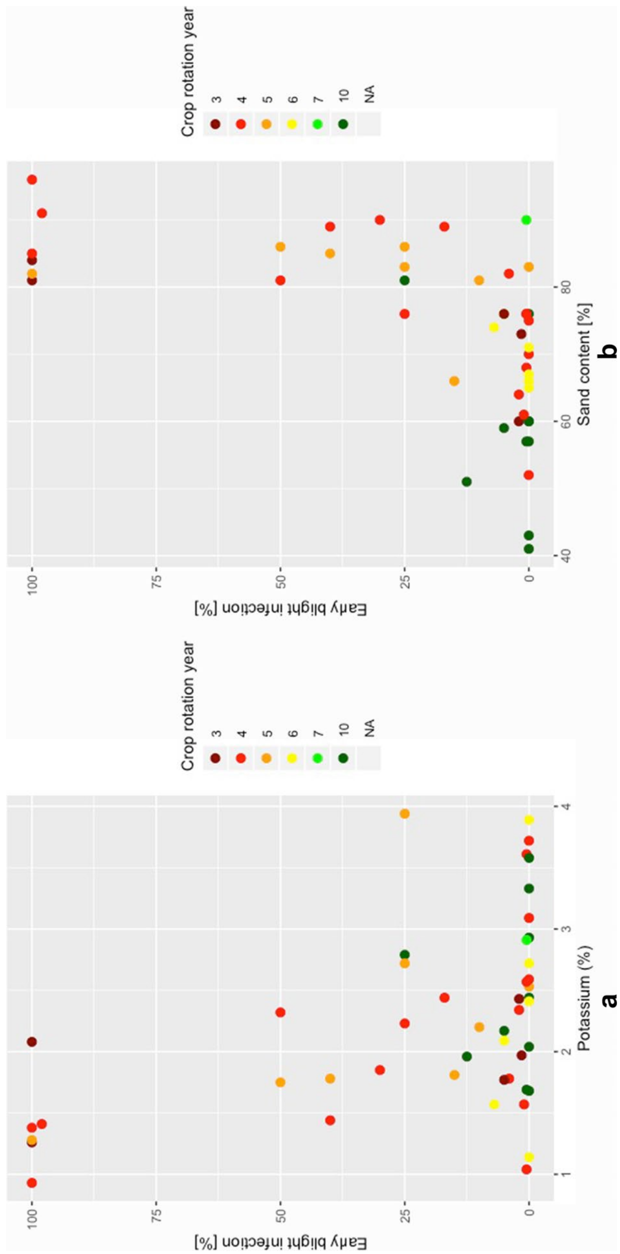

Fig. 8 **a**, **b** Crop rotation in colour and leaf potassium content/soil sand content versus infection

Table 3 Soil potassium (K-AL) analysis before planting and application of fertilisers. Level of K (potassium sulphate) fertiliser applied and resulting leaf potassium content (September 6th in 2021 and 25th of August 2022) in the four field trials 2021–2022

K-AL [mg K/100 g soil]	K level	Applied K fertiliser K [kg K/ha]	Resulting K content in leaf [%]
2021 Nymö			
18	1	20	2.75 ± 0.36
	2	120	2.62 ± 0.05
	3	220	2.39 ± 0.68
2021 Gärds Köpinge			
14	1	100	2.01 ± 0.01
	2	200	2.75 ± 0.10
	3	300	2.93 ± 0.21
2022 Lyngby Gård			
5,7	1	0	0.86 ± 0.13
	2	133	1.22 ± 0.13
	3	265	1.66 ± 0.37
2022 Åsums boställe			
11	1	0	0.74 ± 0.16
	2	133	1.26 ± 0.17
	3	265	2.11 ± 0.23

Irrigation There was no significant correlation between total volume of irrigation and early blight infection rate. However, farms that lacked any irrigation system had lower early blight infection. These farms also had heavier soils (lower sand content) that were less prone to early blight infection (see the "Soil Analysis" section). The type of irrigation used by the farmers was mostly water canon (85%) and therefore no conclusion on the effect of irrigation type can be drawn.

Yield The tuber yield data that was collected in the survey was not complete. Not all farmers filled it out, and for those who did, it was based on estimates and guesses. Therefore, the yield data was excluded from the results.

Potassium Field Trials

In the potassium field trials the effect from different levels of potassium fertilisation (Table 2) on the rate of early blight infection was evaluated. Higher level of potassium fertilisation resulted in lower rate of early blight infection (Table 3, Fig. 9). However, in 2021, the significant effect of treatment (Df = 5, $F = 47.2$, $p < 0.0001$) was only due to a difference between fungicide treated and untreated but no significant effect of potassium fertiliser (Table 3). The lower levels of fertiliser did not lead to potassium deficiency, and the leaf potassium concentrations were all above 2% (Table 2) this year. Fungicides reduced the infection rate significantly (Table 3). In 2022, when the ladder used started at zero K added for K1, large differences in leaf K concentrations were obtained and there was

Table 4 Infection rate (rAUDPC), defoliation rate (rAUC) and yield data, compared between untreated and fungicide-treated plots with three different levels of potassium fertilisation (K1–3, where 1 is the lowest) at two field trial sites in 2021

K level	Treatment	rAUDCP*		rAUC*		Yield [ton/ha]		Starch content [%]		Starch yield [ton/ha]	
Nymö 2021											
1	Untreated	4.56E − 02	a	1.98E − 01	ab	72.6	a	18.7	a	13.5	a
2		5.35E − 02	a	2.48E − 01	a	58.6	a	18.3	a	10.7	a
3		3.62E − 02	a	1.84E − 01	ab	74.7	a	19.1	a	14.3	a
1	Treated	5.39E − 03	b	1.59E − 01	ab	63.8	a	18.6	a	11.9	a
2		2.61E − 03	b	1.30E − 01	b	64.6	a	18.9	a	12.3	a
3		2.81E − 03	b	1.48E − 01	ab	70.2	a	19.1	a	13.4	a
Gärds Köpinge 2021											
1	Untreated	2.09E − 01	a	4.85E − 01	a	69.4	a	19.6	a	13.6	a
2		1.29E − 01	a	3.54E − 01	a	73.3	a	18.9	a	13.9	a
3		1.66E − 01	a	4.19E − 01	a	71.1	a	19.3	a	13.7	a
1	Treated	1.66E − 02	b	1.26E − 01	b	81.9	a	19.9	a	16.3	a
2		1.62E − 02	b	1.28E − 01	b	77.1	a	19.2	a	14.8	a
3		1.83E − 02	b	1.42E − 01	b	76.7	a	19.1	a	14.7	a

*rAUDPC and rAUC were evaluated 9th of August–13th of September at both sites. Different letters indicate significant difference according to Tukey test

Table 5 Infection rate (rAUDPC), defoliation rate (rAUC) and yield data, compared between untreated and fungicide-treated plots with three different levels of potassium fertilisation (K1–3, where 1 is the lowest) at two field trial sites in 2022

K level	Treatment	rAUDCP*		rAUC*		Yield [ton/ha]		Starch content [%]		Starch yield [ton/ha]	
Lyngby gård 2022											
1	Untreated	7.50E−02	a	0.765	a	43.5	a	19.6	a	8.5	a
2		2.87E−02	ab	0.655	a	51.1	bc	19.6	a	10.0	bc
3		1.31E−02	bc	0.633	a	52.9	c	19.7	a	10.4	c
1	Treated	1.50E−02	bc	0.731	a	45.6	ab	19.6	a	9.0	ab
2		7.66E−03	c	0.599	a	55.1	c	19.3	a	10.6	c
3		5.81E−03	c	0.593	a	55.3	c	19.8	a	11.0	c
Åsums boställe 2022											
1	Untreated	8.39E−02	a	6.17E−01	a	59.1	a	22.3	a	13.2	a
2		4.00E−02	a	4.13E−01	bc	68.4	b	22.4	a	15.3	b
3		3.38E−02	ab	3.62E−01	c	70.0	b	22.2	a	15.5	b
1	Treated	1.20E−02	b	5.19E−01	ab	61.1	a	22.6	a	13.8	a
2		2.76E−03	c	3.25E−01	c	70.1	b	23.1	a	16.2	b
3		6.15E−04	d	2.34E−01	d	73.0	b	22.6	a	16.5	b

*At Åsums boställe rAUDPC and rAUC were evaluated 9th of August–12th of September; at Lyngby gård due to early defoliation, rAUDPC was evaluated 18th of July–22nd of August and rAUC 8th of August–5th of September. Different letters indicate significant difference according to Tukey test

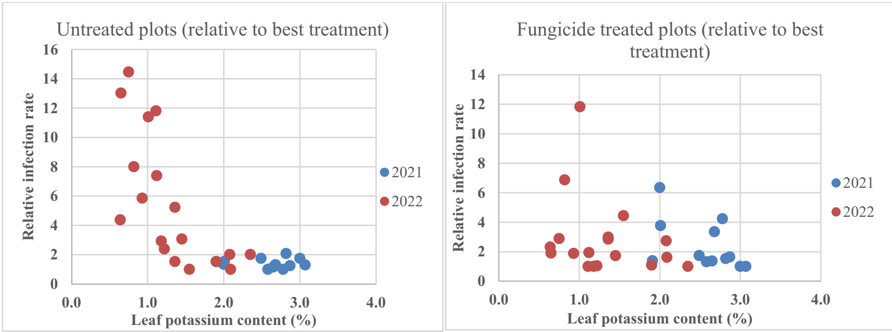

Fig. 9 Graph showing the relationship between the leaf potassium content (%) and the relative infection rate of early blight in the field trials 2021 and 2022 for untreated and fungicide-treated field plots

a significant effect of fertiliser level on the early blight infection rate (Table 3, Df = 5, $F = 53.6$, $p < 0.0001$). There was also a significant effect of potassium on the starch yield. There is a known connection between the tuber starch content and amount of potassium fertiliser where a higher potassium fertilisation tends to give a lower starch content. This effect was not significant for our trials when all treatments were pooled for both sites, but since the effect on tuber yield was positive with a higher K fertilisation, the overall starch yield was affected by the potassium fertilisation levels (Tables 4 and 5). In Fig. 9 the levels of leaf potassium are compared with the 'relative infection rate' instead, where the relative infection is calculated by dividing the rAUDPC value with the lowest rAUDPC value scored, within untreated or fungicide-treated plots, at each specific site and year. These figures show that there was a seasonal difference but more importantly that there was a relationship between the leaf potassium content and early blight infection rate. The effect was most clear in the untreated plants.

Discussion

We found large variation in early blight infection rates among 51 investigated farmer's fields in southern Sweden over three years. We also found large variation in several soil and plant parameters analysed that could explain some of the variation in infection rate. Soil sand content and leaf potassium concentration were clearly negatively associated with higher early blight infection rates. The effect of potassium was confirmed in field trials where we found that low levels of potassium fertilisation, leading to deficiencies at the end of the season, resulted in significantly higher infection. Also, other parameters, e.g. leaf copper content and soil phosphorous levels and crop rotation, may be associated with infection rate but need further studies to be confirmed.

Soil Composition

Our results indicate that the sand and clay content of the soil is important for the risk of severe early blight infection. To explain that we suggest two different hypotheses that may be linked with each other. The first is that plants in a lighter soil will more easily experience drought stress and nutrient depletion because the soil cannot hold as much water and nutrients will easily leak out leading to deficiencies. Secondly, heavier soils have different soil microbiome composition, a composition that could offer more competition with *A. solani* from other microorganisms. Thereby, the survival of *A. solani* could be affected and result in decreasing soil inoculum concentration or reduce the sporulation rate of the pathogen. Future studies to test these hypotheses would be of importance.

Another soil factor that requires some explanation is the P-AL content. This parameter shows how much phosphorus is available in the soil for the plant to take up, and there was a positive correlation between P-AL and early blight infection. However, P-AL also correlated with the sand content so that heavier soils appeared to have less P-AL (Table 2). By consulting agronomic experts we hypothesise that farms with lighter soils in a historical perspective have had a need of animals at the farm to achieve and maintain fertile soils with positive economic results. These farms have most likely used more manure in their fertilisation strategy leading to higher P-AL. The P-AL values on some of the farms with light soils were extremely high, and those values would not be preferred by the farmer but is most probably a result of having lots of manure at the farm to use (Liu et al. 2012). Thus, it cannot be concluded from this study if the soil available phosphorus has anything to do with the severeness of infection, or if the P-AL parameter is just linked to higher use of manure on sandy soils.

Leaf Nutrient Components

The supply of nutrients affects the plants' resistance to pests and pathogens (Huber et al. 2012) and therefore leaf nutrient parameters were studied. The leaf analysis was conducted in the middle of August for all three seasons included in this study. The sampling date is later than what is usually done to correct deficiencies in potato production, but since this was an observational study, we did not want to fertilise to support the plants, and it was decided that a later sampling would give more information about deficiencies related to early blight. There is most probably a correlation between nitrogen content and early blight infection (Dordas 2008), but we did not find any significant effect in our study. This might be explained by the intense nitrogen fertilisation strategy that is practised in most farms. Most plots had a good nitrogen value, and no deficiencies were observed (Table 1). However, in many fields the potassium levels were lower than what would be recommended, which might explain why a deficiency-related infection could be seen. Further, the negative correlation between leaf potassium and infection was mainly found on sandy soil, which is not surprising since sandy soils often are inherently low in K (Zörb et al. 2014). Amtmann et al. (2008) state that K application is beneficial for plant defence against most fungal diseases. This has been explained by the effect K has on primary metabolism by helping to synthesise high molecular weight compounds that is reducing the concentrations of low molecular weight compounds that are feeding the pathogens (Römheld & Kirkby 2010). According to Huber et al. (2012), potassium addition is only effective in disease control if there is a deficiency in the plant and is related to the metabolic functions of K and several other biochemical and physiological processes (Amtmann et al. 2008; Dordas 2008). This agrees with the results in our potassium field trials where increased infection rates were only found at low potassium levels in the leaves, i.e. below 2%. Higher potassium levels than 2% did not lower the infection rate. However, in a review by Jindo et al. (2021), potassium was not considered as important for early blight infection in potato, but it does not seem to be well investigated and further research is needed for clarification. Blachinski et al. (1996) reported no effect from potassium foliar treatment in lowering early blight infection rate; however the K depletion was not as high in their fields as it was in our study. On the other hand, they mention that the developmental stage of the potato plant is related to the potassium leaf content and could therefore influence the amount of early blight infection, since the disease is related to senescence. We could not find any relationship between the planting or emergence date and potassium leaf levels in our study. In some cases, it has been observed that potassium-deficient plants were less infested by insects and necrotrophic pathogens (Amtmann et al. 2008). This was explained by the fact that potassium deficiencies may induce the jasmonate signalling network (Armengaud et al. 2004) that classically is supposed to trigger defence responses to insects and necrotrophic pathogens, while salicylic acid signalling is important for defence against biotrophic pathogens. *A. solani* is a necrotrophic pathogen and according to this potato plants should be more resistant at lower potassium levels. However, that seem to be different in different plant species. Brouwer et al. (2020), reported that in potato instead an intact salicylic acid signalling is required for defence against *A. solani* and that has also earlier been reported in tomato (Sarkar et al. 2017). Thus, a possible increase in jasmonic acid levels at low potassium level does not contradict our results.

Potassium also influences potato starch content. Too high potassium fertilisation may reduce the dry matter starch content (Miča, 1988), and potassium can also affect the physiochemical properties of the starch (Zhang et al. 2018). But the effect on the decreasing starch content is cancelled out by a higher tuber yield, making the effect less relevant. It is also mentioned by Miča (1988) that this phenomenon might be explained by errors in the methods of how to measure starch content. If the starch content calculation is done on dry or fresh weight, the results differ, meaning that it might not be that the actual starch content in the tuber goes down, rather the water content of the tuber goes up as a result of higher K, giving false values on starch content when using fresh weight calculations. In this study no effect on starch yield from potassium was observed and the physiochemical properties of the starch were not evaluated. Since increased infection rate of early blight only seems to happen when there is a clear depletion of potassium, and no further correlations with infection and higher potassium fertilisation levels that are exceeding the depletion threshold, the conclusion would be that if the recommended potassium fertilisation guidelines are followed, nor infection or starch yield will be affected.

Micronutrients

Many of the nutrients are correlated with each other which can be explained by two factors. First, a richer soil will have more micronutrients in general to supply the crop (Montgomery et al. 2022). Secondly, a farmer that is careful with micronutrient fertilisation will make sure that there are enough of all micronutrient components available for the plants (these fertilisers are often sold as mixes with many micronutrient components). There were positive correlations between infection and boron and magnesium, and high magnesium content is correlating with low potassium. A possible explanation for this phenomenon is that Mg ions and K ions are competing in the uptake of the plant (Senbayram et al. 2015; Taiz and Zeiger 2002; Tränkner et al. 2018). High levels of Mg also compete with the uptake of Ca which also may increase the rate of disease (Huber & Jones 2013). Boron on the other hand is not taken up in its ionic form (Taiz and Zeiger 2002) and is the least understood plant micronutrient. Still, it is the most widespread micronutrient deficiency in the world (Dordas 2008) and we have no explanation for the correlation with infection rate. The negative correlation between copper and infection might be explained by the leaf copper content being related to the soil composition, i.e. sandy soils generally led to lower copper values. Copper also has a role in plant defence against pests as it is important for detoxification of oxygen radicals and hydrogen peroxide (Huber et al. 2012). According to the leaf analysis, a copper value below 7 ppm would indicate a deficiency (Yara 2022) and in most cases field plots with high infection had lower than 7 ppm copper (Fig. 6).

Seasonal Differences

The seasonal weather difference is the single most important factor when it comes to disease severity; however it is also one of the factors that we cannot control (Meno et al. 2022a, b). Work on decision support systems for early blight in Sweden is ongoing and as a part of an integrated pest management strategy these interpretations of weather data and the direct weather effect on disease are of great importance to include in the farm strategies when reliable models are available. Weather models were not evaluated in this study, but the differences in observed infection rates over the three seasons indicate that the seasonal weather parameters are of great influence. In 2020 the total precipitation was higher than in 2019 and 2021, but most of that rain came early in the season. In 2019 there was more rain in July than the other two years which might have facilitated sporulation. This may explain the general higher infection rate in 2019 and 2020 compared to 2021. However, to draw conclusions about weather data and its influence on infection of early blight, more specific studies must be carried out.

Management Strategies

Of all the management strategies that were evaluated in this study: seed tuber treatment, irrigation, cultivar, planting date, emergence date and crop rotation, there was only an indication that crop rotation affected the disease severity. Early blight is a soilborne pathogen; hence the survival rate in the soil is an important factor related directly to the amount of infection (Suganthi et al. 2020). In Fig. 8b it appears that farms with a longer crop rotation also seem to have heavier soils. This is most probably due to heavier soils being better at delivering economic advantageous yields for other crops, like cereals, in between the potato, and further the farmers are not pushed to have short time in between their potato years. Since the economic calculations are most crucial for the survival of a farm, it is hard to find farms with long potato rotation on lighter soils and vice versa. It can be assumed from the graphs (Fig. 8a, b) that a four-year (or shorter) crop rotation is not making a big difference on the severity of disease later in the season. Previous studies like Shtienberg and Fry (1990) and Abuley et al. (2018) have been studying shorter crop rotations and linkage with potato early blight, from zero to four years, concluding that the initial appearance of infection comes earlier with a very short (<2 years) or no rotation of crops. Crop rotations below three years are not present in our study since it is not recommended for potato and further this statement could not be verified in this observational study. Moreover, the true onset of infection was not scored in our fields but only the later severity of disease. It would be of interest in future studies to find the point in crop rotation years where soilborne onset of infection and following disease severity seems to decrease, and this breaking point is most likely different for heavy and light soils and also how the onset of infection links with the disease severity.

Conclusion

By using an observational study on a large number of commercial farms and a follow-up field trial, the most important message is the significance of the soil composition for early blight infection. Sandy soils imply higher risk for severe infections. Therefore, when deciding on disease management strategies, for example fungicide treatment against early blight, the soil composition should be evaluated beforehand and taken into consideration when planning treatments. We have also seen an interesting association between potassium and early blight infection that needs to be further investigated in a more controlled environment. Potassium deficiencies will occur more easily on sandy soils and therefore extra care must be taken to optimise applications of fertilisers. If there is a lack of potassium available for the potato plant, the rate of early blight infection is increasing. This study highlights the extreme complexity of agricultural systems, but also the importance of conducting studies at farms as alternative plant protection methods and IPM would need to be adapted to the farm conditions. When studying what factors are directly relevant for a plant disease, there will be multiple parameters directly or indirectly correlating with each other and to separate them is a demanding task. As in this study, the soil parameters, e.g. sand content, will closely correlate with the plant nutrient uptake which further makes it complicated to decipher what factors from the leaf nutrient composition are related to the severity of disease and what could be explained by the soil composition.

Future Work

It would be of interest to incorporate soil structure in the development of decision support systems for diseases like early blight, since soil type has great influence on infection rate. Since we did not observe a drop in infection rate after four years, as other studies have suggested, but a slower decrease of infection over many years, it would also be valuable to further investigate if there is a minimum crop rotation time where the soil inoculum decreases enough to result in a slower development of disease. Studies on inoculum survival in different soil types would be important in this context. The role of potassium, especially on potassium-depleted soils, and soil phosphorus content for early blight development also needs further research. In an IPM perspective it is also important to consider differences in host plant resistance among available cultivars and for the future breed cultivars with improved resistance.

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Author Contribution All authors contributed to the study conception and design. Data collection and analysis were performed by LS. ÅL performed statistical analysis and EL helped with visual disease evaluation. The first draft of the manuscript was written by LS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The data generated during this study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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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

			rAUDPC				rAUC			
Treatment	Brand name	Active component	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.

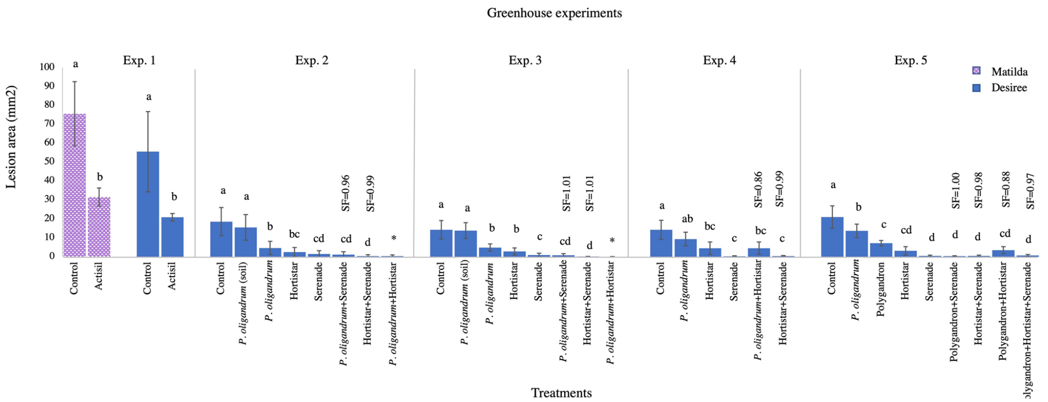

Fig. 1 Control of early blight disease of potato (cultivar Désirée and Matilda) caused by *Alternaria solani* using *Pythium oligandrum*, Polygandron®, Serenade®, Actisil® and HortiStar® in greenhouse experiments. Treatments were applied 48 h (Exp. 2–5) or 24 h (Exp. 1) before inoculation of plants with *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 ($0.96 \le SF \le 1.01$) 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

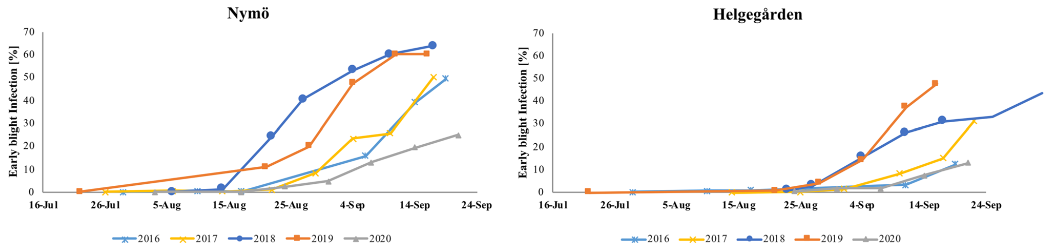

Fig. 2 Infection development disease progress curves for untreated plots during the different seasons and sites of the large trials

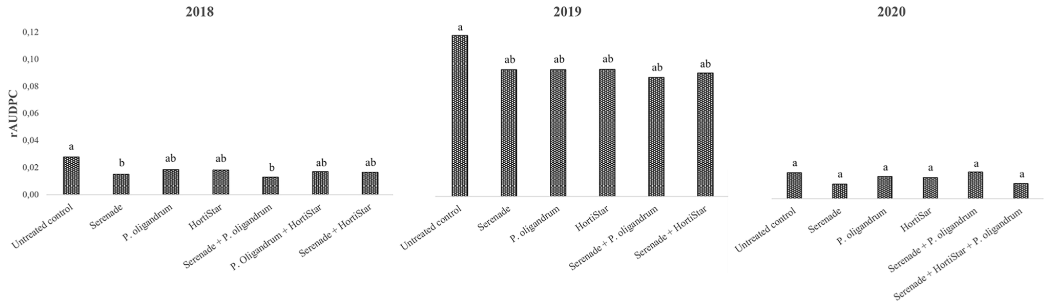

Fig. 3 rAUDPC values for the different years and treatments in the small trials

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

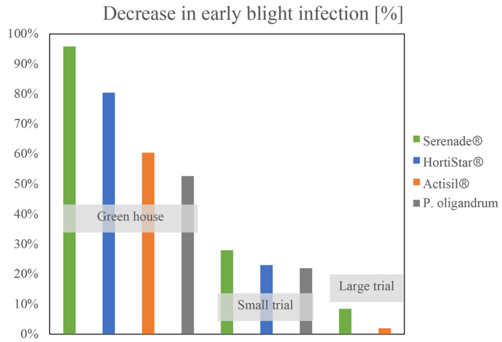

Fig. 4 Comparison between average reduction in early blight infection between the different trial setups for the four different agents. To compare the results from the different trial settings, the percentage of infection reduction from the treatments compared to control is used. For the greenhouse trial, 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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A more sustainable agriculture demands less pesticide usage. This is possible to accomplish by using Integrated Pest Management, IPM, to help control plant diseases. In this thesis, it is shown how early blight in potato can be battled with more knowledge and less fungicides.

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