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Master Thesis

The potential of alternatives to traditional fungicides in the control of late blight (*Phytophthora infestans*) in potatoes

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The potential of alternatives to traditional fungicides in the control of late blight (*Phytophthora infestans*) in potatoes

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Preface and Acknowledgement

My interest for the potato crop as well as the pathogen *Phytophthora infestans*, with the coupled late blight disease, have followed me through my entire education. Therefore, there was no doubt in my mind that my thesis should be about this certain area. The fact that late blight is still one of the most destructive diseases today is astonishing, and I believe that its future control, in a more environmentally friendly approach, is the next logical step.

I would like to thank my supervisor Isaac Kwesi Abuley for his tireless support and guidance in most aspects of the subject, which I would not have been without. I am grateful to my supervisor Jens Grønbech Hansen for his expertise in the use of BlightManager DSS, which have benefitted me greatly. I would like to thank my supervisor Sabine Ravnskov, for her expertise surrounding the technical aspect of the thesis, they were greatly appreciated.

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1 Abstract

The disease late blight (*Phytophthora infestans)* is a major problem in the modern potato production, which is one of the most important arable crops today. Causing substantial yield losses as well as the consequently economical strain due to the need for extensive control measures, here primarily relying on traditional fungicides. Nevertheless, due to the increasing focus on reducing the fungicide usage in Europe as well as the growing problems with fungicide resistance, the need for alternatives to traditional fungicides has never been more pressing.

Therefore, this thesis objective is to identify and optimize alternatives to traditional fungicides for the control of potato late blight. This study took three approaches, firstly a literature survey to identify the possible alternatives and their efficacy against late blight. Consequently, yielding a wide arrange of different alternatives to fungicides, with varying mode of action and efficacy, demonstrating the availability and potential of these compounds, but better alternatives need to be found and further studied.
Moreover, this was followed by an analysis and statistical processing of experimental results carried out under field conditions to determine the efficacy of alternative products as a standalone treatment (experiment 1) and in combination with fungicides or other alternatives (experiment 2). Lastly, the BlightManager DSS was used to develop simulation studies aiming to optimize the application of alternatives in cultivars with different levels of susceptibility as well as illustrating the potential for reducing fungicide dosage.

Conclusively, none of the tested alternatives to traditional fungicides, on their own, was able to suppress the late blight considerably. With the phosphite-based compound Resistim having the highest control efficacy and still being far less effective than traditional fungicides, suggest they are inferior replacements. However, better disease control was obtainable when the alternatives (Resistim and AgriCHOS) were combined with fungicides, indicating the best use of such product is by replacing some fungicide treatments with these alternatives, reducing the number of fungicides applications. Although based on the simulation it was demonstrated that the best efficacy of the alternatives and reduction in fungicide dosage is best achieved in combination with resistant cultivars, as well as the importance of timing the use of the alternatives correctly, under low infection pressure.

2 Resume

Kartoffelskimmel (*Phytophthora infestans*) er en alvorlig sygdom i den moderne kartoffelproduktion, som er en af de mest betydningsfulde afgrøder i dag. Kartoffelskimmel er skyld i betydelige udbyttetab samt økonomiske udgifter på grund af den omfattende skimmelbekæmpelse, der primært er bygget op om gentagne fungicid behandlinger. På grund af det stigende fokus på at reducere brugen af fungicider i Europa, samt det voksende problem med fungicidresistens, har behovet for alternativer til fungicider aldrig været mere relevant.

Derfor er målet med denne specialeopgave, at identificere og optimere alternativer til fungicider i bekæmpelsen af kartoffelskimmel. Dette studie har fulgt tre fremgangsmåder. For det første blev forskellig litteratur undersøgt for med henblik på at identificere potentielle alternative bekæmpelsesmidler og deres effekt overfor kartoffelskimmel. Denne litteratursøgning resulterede i en bred skare af forskellige alternative bekæmpelsesmidler med varierende virkningsmekanismer og effektivitet. Disse demonstrerede tilgængeligheden og potentialet af denne type bekæmpelsesmidler, samt tydeliggjorde manglen på mere effektive alternative bekæmpelsesmidler og nødvendigheden af yderligere forskning inden for området.
Dette var efterfulgt af statistiske analyser af forsøgsresultater fra markforsøg, som havde til formål at bestemme effektiviteten af alternative bekæmpelsesmidler, som er anvendt enkeltvis (eksperiment 1) i bekæmpelsen af kartoffelskimmel samt i kombination med fungicider (eksperiment 2). Afslutningsvis blev BlightManager DSS brugt til at konstruere simulationsstudier, som havde til formål at optimere tildelingen af alternative bekæmpelsesmidler til kartoffelsorter med forskellig grad af resistens, samt illustrere potentialet for at reducere mængden af fungicider.

Ingen af de testede alternative bekæmpelsesmidler, anvendt enkeltvis, var i stand til at bekæmpe kartoffelskimlen i særlig høj grad. Endvidere sås det, at det bedst præsterende alternative bekæmpelsesmiddel Resistim (phosphit baseret), fortsat var betydelig lavere i bekæmpelseseffekt af kartoffelskimmel end fungiciderne, hvilket indikerer, at de er utilstrækkelige som erstatninger til fungiciderne. Imidlertid kunne en bedre bekæmpelse opnås, når de alternative bekæmpelsesmidler (Resistim og AgriCHOS) blev brugt i kombination med fungiciderne, hvilket indicerer, at den bedste effekt af midlerne opnås som supplement til fungiciderne, hvor de i stedet kan reducere antallet af fungicidsprøjtninger samt det generelle fungicidforbrug.
Baseret på simulationerne viste det sig, at den bedste bekæmpelseseffekt af de alternative bekæmpelsesmidler og reducering i fungicidniveauet blev opnået i kombination med resistente kartoffelsorter (Nofy). Endvidere blev vigtigheden af korrekt timing af tildelingen af de alternative bekæmpelsesmidler under lave infektionstryk tydeliggjort.

3 Introduction

Potato late blight (*Phytophthora infestans*) is one of the biggest problems in potato production (*Solanum tuberosum),* resulting in severe yield reductions worldwide (Cooke et al., 2011) in worst cases up to a 100% yield reduction (Zhang et al., 2020). Furthermore, late blight control often requires repeating preventative control with fungicides, leading to a substantial amount of fungicide use (Haverkort et al., 2008). The management with fungicides and yield losses, summarize to over $6 billion in costs annually (Fry et al., 2015). In Denmark the potato have an export value at 2 Billion DKr annually, where 130 million DKr are used in the control of late blight and early blight (Hansen, 2020). With an increasing focus on reducing pesticide usage and with a rising preference for organic produce (Đurić et al., 2019), as is the case for the European union's Green Deal - Farm to fork strategy where one focus area are to reduce pesticides, with up to a 50% reduction of certain compounds (European Commission, 2020). In Denmark, projects like BlightManager strive to reduce the fungicide use in potatoes with up to 30% (Hansen, 2020). Furthermore, due to the fact that *P. infestans* are able to overcome multiple R-genes rapidly, complicating the use of host resistance (Cooke et al., 2011), as well as its recurring development of resistance against certain traditional fungicides (e.g. Fluazinam and Metalaxyl) (Hansen, 2018), results in increasing demand for alternatives to traditional fungicides in the control of late blight.

Alternatives to fungicides such as biological control agents (BCAs), Plant strengtheners and plant resistant inducers (PRIs) are considered an environmentally friendly control method for plant disease control (Köhl et al., 2019). However, there has not been much success on the use of alternatives to control late blight under field controls (Köhl et al., 2019), albeit their proven efficacy under controlled conditions (e.g. greenhouse)(Nechwatal and Zellner, 2015). Some of the problems with the use of alternatives include the poor persistence of alternatives to traditional fungicides, under field conditions compared to traditional fungicides (O'Brien, 2017). Therefore, it has become extremely relevant to identify and determine the efficacy of alternatives to traditional fungicides, as well as establishing how to best combine them with resistant cultivars and chemical fungicides to get the best late blight control in potato production, and the highest reduction in traditional fungicides.
In this project, the following are hypothesized:

1. Alternatives such as BCA's, plant strengtheners, PRI's and other, can in combination with resistant cultivars and chemical control agents offer an effective control of late blight in potatoes, compared to the standalone effect of the alternatives.
2. A simulation is an effective tool to determine the best control course of action in different scenarios as well as illustrating the potential for reducing the dosage of traditional fungicides.

The overall objective of this project is to identify and optimize alternatives to traditional fungicides for control against late blight. The specific objectives of this project are to (1) survey literature for alternatives to traditional fungicides for late blight control; (2) analyze disease and yield data from a test of different alternatives to fungicides; (3) determine the optimal application of alternatives to traditional fungicides as well as the potential for reducing the usage of traditional fungicides via simulation studies with the BlightManager simulation platform.

4 Literature survey

To fully understand the need for effective alternatives such as biological control agents (BCA), plant resistant inducer (PRI), plant strengtheners and resistant cultivars, as well as the advantages and disadvantages surrounding the use of such compounds, it is essential to briefly describe the motivators behind the need for alternative control measures. As well as the pathogen *P. infestans* origin, morphology, disease cycle, and symptoms. Furthermore, the alternative control options as well as the challenges which counteract an effective control are also surveyed in this chapter.

4.1 The potato crop

The potato crop *(Solanum tuberosum)* from the family Nightshade *(Solanaceae)* was introduced to Europe from South America around 1570 A.D (Agrios, 2005). Even with the lack of popularity (in Europe), at the beginning, it quickly grew popular and its cultivation spread rapidly. The crop became popular in Europe partly because of the lack of diseases affecting the potato, but also the higher yield of edible food per unit land, when compared to the cereals used at that time (Agrios, 2005). The potato crop consists of great diversity with an abundance of different cultivars which differs in properties and morphology (DeFauw et al., 2012). An example of potato diversity, especially color differentiation, is seen in picture 1.

Picture 1:Different South American potato varieties, from a food market in Arequipa, Peru (photo credit: Tobias Hove Jensen, 2019)

Today potato is the second most important arable crop in EU, with production at around 6 Mha, representing a value of €6 billion (Haverkort et al., 2008). Globally potato is the leading non-grain commodity and has a production of over 329 million tonnes from 18,6 Mha, in 2009 (DeFauw et al., 2012). And the positive properties, such as its adaptability, easy cultivation, nutritional properties, and high potential yield, have made it a crop in "growth", both in area and consumption wise (DeFauw et al., 2012).

In Denmark, the area with potatoes is 62.743 ha in 2020 compared to 53.589 ha in 2019 (Danmarks Statistik, 2020), which is a substantial increase. There are three common classifications of potatoes, manufacturing (e.g. potatoes for starch, chips and pommes frites), seed potato, and potato for consumption (Danmarks Statistik, 2020). The division with the biggest area in 2020 is the potatoes for manufacturing, primarily starch production, with 42.351 ha whereas the potatoes for consumption constitute 11.187 ha and the seed potatoes 9.205 ha (Danmarks Statistik, 2020). Potato is an important crop, especially when facing future challenges, such as the increasing human population and thus increasing demands for food (DeFauw et al., 2012). Furthermore, with FAO estimating the need for a 70% growth in global agricultural production, between 2005 to 2050 (DeFauw et al., 2012), potato production is only getting more relevant. Therefore, the estimated $6 billion in losses and management cost yearly, due to late blight (Fry et al., 2015), makes the control of *P. infestans,* in an economical and environmentally reliable way, the next logical step.

4.2 Phytophthora infestans

4.2.1 Origin and History

The pathogen *Phytophthora infestans* (meaning infectious plant destroyer (Agrios, 2005)), causing the disease late blight in both tomatoes and potatoes, are without doubt one of the most devastating plant pathogens worldwide, both in terms of yield and economical decline (Luis et al., 2007). As well as being one of the more recognized pathogens, known to be the culprit behind the Irish potato famine in the 19th century, resulting in the death of more than one million Irish people and further forced a large part of the population to migrate to North America (Luis et al., 2007). This happened around 1845 to 1847 when the first European epidemic of *P. infestans,* became a reality. Up until that time, the growing of potatoes had been without any major complications, until the growing season of 1845 when the weather became wetter and temperatures declined, for several weeks, favoring the distribution of the pathogen (Agrios, 2005). What started as a promising year with good growing conditions, became the opposite, with substantial disease spread from the European epidemic ground-zero in Belgium (Agrios, 2005). This is shown in fig 1, illustrating the spread of the potato late blight, from Belgium to the surrounding countries in only a few months, and in the middle of October, it was distributed as far as Scandinavia, Spain, Poland, and Ireland, from June to October 1845.

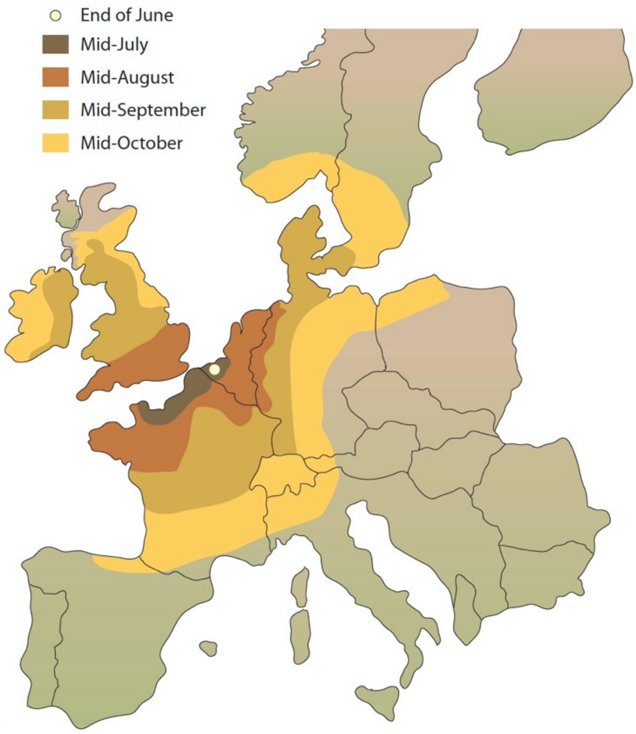


Figure 1: The spread of potato late blight in Europe in the summer and autumn of 1845. The colors from dark to light respectively, signifies the spread from July to October (Agrios, 2005).

With *Phytophthora infestans* present the symptoms followed, and especially in northern Europe the potato fields all became blighted, rotted, and died (Agrios, 2005). With the limited resources and control options available for the farmers, led to substantial yield reductions, further worsened when the few harvested tubers with no visible symptoms, later rotted as well, later resulting in the beforementioned potato famine (Agrios, 2005). Even though it was, at that time, believed by the farmers to be the work of the devil, Dr. Miles Berkeley hypothesized that the course of late blight was some kind of "fungi" *(Oomycetes)*. This was later confirmed by Anton deBary, who inoculated several potato tubers with spores obtained from late blight, and the later symptomatic reaction seen on the potato plant, compared to healthy non-inoculated control, confirmed the theory (Agrios, 2005).

The Origin of the pathogen has been widely discussed, with two main theories, respectively the Andean and Mexican theory, depending on an origin in the Andean mountains or the highlands of Mexico (Andrivon, 1996). Convincing evidence, such as the low number of clonal lineages of *P. infestans* seen in the Andes compared to the higher genetic diversity seen in the Mexican highlands, favors the latter of the two theories (Fry and Goodwin, 1997a). Though proposed by Luis et al. (2007), *P. infestans* are most likely not from central Mexico (based on their data) but have a South American origin. Hence the unclarity surrounding the origin of the pathogen. Most likely, it later spread from its origin by the transport of infected tubers to North America and over the Atlantic to Europe, sometime after the introduction and distribution of the potato (Fry and Goodwin, 1997a).

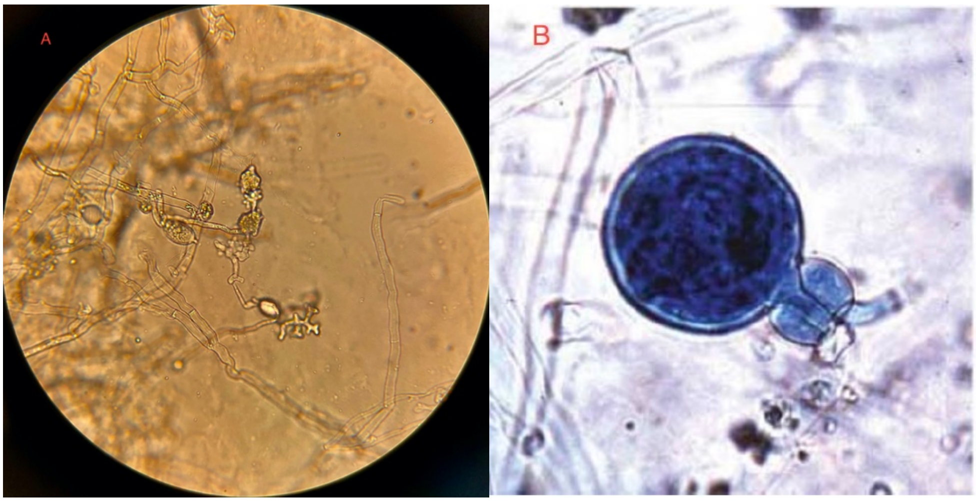
In newer times, one of the more significant changes in the development and distribution of *P. infestans* was the introduction of the A2 mating type (Fry and Goodwin, 1997a). Before 1980, only the A1 mating type was outside of Mexico, and the A2 mating type was more or less non-existing elsewhere (Fry and Goodwin, 1997a). After the distribution of the A2 mating type outside Mexico, it has opened up for the possibility of sexual reproduction between the two mating types of *P. infestans (Fry and Goodwin, 1997b),* thus creating a shift from a low to high genetic diversity in areas with both mating types (Luis et al., 2007), significantly complicating the control.

4.2.2 Taxonomy and Morphology

Phytophthora infestans an oomycete, which is heterotrophic organisms earlier regarded as part of the fungi kingdom because of them being "fungus-like" in their similarities (Fry and Goodwin, 1997a). Instead, they are part of the kingdom of stramenophila belonging to the phylum Oomycota together with approximately 700 other species (Susan and Ray, 2013). The group differentiates themselves from "true fungi" by its diploid vegetative cells and the lack of chitin in their cell walls (Fry and Goodwin, 1997a), and like many other stramenopiles, are instead composed of cellulose or polymers resembling cellulose (Susan and Ray, 2013).
Most of the species can reproduce both sexually and asexually and the group is further characterized by its asexual zoospores with flagella where one is whiplash and one tinsel (heterokont) (Fry and Goodwin, 1997a). As well as its oogamous sexual reproduction where the female gamete is large (no flagella), in contrast with the smaller male gamete with flagella (Susan and Ray, 2013). Here the oospores, a thick-walled zygote, are a product of the fertilization of the eggs produced in the antheridium (male) and the oogonium (female) (Susan and Ray, 2013).

A substantial number of the phylum is the water molds, which are aquatic Oomycota, including species of *Achlya* and *Saprolegnia*, and another group is terrestrial oomycetes, containing important plant pathogens (Susan and Ray, 2013). Here the most important plant pathogens belong to the order *Peronosporales* and *Saprolegniales* with the genus *Aphanomyces* (Saprolegniales) causing root rot disease in e.g. sugar beets. Belonging to the Peronosporales, two distinguished genera, are the *Pythium* and *Phytophthora*, both including species of potent plant pathogens (Agrios, 2005). Of the approximately 35 species belonging to *Phytophthora*, the most infamous is the species *P. infestans*, the pathogen behind potato late blight disease (Susan and Ray, 2013).

Phytophthora infestans develops by the beforementioned sexual (oospores) and asexual (mycelium, sporangia, and zoospores) reproduction seen in the oomycetes (Andrivon, 1995). After a period with a vegetative growth of the hyphae, sporangiophore develops, later producing lemon-shaped sporangia containing motile zoospores (Judelson and Blanco, 2005). Refer to picture 2, where A shows an example of *P. infestans* hyphae, sporangiophore, and sporangia and B shows an example of an oospore.



Picture 2: A) Different sporangiophores with sporangia (asexual organs) growing (photo credit: Tobias Hove Jensen, 2019) B) A thick-walled resilient oospore (sexual organ) (Judelson and Blanco, 2005).

Asexual reproduction can germinate using two pathways, depending on different factors. At temperatures higher than 14 degrees Celsius, the sporangia can germinate directly by producing a germtube, infiltrating the host through a wound or stomata or by the production of appressoria (Judelson and Blanco, 2005). At colder temperatures the germination will often happen indirect (zoosporogenesis) (Judelson and Blanco, 2005), by releasing around 6 motile zoospores that, with their flagella, moves through water, before germinating by the means of a germ tube (Agrios, 2005).

Because the pathogen is heterothallic, when mating type A1 and A2 of *P. infestans* are both present, the female hyphae (one mating type) can grow through the male antheridium (another mating type) creating a globose oogonium above the male reproductive organ (Judelson and Blanco, 2005). Then the antheridium will fertilize the oogonium thus producing an oospore (picture 2 B). Under the right conditions, the oospore will germinate by germ tube, creating a sporangium (Agrios, 2005).

4.2.3 Disease cycle

To further understand how the polycyclic *P. infestans* spread and infect the potato crop, in practice, a review of its disease cycle is essential. Therefore, refer to figure 2, illustrating the complete disease cycle of *P. infestans*. The review of figure 2, will be in three parts, depending on the primary inoculum and the time of infection in the growing season.

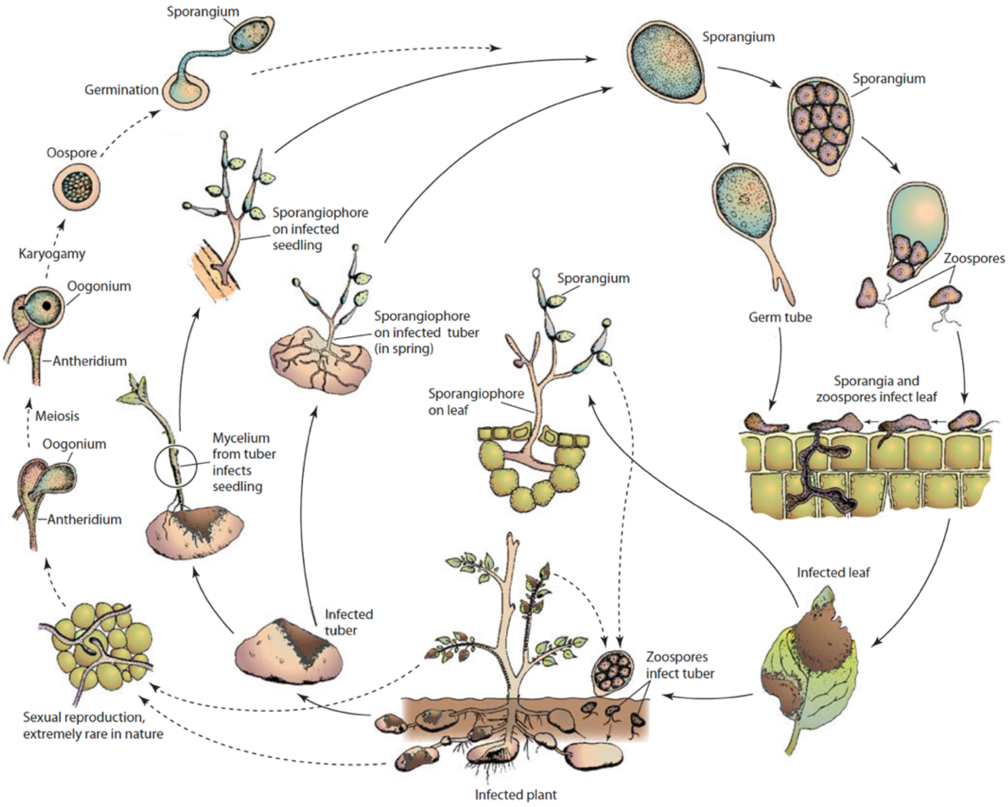


Figure 2: The complete disease cycle of Phytophthora infestans, showing its different ways of infecting the host plant, either by zoospores/sporangium infecting leaves or zoospores infecting tubers or by oospores (Agrios, 2005).

The first part in focus is the "foliar infection" by the asexual reproduced zoospores and sporangia that are infecting the host leaves (by wind etc.), under the right temperature and humidity (figure 2). Afterwards, the inoculum germinates and penetrates the leaves directly via the stomata or by means of a appressorium formation., the mycelium will spread through the plant cells creating haustorium's inside the cells, that functions as a feeding organ (Judelson and Blanco, 2005). Thereby slowly killing the foliar biomass, and later new sporangiophores with sporangia, will germinate from the stomata and spread the spores to new hosts and leaves. Thereby being the primary form of infection and spread, throughout the summer (Fry et al., 2015).

The next essential part of the disease cycle (figure 2), is the "tuber infection", which is also by asexual reproduction. Here the zoospores of the sporangia can drop from the infected leaves, and if the soil is wet enough, the zoospores can either lay dormant in the topsoil/or on top of the soil, infecting the tubers if they come in contact during harvest or by rain, infiltrating through cracks in the soil infecting the tubers this way (Judelson and Blanco, 2005). The pathogen can then survive through the winter as mycelia in the infected tubers (or plant material), and during the next growing season, it will become the primary inoculum in the spring (the early part of the growing season) (Fry et al., 2015). Moreover, it either spreads by creating a sporangiophore directly on the tuber, or by migrating with the germinating potato sprout (endophytic) and later create a sporangiophore on the potato stems (figure 2). Thereby, spreading the sporangia and zoospores, starting the “foliar infection” step of the disease cycle, anew (figure 2).

The final part of the disease cycle (figure 2), is the “soil infection”, which is the sexual reproduction and creation of the oospores (Fry et al., 2015). Here, when both mating types are present on either the leaves or the tubers, the oospores can be produced (as described earlier). Therefore, this part is happening later in the growing season, when the crops defenses are weakened and both mating types have had time to “arrive” and interact. The oospore can overwinter in the soil (at least up to four years (Agrios, 2005), and when triggered by e.g. potato root exudates, sporangia will germinate from the oospore and function as one of the primary inoculums at the start of the growing season (spring) (Judelson and Blanco, 2005). Often infecting the germinating potato sprout, newly protruded from the soil, or later the leaves touching the soil, (Cooke et al., 2011) hence the term “soil infection”. This contributes to the spreading of zoospores, thus connecting to the “foliar disease” part of the disease cycle (figure 2).

4.2.4 Symptoms

The symptoms are primarily seen on the leaves (especially during “foliar infection”), but also on the stems (Ghita Cordsen and Jørgen, 2011). In the beginning, the symptoms are present as light-brown necrotic lesions as seen in picture 3 B, and will after a short period become dark-brown as seen in picture 3 A (Ghita Cordsen and Jørgen, 2011). Furthermore, on the underside of the infected potato leaf (picture 3 A), sporulation (the development and spread of spores) can be seen as a white layer between the infected and healthy plant material, which is the sporangia spreading (Ghita Cordsen and Jørgen, 2011).

Picture 3: A) Late blight symptoms with active sporulation on the underside of an infected potato leaf (photo credit: Tobias Hove Jensen, 2020) B) Early, late blight symptoms, on a potato stem and leaf (photo credit: Tobias Hove Jensen, 2016) C) Tuber infected with late blight (photo credit: Tobias Hove Jensen, 2020)

As mentioned earlier, the potato tubers can also be infected, and the symptoms present themselves both on the surface of the tubers and the inside. On the outside, the symptoms can be identified by their irregular red-brownish spot on the skin, and inside the tuber, it is dark necrotic lesions, as seen in picture 3 C, easily distinguishable from the healthy tissue. These symptoms often develop during the storage of the tubers (Ghita Cordsen and Jørgen, 2011).

4.2.5 Environment and Genetic Diversity

It is important to briefly mention other factors surrounding *P. infestans* and its late blight disease, which can have a substantial effect on the pathogen's virulence, aggression, and spread, complicating its control.

Environment

One factor, not mentioned in the disease cycle (figure 2), is the importance of temperature, humidity, and leaf wetness in the development and spread of *P. infestans*. Depending on the stage of the disease cycle, the sporulation (as seen in picture 3: A) is greatest with temperatures ranging from 15 to 22 degrees Celsius and at RH (relative humidity) nearing 100% (Fry et al., 2015). At higher temperatures, over 30 degrees Celsius, the sporulation will come to a halt and resume when conditions become more favorable again (Agrios, 2005). This temperature/humidity dependent life cycle of the pathogen also allows for the use of forecasting models, that based on e.g. weather forecasting and geography can suggest when and with what dosage to control late blight (Narouei-Khandan et al., 2020).

Genetic diversity

After the introduction of the, before mentioned A2 mating type, some of the developments have been the pathogens option to survive in the soil for a longer duration of time, as an oospore (Judelson and Blanco, 2005). Moreover, the sexual reproduction and thus the genetic recombination between different mating types with different genotypes, have resulted in a much higher genetic diversity and therefore also a bigger assortment of more virulent and aggressive *P. infestans* populations (Hansen, 2018). An excellent example of a genotypic specific advantage is the *P. infestans* genotype called blue-13 (EU_13_A2), that for years was a dominant genotype in Europe, and in 2018 it was still the most frequent genotype found when sampling in 10 countries, with 25% of all samples being blue-13 (Hansen, 2018). A big part of this isolates success, is because of its reduced sensitivity to the active compound metalaxyl, used in some fungicides in Europe, thereby selecting for blue-13, allowing it to spread (Hansen, 2018).

4.3 Traditional Late Blight Control

Before going further with the alternative control options, some of the more traditional and frequently used methods will be in focus. This includes the five most effective areas of control as stated by Cooke et al. (2011), control of the primary inoculum sources, use of resistant cultivars, the use of fungicides, and decision support systems, and the prevention of tuber blight. Some of these would be ideal, in combination with some of the alternatives, for example, one of the more important control measures, the use of resistant cultivars.

4.3.1 Primary inoculum

Some of the more basic sanitary measures are the use of certified seed material or *P. infestans* infection-free, which will reduce the primary inoculum at the start of the growing season (Cooke et al., 2011). And as stated by Cooke et al. (2011), up to 39% of the early infection, can be due to infected seed tubers. Another preventative measure is the removal of any potential inoculum that could help the pathogen in overwintering, here, both potato regrowth's/ leftover tubers in the field, potato dumps, or in general any potato plant material (Ghita Cordsen and Jørgen, 2011).

Also, make sure to remove infected plant material before harvesting the potatoes, to prevent any contact between the pathogen and the tubers (Ghita Cordsen and Jørgen, 2011).

Moreover, to reduce both the leftover tubers and the oospore, good crop rotation with at least 3 potato-free years (preferable more), is needed to reduce some of the oospores (Cooke et al., 2011). This is important, because of the oospore's survivability, and their potential function as primary inoculum in the spring (up to 18% infection from oospores) (Cooke et al., 2011). These measures are some of the only effective control measures available for organic potato farmers, but should, in general, be the "baseline" for any potato production, to reduce the need for other treatments, especially fungicides (Cooke et al., 2011). Also, these preventative measures could help reduce the disease pressure, making the alternative control options, more viable.

4.3.2 Resistant cultivars

Earlier the breeding of late blight resistant cultivars was based on the 11 R genes, derived from the Mexican wild species *Solanum demissum (Elzbieta Zofia, 2012)*. In Europe, especially R1 - R4 and R10 have been used, but because of *P. infestans* rapid adaptability to host resistance and the emergence of more virulent populations*,* they have been overcome (Elzbieta Zofia, 2012). Later, other R genes have been found and incorporated into the breeding programs and cultivars with race-nonspecific resistance (horizontal) have been breed (Cooke et al., 2011).

The R genes can work in different ways. As described in the gene for gene model it works by reacting to the effectors, released by *P. infestans* when it infects the host and penetrates the cells. Here the hosts R genes will react to the corresponding avirulence (Avr) effectors, when the R protein recognizes them, a reaction is triggered often in the form of hypersensitive response (HR) (Elzbieta Zofia, 2012). This is usually followed by a systemic acquired resistance (SAR) (horizontal), which is an enhanced defense both systemically and at the infection-site in the host, by increasing the expression of some genes connected to photogenesis (Elzbieta Zofia, 2012). The SAR delivers a long-lasting resistance, with effect against most of the *P. infestans* genotypes. One option to prolong the host resistance, is by incorporating different R genes into each cultivar, thereby increasing the mutations needed to break the avirulence genes (Elzbieta Zofia, 2012).

First and foremost, in the control of late blight, one should strive to only use the most resistant cultivars, because of their ability to reduce the development and onset of the disease (Cooke et al., 2011). This presents some problems, because the availability of commercial cultivars with strong resistance, are limited (Agrios, 2005). Also, in western Europe, many of the used and imported cultivars have prioritized traits, such as earliness, quality, and yield, which is often not combined with resistance against late blight (Cooke et al., 2011).

Furthermore, the beforementioned genetic diversity seen in the *P. infestans* populations and their polycyclic nature, makes some of the resistant genes (R-genes) in the potato cultivars, less likely to work on all the different isolates (Fry et al., 2015). While also being susceptible to having their resistance broken. Therefore, necessitating the use of fungicides (or potentially, biological alternatives) to supplement the resistant cultivars, to prolong the resistance (Agrios, 2005). So, using a potato cultivar with partial resistance in combination with e.g. fungicides or alternatives can also be effective, by reducing the fungicide doses and application frequency (Hansen et al., 2019).

4.3.3 Tuber blight

As clarified in the disease cycle (figure 2), the tuber infection is one of the main sources for the late blight infection, and therefore it is very important to reduce this primary inoculum. When sporulating the zoospores or sporangia, can be swept from the leaves, down the stems, and into the soil, where it can survive for up to 2 months, and have plenty of opportunities to infect the tubers, especially tubers near the soil surface (Cooke et al., 2011).

To prevent any infection (if the risk is high) at the end of the growing
season, it can be useful to destroy the haulm completely, e.g. by chemical or burning (if organic) destruction (Cooke et al., 2011). Furthermore, tuber distribution can also be cultivar specific, with some placing tubers in the topsoil, or are prone to cluster the tubers together, etc. (Cooke et al., 2011). This makes it worthwhile choosing the right cultivar.
Another factor worth mentioning is the soil type. Here it is important to grow the crop on soils with sufficient drainage, and if possible, not to irrigate if the disease pressure is high in the field, because it promotes sporulation (Cooke et al., 2011).

4.3.4 Fungicides

Late blight is often controlled with fungicides such as, Ranman Top (Cyazofamid), Revus (Mandipropamid) and Shirlan (Fluazinam) (SEGES, 2020). With the increasing focus on the negative aspects of using pesticides, the potential of fungicide reduction, are in focus (European Commission, 2020). Also, the environmental effects can be significant, with the cost at around 25 GJ in energy, to produce 1 ha of potato where the spraying constitutes around 9% of this (Haverkort et al., 2008). With approximate 155 MJ per spray (Diesel and machine maintenance etc.) and 2.25 GJ when spraying 15 times (Haverkort et al., 2008). Moreover, as mentioned earlier, together with the immigration of the A2 mating type, and thus the increased genetic diversity in the *P. infestans* populations, and the excessive use of fungicides, have selected for fungicide resistant population (Doležal et al., 2017) as with the Blue-13 and metalaxyl, or the more recent

EU_37 with resistance against fluazinam (Hansen, 2018). Thereby, complicating control with fungicides.

There are different fungicides available, against late blight, with a different mode of action (MOA), working curative, protectant, or eradicant (Cooke et al., 2011). Another important element is the effect of the fungicides on stem blight, leaf blight, and tuber blight, which can vary,(Cooke et al., 2011) as well as the compound's mobility and rain fastness (SEGES, 2020). All factors should be taken into consideration when controlling late blight with fungicides.

Firstly, when controlling late blight, it is essential to be aware of the best application frequency and timing. This is depending on parameters, such as the potential foliar resistance in the cultivar, and when this resistance will "break" (Hansen et al., 2019). Also, the type of fungicide and crop growth rate can affect what the best application strategy , this is also the case, with weather, irrigation, and disease pressure, where the beforementioned forecasting models, can help decide the specifics around the application (Cooke et al., 2011). So, e.g. if the cultivar has late blight resistance, for a duration of the growth-season, then fungicide application should be absent or used in a lower dose, in periods with no risk, and prioritized when the risk Is greater (Hansen et al., 2019).
When that is said, it is often the normal practice (in Nordic countries), to rather be safe than sorry, and apply fungicides in the first period with late blight favorable conditions, after the crop has emerged, and then continue applying protectant fungicides, at a 5 to 7 days interval, when infectious periods occur (Cooke et al., 2011). Otherwise, a used strategy, is a longer interval (between 5 to 14 days) between sprays, where systemic or translaminar fungicides are used with contact fungicides, in collaboration with DSS's (Cooke et al., 2011). Some active compounds with different MOA can vary in effect based on application time. E.g. metalaxyl (better alternatives today) or cymoxanil, are effective in the early growth stages, with rapid growth, where the primary inoculum is from the tuber and oospore infection (Cooke et al., 2011). Contrasting, cyazofamid and fluazinam, which works best in the later part of the disease cycle, where these compounds can be applied against tuber blight (Cooke et al., 2011).

An option to diminish the possibility of fungicide resistance in the pathogen could be by using low-resistance-risk fungicides, with high-resistance-risk populations, and vice versa (Doležal et al., 2017). By mixing/or changing between fungicides with a different mode of action, like single-site and multi-site inhibitors (Doležal et al., 2017). Also, relevant to the thesis topic, would be the reduction of the fungicide dose, by supplementing with "alternatives".

4.3.5 Decision Support Systems

This type of tool (computerized system), used in late blight control is meant to incorporate information like weather (e.g. precipitation and humidity), life cycle, host resistance, disease pressure, and plant growth, etc. to help make the best decision, in controlling late blight (Cooke et al., 2011). Different models have been used in this regard, most common are the forecasting models like BLITECAST, that model works by accumulating the late blight risk units based on the daily temperature and hourly humidity data, assessing the potential late blight risk. The DSS/models can be used to regulate the application of fungicides to reduce the amount of fungicides, e.g. with reduction between 8 to 62%, compared to the regular treatments (Cooke et al., 2011), making it a valid supplement in the management of late blight.

The DSS, most used in Denmark, is called Skimmelstyring (Skimmelstyring, 2020) (Late blight control). Which uses data such as temperature, RH, and also geographical data from a disease surveillance network, with locations of late blight infection cases, from all over the country (Hansen et al., 2019). A further developed DSS of Skimmelstyring, BlightManager, will be used later to simulate different scenarios. An example of the output of the Skimmelstyring DSS forecasting model can be seen in figure 3. The simulation is based on July 2020, in the area around the city Grindsted, the text "Nedbør" on the right is translated to "Precipitation [mm]" and the vertical text on the left is translated to "Infection pressure [Risk Hours].

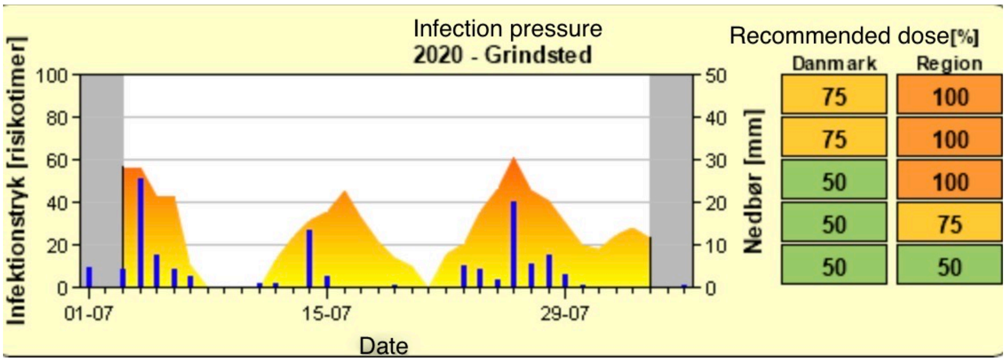


Figure 3: Displays the model's illustration of the infection pressure, where it is based on the weather data (precipitation, etc.) and the accumulated hours of late blight risk, estimates the infection pressure. This is seen as the blue bars (precipitation) and the corresponding yellow-red spectrum (infection pressure). Based on the severity of the infection pressure, and if late blight is present in the same region of the user, or just in the country, a fungicide dose is recommended. Respectively, 50%, 75%, or 100% of a full dose. (Skimmelstyring, 2020).

Figure 3, shows a relative low infection pressure in Grindsted in the chosen time interval, with the infection pressure reaching just above a 60% infection pressure in late July.

4.4 Alternatives to traditional fungicides

This chapter focus on the general aspect of the alternatives, such as the MOA and the classifications of the alternatives, as well as the challenges they present. Followed by the introduction and analysis of selected alternatives to traditional fungicides (active compound), from each "group", with corresponding examples of products, in use. In this thesis, the alternatives have been divided into the following four groups, biological control agents (BCA's), plant resistant inducers (PRI's), plant strengtheners/biostimulants, and "other" alternatives.

4.4.1 Classification

Alternatives to traditional fungicides is a wide term, with many different classifications and differentiations, that can vary between countries and research institutions. In this thesis, a part of the focus area will be on biological control, which is often the case with BCA's and partly PRI's and Biostimulants (Alabouvette et al., 2006). These are often biological compounds or organisms (but not exclusively), whereas the "other" alternatives can be nutrients or other inorganic compounds, or a mixture (Borza et al., 2017). Though, this is not always the case, because PRI's are not necessarily organisms (Alabouvette et al., 2006), therefore the four groups in this thesis (BCA's, PRI's, Plant strengtheners and others), are primarily classified based on their primary MOA and how they affect the pathogen. Therefore, this classification is not necessarily the correct differentiation of the compounds, and are partly a construct of the author's opinion.

4.4.2 Biological control of plant diseases - Definition

Biological control can be defined as the using of organism (not man), to reduce disease producing activity or inoculum of a pathogen (Alabouvette et al., 2006). This can be done with a wide range of different MOA, such as antagonism and induced resistance etc. and not only from microorganisms, but also natural products from plants, or secondary metabolites etc. that can acts as elicitors for plant stimulating (Alabouvette et al., 2006).

4.4.3 Inorganic control agents - Definition

Inorganic control agents are compounds such as minerals with no carbon structure (Borza et al., 2017). These types of chemical alternatives, have been used for many years, and have often been copper-based. But due to negative effects, such as potential accumulation, this practice has been banned in many places (Patinha et al., 2018). This is also the case with Phi-based (phosphite-based) fungicides, which can have an effect on the pathogen but can accumulate in the crop (Borza et al., 2017).

4.4.4 Classification - Direct MOA - Biological control agents (BCA)

The focus will mainly, be on the microbial biological control agents (MBCA). Furthermore, as mentioned earlier, many compounds with different MOA can be classified as BCA or MBCA, also e.g. organisms whose main focus is to induce resistance in the plant (Köhl et al., 2019). These types will not be included in BCA's but PRI's (so based on their MOA), and this section will be about organic compounds or antagonistic organisms, that in some form or another, directly interact and control the pathogen.

In the control of plant diseases, the BCA's are usually, either bacterial, fungal, or viral strains (mycovirus and bacteriophages), which is applied to the soil or the plant, depending on the host-pathogen (O'Brien, 2017).

Examples of the more direct control of the pathogen, which is the primary identifier for this classification, could be hyperparasitism and different forms of antibiosis (Köhl et al., 2019). Hyperparasitism is the parasitism between two parasites, where one of the organism's gains nutrients from the other organism, this could be the interaction between a BCA, and the plant pathogen, where the latter parasitizes on the other (Köhl et al., 2019). Hyperparasitism happens primarily between fungi, and is uncommon between bacteria, though predatory bacteria that feed on cytoplasm from other bacteria do exists (Köhl et al., 2019). The primary mechanism of the hyperparasites is to release cell wall degrading enzymes (CWDE) and at times together with secondary metabolites (O'Brien, 2017). These will degrade the cell wall (and other fungal organs), creating an opening to predate, using CWDE such as chitinases, proteases, glucanases, and in the hyperparasitism of oomycetes, cellulases (O'Brien, 2017).
One hyperparasitism strategy is the biotrophic mycoparasitism's (parasitism on fungi), that obtain nutrients from fungi cells, by using haustorium, which continues taking nutrients while keeping the host alive (Köhl et al., 2019). Here, a hyperparasite with a necrotrophic lifestyle is a better for commercial mass production, due to it using an effective strategy, where it attacks and kills the pathogen's spores and hyphae, before obtaining the nutrients (Köhl et al., 2019).

Another type of direct interaction with the pathogen, are antibiosis by antimicrobial metabolites. Here, there is an antagonistic interaction between the metabolic substances from the BCA organism, and the pathogen (Köhl et al., 2019). E.g. a wide array of fungi and bacteria produces antibiotics, giving them a competitive advantage, that will directly eliminate the competing organism or plant pathogen (O'Brien, 2017). Broad-spectrum antimicrobial metabolites (e.g. lipopeptides and pyrrolnitrin), proven suitable for biocontrol, can be found in bacteria such as *Pseudomonas, Bacillus, Streptomyces,* and *Agrobacterium*, to name a few (Köhl et al., 2019). Some fungal antagonist, that produces certain metabolites, are *Clonostachys* and *Trichoderma*, which e.g. can produce gliotoxin and gliovirin (Köhl et al., 2019).

4.4.5 Classification - Plant resistant inducing MOA - Plant resistance inducers (PRI)

This section is classified as PRI's and will focus on both chemical compounds, plant extracts, and organisms. Which mode of action, is to indirectly control the pathogen, by inducing resistance in the plant or priming it, thereby using the plants own defense mechanisms (Alexandersson et al., 2016).

PRI's are compounds that can improve the plants' protection against pathogens, such as fungi, bacteria, viruses, and oomycetes, by inducing its own defense mechanisms (Alexandersson et al., 2016). They are also known as plant resistant/defense activators (Alexandersson et al., 2016). The induction of the host defense mechanism happens when elicitors such as, volatiles, antibiotics, and proteins, are released by the PRI, which induces the expression of genes in different pathways (O'Brien, 2017). The defense system can also be activated by the plants' recognition of pathogen-associated molecular patterns (PAMPs) (M. Sandroni, 2020). The induced resistance can be local and systemic and can be further divided into two groups, which are different in the signaling pathway, respectively the system acquired resistance (SAR) and induced systemic resistance (ISR) (M. Sandroni, 2020).
SAR is induced by the salicylic acid pathway and happens when pathogens directly attack the host in distal parts of the plant (M. Sandroni, 2020). The ISR is induced by the jasmonic acid or ethylene pathway, where the whole plant defense mechanism is enhanced against multiple pathogens (M. Sandroni, 2020). They are often induced by beneficial organisms (Köhl et al., 2019) e.g. Rhizobia and mycorrhiza colonizing the roots (Alexandersson et al., 2016).

The plant can react differently to PRI's stimulation, for example, by altering its cell walls, as well as producing antimicrobial proteins or by reacting with hypersensitive response (rapid cell death, around the infection site) (Alexandersson et al., 2016). Inducing of the plant's defense mechanisms can result in a fitness penalty, with varying severity, depending on the type of PRI and the environment in which it is used (Alexandersson et al., 2016). Both the SAR and ISR state will slowly fade when the stimulation/inducing is no longer happening (Köhl et al., 2019). Contrasting this is the term priming, which is used to describe a special case of induced resistance, happening after the application of PRI's. Here, the plant will react more rapidly to an attack or stress, because it is in a prolonged induced state, even after the stimulus has disappeared (Alexandersson et al., 2016). Conclusively, these harmless stimuli by the PRI's prepare and increases the plants' natural defense mechanism, for when the pathogen infects.

4.4.6 Classification - Plant promoting MOA - Plant strengtheners/biostimulants

The plant strengtheners/biostimulants (metabolic enhancers, positive plant growth regulators and biofertilizers, etc.) classification, are in this thesis, organisms, or compounds (partly organic) which promotes plant growth or plant productivity, thereby indirectly controlling the pathogen (Yakhin et al., 2017). This can be microorganisms, algae, plant extract from higher plants (exudates, seeds, and leaves, etc.) and amino acids as well as protein hydrolysates from animals (Yakhin et al., 2017). This classification tends to overlap with fertilizers, with some compounds being partly composed of certain nutrients as well as having a non-definable/broad MOA, with somewhat the same effect.

More commonly, biostimulants are used to regulate and modulate the physiological processes in the plants, thereby promoting growth, as well as reducing stress factors, that will limit the plant and the production of yield (Yakhin et al., 2017). This can be by MOA that affects plants' antioxidant and hormonal systems, thereby increasing the plant's general competition and the chance of resisting the pathogen (Yakhin et al., 2017).

An example of a biostimulant organism are bacterial or fungal endophytes (e.g. rhizobia) (White et al., 2019). That are often seed transmitted, and can from the start of germination promote plant health by increasing nutrient uptake, defending the host against pathogens due to increased growth, and increasing stress tolerance, thereby promoting the plant's general growth (White et al., 2019). Here, one nutrient enhancing effects by some microbes can be that they are equipped with high-affinity transporters, that enables them to find and take up the metals (Fe, Zn, and Mg, etc.) that are released by the organic acids exudated from the plant roots (White et al., 2019). An example of stress decreasing by some endophytes is their ability to reduces the host reactive oxygen species (ROS), produced by the plant when under stress, and can do oxidative damage to plant - nucleic acid, membranes, and proteins (White et al., 2019).

4.4.7 Classification - Other alternatives

This classification is more diverse than the other groups. Primarily it is reserved inorganic compounds with a dominantly direct MOA, and nutritional value (like Cu-based products) (Borza et al., 2017), though not exclusively. It can also be salts (assembly of anions and cations), which can both be organic or inorganic (Borza et al., 2017), or completely organic compounds, like e.g. compost (Al-Mughrabi, 2006). So, the classification here is also based on the "alternative nature" of the product/compound, which does not easily fit under the other categories. Therefore, due to the indefinable nature of this group, it will only be briefly introduced.

An example of this type of classification is the Phosphite-based product, which is also a type of fertilizer. They are composed of e.g. H_3PO_3, which is an anion/salt of phosphorous acid, that have shown an effect against oomycetes, making them a possibility in the control of potato late blight (Borza et al., 2017). Phi based products work by systemically inhibiting the development of e.g. late blight, both directly and indirectly MOA, by having a toxic effect on the pathogen at high enough doses (Borza et al., 2017). Other MOA by Phi based compounds, can be inhibition of phosphorylation, disrupting fungal metabolism and growth as well as stimulating tolerance against abiotic factors and inducing resistance, in the plant (Achary et al., 2017).

4.5 Examples of alternatives for late blight control

Five specific active compounds, from each classification group, have been chosen to be presented and further analyzed. Some of the active compounds/products have different MOA, across the classifications. Therefore, they will be assigned a group based on the "primary" MOA (most dominant trait) or how the active compound has been marketed as before. Some of the compounds presented in this section of the thesis are from trials with tomatoes. This is due to the lack of trials with potatoes. However, due to the closeness of tomato and potato, these trials with tomato will give a good indication of the usability of these compounds in potato. Furthermore, some of the analyzed compounds are based on only 1 article, due to the lack of other available peer-review literature.

4.6 Examples - Biological control agents (BCA)

4.6.1 Bacillus amyloliquefaciens /Serifel

Different strains of *Bacillus amyloquefaciens* have been shown to have antagonistic properties, capable of creating volatile compounds with controlling effect on certain plant pathogens (Yuan et al., 2012). One of the strains is *B. amyloliquefaciens* MBI600, which is the active compound in the commercial product Serifel. The MOA of this strain is the production of secondary antimicrobic metabolites, general competition as well as inducing the ISR and SAR defense mechanisms of the plant (Dimopoulou et al., 2019).

To evaluate the specific efficacy of *B. amyloliquefaciens* against late blight, literature, and trials by Caulier et al. (2018), are in focus. Here different strains of *Bacillus spp.* (and *Pseudomonas spp.)*, including *B. amyloliquefaciens,* will be examined to determine their potential as BCA's. Both an *in vitro* trial was made, to assess the effect of the antifungal metabolites, then the most promising strains, in inhibiting *P. infestans,* were selected for further in planta potato (Bintje) trials under greenhouse conditions and a field trial, by Caulier et al. (2018). To see the results from the greenhouse assay, refer to figure 4, illustrating the protection index (%) of *Bacillus spp.* and *Pseudomonas spp.*

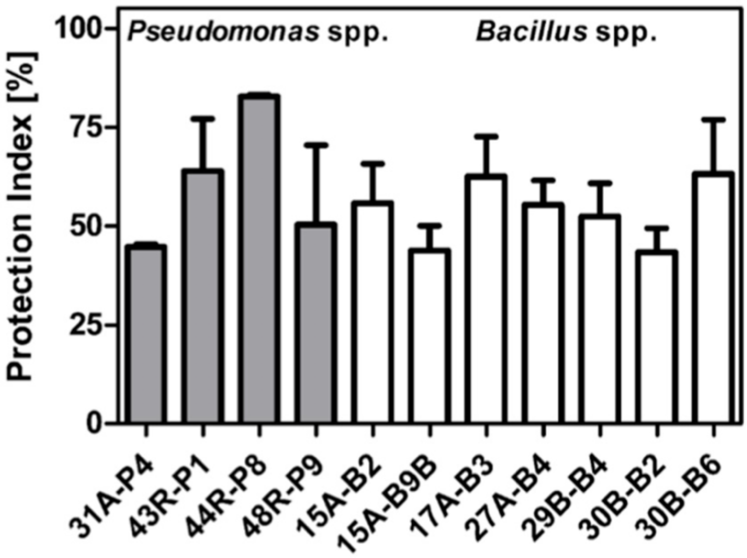


Figure 4: Mean of normalized protection index (PI) against late blight, in Bacillus spp. and Pseudomonas spp. 17A-B3 = Bacillus amyloliquefaciens, 30B-B6 = Bacillus subtilis 44R-P8 = (Caulier et al., 2018).

In figure 4, *B. amyloliquefaciens* had a protection index (PI) at 62%, which was a significant protection. *B. subtilis* had an index at 67%, but the highest PI was by *Pseudomonas protegens* at 83%. Under field conditions (natural high infection pressure), here the fungicides had a disease severity reduction at 83% and 98% compared to the control, whereas *B. subtilis,* at the end of the trial, significantly reduced the late blight severity with a PI at around 70% (day 3), 41% (day 16) and 22% (day 56). With the best results (excluding the fungicides), also compared to the pseudomonas spp. strains. At the end of trials *B., amyloliquefaciens* did not significantly reduce the symptoms, and had a PI at approximately 33% (day 3), 10% (day 16), and 0-2% (day 56), with the least efficacy of all the bacterial products tested (Caulier et al., 2018).

It seems that *B. amyloliquefaciens* can have a mediocre efficacy under controlled conditions, but under field conditions the effect fails, and are no way near the fungicide's efficacy. Even compared to the other *Bacillus* strain *(B. subtilis),* it cannot attain the same control efficacy.

4.6.2 Bacillus subtilis/Serenade ASO

Bacillus subtilis is an endospore-forming rhizobacterium, that primary during endospore formation produces several antimicrobial metabolites/mycotoxins (Reiss and Jørgensen, 2017). The MOA of this genus is normally the destruction of pathogen cell membranes as a direct effect of the antibiotics. Furthermore, there is also an indirect effect on pathogens, by the resistant inducing MOA which is also seen in *B. subtilis (Reiss and Jørgensen, 2017)*. One known strain with anti-plant pathogenic properties is the *B. subtilis* strain QST713 which is also the active product in the commercial product Serenade ASO (Reiss and Jørgensen, 2017). This strain has been shown to affect a wide range of plant pathogens.

To establish the efficacy of *B. subtilis*, refer to the beforementioned trials by Caulier et al. (2018), explained in the example with *B. amyloliquefaciens*/Serifel (including figure 4). Here *B. subtilis* strain 44R-P8, showed significant positive results, especially under field condition, with superior efficacy to the *B. amyloliquefaciens* in field trials.

In another trial by Kumbar et al. (2019), they assessed the efficacy of different *B. subtilis* isolates against late blight, under field conditions. A fungicide Mancozeb was used as the positive control, and the treatments were applied both by foliar spray and soil drenching on potato plants. The Late blight disease incidence (%) was significantly reduced by the bacterial treatments, specifically after 60 and 80 days, when compared with the untreated control. After 60 days the occurrence of disease was 67.7% in the control, 49% in the fungicide, and between 53 and 59% for the bacterial treatments. After 80 days, the control was at 100% disease incidents and approximately 62% for the fungicide and between 59 to 72% for the bacterial treatments.
This indicates that there is a potential for a solid protection against late blight, by using different strains of *B. subtilis*.

4.6.3 Trichoderma spp.

Trichoderma spp. is a genus of Ascomycota, living in the soil, and belonging to this genus are many different strains with control effect against plant pathogens, with some being the active compound in a wide range of commercial biocontrol products (Bae et al., 2016). Examples include *Trichoderma virens, T. viride,* and *T. harzianum (Bae et al., 2016)*. The MOA is both more direct with mycoparasitism (cell-wall-degrading enzymes) and antibiosis in addition to a more indirect plant effect by general competition/niche exclusion and resistant inducing properties (Bae et al., 2016). One of the most prominent MOA is the antibiosis with the production of antimicrobial metabolites, which inhibits the plant-pathogens growth. This is further outlined by the +100 antimicrobial metabolites, that have been found in connection with this genus (Bae et al., 2016).

In the trials by Bae et al. (2016) 128 *Trichoderma spp.* strains were tested to evaluate the efficacy of their metabolites, against 7 *P. infestans* isolates, on agar plates. Here metabolites from the isolate *T. atroviride/petersenii* and *T. virens*, inhibited the pathogen isolates the most. *T. virens* had a broad inhibitory effect, against all the isolates with between 18-100% inhibition. *Trichoderma atroviride* showed an inhibitory effect against 5 of the 7 pathogen isolates.

In another study by Yao et al. (2016), they did in vitro assays to examine the effect of different *Trichoderma spp.* isolates (linear growth of pathogen) against *P. infestans* isolates. Followed by a potato in-planta assay (greenhouse and field conditions) where the isolates efficacy (disease index), was further evaluated. In both cases, one of the most promising isolates as the

Trichoderma HNA14, which significantly inhibited the pathogens' linear growth, in the *in vitro* assay, by 56.8% reduction. The next most promising strain of Trichoderma was HNQ11, with a 60.4% reduction. Under greenhouse conditions, isolate HNA14 had the second-lowest disease index at 11.5, where isolate HNA12 had a disease index at 10.0, compared to the untreated control. Under field conditions, many isolates had a statistically significant reduction of disease severity, compared to the control. 21 days after treatment the plots treated with isolate HNA14 had the lowest disease index (excluding the fungicide) at 16.0 compared to the control with an index at 29.9. Moreover, there was no significant difference between the isolate HNA14 than the fungicide Thiram that had a disease index at 15.5, 21 days after treatment.

The trials indicate that there is great potential for finding *Trichoderma spp.* isolates with a robust control against late blight. The efficacy seems to be broad against different *P. infestans* isolates, and can under field conditions compete with some fungicides.
Furthermore, there is potential for the use of volatile organic compounds from *Trichoderma spp,* as biofumigation against late blight. This is explored by Elsherbiny et al. (2020), where the volatile organic compounds emitted by especially *Trichoderma atroviride*, with the greatest disease inhibition seen at 81.4%, in in vitro trials.

4.6.4 Aureobasidium pullulans

Aureobasidium pullulans is a yeast-like species that have biocontrol properties against a different array of pathogens often seen in apple and citrus production (Di Francesco et al., 2017). The MOA is broad, with the dominant features being antibiosis and parasitism as well as nutrient/space competition and resistance inducing (Di Francesco et al., 2017).

In the study by Di Francesco et al. (2017), it was assessed if the positive efficacy of *A. pullulans* on other pathogens, could be imitated in the control of late blight. This was done by inoculating the *A. pullulans* strain L1 and L8 on tomatoes, under greenhouse conditions, 24 hours before or 16 hours after pathogen inoculation. It was established that *A. pullulans* worked best preventative, with a significantly better effect with the application before pathogen inoculation, compared to after. The strain L8 applied before inoculation reduced the disease (average AUDPC reduction) 60% and L1 reduced it by 36.7%, compared to the control. In the case of the application 16 hours after inoculation, the disease reduction was on average 32.2%. All in all, *A. pullulans* showed a good result, with a substantial reduction in disease.

This efficacy was not reflected in the trials by Nechwatal and Zellner (2015), who tested 20 alternative products, one of which was *A. pullulans*. Both lab, pot, and field trials were commenced. *A. pullulans* were only tested in the in vitro leaf assay because it did not fare well in the initiating tests. In these first tests, it scored a high infection efficiency (% leaves infected), at

98.1 and 100% compared to the 96.1% control. To further compare, copper hydroxide had an 11.2% mean disease incidence. The authors speculated that the bad efficacy by *A. pullulans* could be due to that its specific requirements were not fulfilled during their experiment.

All in all, *A. pullulans* showed good results in the first trials, with a substantial reduction in disease. This was not reflected in the trials by Nechwatal and Zellner (2015), where the mean disease incident was even higher than the control. This indicates that this product is not reliable in the control of late blight, or there is a need for further understanding of which requirements are needed for stable control.

4.6.5 Chaetomium spp./Chaetomium globosum

Chaetomium globosum is one of the about 100 species found in the genus *Chaetomium spp.* species in this genus, primarily lives in the soil, or endophytic in the plant (Madbouly and Abdel-Wareth, 2019). *C. globosum* is a saprophyte that exists both in the phyllosphere and rhizosphere of the plant, and has been used in the control of many pathogens of fruits (Madbouly and Abdel-Wareth, 2019). A noteworthy characteristic of this fungus is its ability to produce species-specific metabolites that can work in a microbial antagonistic fashion (Madbouly and Abdel-Wareth, 2019). The MOA can be antibiosis, mycoparasitism, and competition (Shanthiyaa et al., 2013).

One species-specific metabolite produced by *C. globosum* is the antibiotic chartomin, which efficacy on late blight, was examined in trials by Shanthiyaa et al. (2013). The efficacy of 8 different *Chaetomium spp.* isolates were tested, both *in vitro* and in two field trials with potatoes. In in vitro, all of the *Chaetomium* isolates tested, inhibited the pathogen, here *C. globosum* showed a statistically significant higher inhibition compared to the other isolates. More specifically, a 72.3% inhibition of *P. infestans* compared to the untreated control, with the other isolates ranging from 44.5 to 64.5%. In general, a promising effect, but with a substantial lead in effect by *C. globosum.* Under field conditions, *C. globosum* was either applied by foliar spray, tuber coating, or soil application, and all of the application treatments resulted in a significant reduction in late blight disease compared to control. An example is in trial 1 where the foliar spray with *C. globosum* by itself, had 75% *P. infestans* infection 90 days after sowing, compared to 90.3% in the control. In the treatment where *C. globosum* was applied in all 3 ways combined, the % infection was 65.7%, with no statistical difference to the fungicide (metalaxyl+mancozeb) treatment with 62.3% infection. This was also reflected in the yield, with 27.3 t/ha in the combined *C. globosum* treatment and 31.3 t/ha in the fungicide treatment, compared to 15 t/ha in the control. *C. globosum* shows positive results with an efficacy significantly different from the control, and close to the fungicide treatment. The inhibiting effect becomes clear when seeing the positive growth in yield when the crop is less affected by the pathogen.

To further support the promising efficacy seen with *C. globosum*, Park et al. (2005) examined the effect of *C. globosum* strain F0142 on tomato late blight, among others. It was discovered that *C. globosum* produces chaetovridins A and B with antimicrobial effects. In vivo trials resulted in a moderate effect of chaetovridins A, on tomato late blight, with a 50% control (disease severity) compared to the control.

4.7 Examples - Plant resistance inducers (PRI)

4.7.1 Chitosan hydrochloride/ChiProPlant

Chitosan by itself is a natural product derived from chitin, and the main-sources derive from different marine arthropods (Malerba and Cerana, 2016). The source will not be deprived, anytime soon, it being the second biggest carbon source worldwide (Malerba and Cerana, 2016). Chitosan is not a specific compound but describes a group of copolymers, with a wide array of different functions and effects, one of them being the inducing of resistance in plants, It can therefore be used as a plant elicitor (Malerba and Cerana, 2016). Other possible MOA, are chitosan's enhancing of plant stress defenses, and its antimicrobial properties, in addition to its boosts in the plants' synthesis of secondary metabolites (Malerba and Cerana, 2016). Chitosan hydrochloride can be made by dissolving chitosan in hydrochloric acid and boil it, this form is appreciated for its water-soluble properties (Seyfarth et al., 2007).

The trial by La Torre et al. (2019), examined different alternatives to copper, in the control of late blight, in organic farming. This was done in both in vitro trials and tomato greenhouse trials, where chitosan hydrochloride was tested as the commercial product Chitoplant. In the *in vitro* chitosan hydrochloride had the best effect on sporangia germination, with one trial at 98.5% overall inhibition after 20 hours and 91% after 40 hours, compared to the control. Which was significantly different from the control. Because of the lack of plant host in this trial, it suggests that chitosan hydrochloride also has a more direct antimicrobial effect on *P. infestans* that is not due to it, inducing resistance. In the greenhouse trial, 15 days after inoculation, it had a disease severity (%) at approximately 61%, compared to 100% severity for the control treated with water. This was a mediocre result compared to the other tested products.

Interestingly, chitosan hydrochloride did quite well in the in vitro trials when it could not utilize its plant resistant inducing properties, but only mediocre in the trials where a host was present. All things considered, this alternative has an acceptable effect with a broad range of MOA to utilize.

4.7.2 COS-OGA/FytoSol

The compound COS-OGA is also based on chitosan, like the above-mentioned example, and share therefore some of the basic properties. Including the MOA's, which is mainly recognized as a plant elicitor (van Aubel et al., 2016). That said, this compound has a different composition and differs in other regards as well. COS-OGA is made up of cationic chitosan oligomers (COS) and anionic pectin oligomers (OGA), and this composition can "impersonate" as a plant pathogen thereby inducing the plants own defense mechanism (van Aubel et al., 2016). More specifically, this compound relies on the systemic acquired resistance (SAR) because it induces the salicylic acid buildup in the plant (Aubel et al., 2018).

The efficacy of COS-OGA was tested by Aubel et al. (2018), in the form of the commercial product FytoSol, against potato late blight. Another product (FytoSave) with a different COS-OGA composition, was tested as well. The compound was applied by foliar spray on potted potato plants, in general, 1, 3, and 7 days before inoculation of *P. infestans*, at different amounts. Depending on if compound persistency or cumulative effect was the focus of the treatments. Disease severity was scored 3 times a week for 3 weeks. The control plants had an AUDPC of 1540 compared to FytoSave at 1087 and FytoSol at 2. This is translated to 29% protection achieved by FytoSave and almost 100% by FytoSol, compare to the untreated control. Based on the big difference between the 2 Fyto-products, the authors speculated that FytoSol may have a direct toxic effect on the pathogen. This was confirmed by an *in vitro* trial where FytoSol significantly reduced *P. infestans* lesions.
Conclusively, Fytosol showed incredible results in controlling the late blight disease, both as a plant elicitor as well as with antibiosis. The efficacy was also better than the other compound, illustrating the importance of the composition of the product, in the control of late blight.

In another experiment by Clinckemaillie et al. (2017), the COS-OGA used in the commercial product FytoSave, was tested against late blight, on potato plants under greenhouse conditions. Here the mean AUDPC for the untreated controls was 3 times higher with 109 (±25) compared to the treated plants with 40 (±19). In the mean leaf area covered by disease, the controls were 73% (±24%) compared to 27% (±12%).
This further confirms the positive efficacy seen in the other trial, and the COS-OGA seems to facilitate a good control against late blight. Even with the less effective product, FytoSave compared to FytoSol.

4.7.3 Pseudomonas spp.

Many different species belong to the genus *Pseudomonas spp.,* with different MOA, some with a more direct effect, more suitable under the BCA classification (Caulier et al., 2018). Because

Pseudomonas also is connected with resistance inducing in plants, they have been placed under the PRI classification. The MOA is seen in *Pseudomonas spp*. production of a with a wide range of secondary metabolites with antimicrobial, resistant inducing, and competition enhancing properties (Caulier et al., 2018). Some of these metabolites with resistant inducing aptitudes are 2,4-diacetylphloroglucinol (DAPG), lipopeptides, and siderophores (Caulier et al., 2018).

In the trial by Caulier et al. (2018), they assessed the production of the resistant inducing metabolites (lipopeptides and siderophores) and the antagonistic activity of different *Pseudomonas spp.* and *Bacillus spp.* strains. Followed by a potato trial under greenhouse conditions, and a field trial. In the in vitro assessment, all the *Pseudomonas spp.* strains tested showed a substantial production of both siderophore and biosurfactants, which was not the case with all the *Bacillus spp.* strains, indicating a difference in the tendency for resistant inducing between the two genera. The result from the greenhouse assay can be seen in figure 4, under the example with *Bacillus amyloliquefaciens/*Serifel, where one *Pseudomonas spp. (P. protegens)* strain had the greatest efficacy with an 83% protection index, compared to the control. In the field trial, the *Pseudomonas* strain 44R-P8 (*P.* protegens) had after 3 days an approximately protection index at 64% and 20% after 16 days and 5% after 56 days, a mediocre result.

In another trial by De Vrieze et al. (2019), 3 *Pseudomonas spp.* strains efficacy was tested against 19 newly collected *P. infestans* isolates of both mating types. In a growth inhibition assay, both the *Pseudomonas spp. (P. brenneri, P. protegens)* strains and *P. infestans* isolates were inoculated on agar plates, together. The strain R47 had a potent inhibition of all the pathogen isolates with a reduction of radical mycelia growth down to 1 to 15%, compared to the untreated control. Some of the other strains had varying inhibitory rates on the isolates, with 17 to 92% depending on the isolate.
All things considered, both trials showed the great potentials in the use of *Pseudomonas spp.* in the late blight control, depending on the strain. Seen by its ability to induce resistance, as well as its antagonistic properties. Though, the effect under field conditions is a bit unclear.

4.7.4 Arbuscular mycorrhiza

Mycorrhiza can form a mutualistic symbiosis with a wide array of plants, by colonizing the plant roots. Overall, the symbiosis benefits the host by the mycorrhizas far-reaching mycelium, supplying water and nutrients (especially P) to the host, and the mycorrhiza benefits by achieving carbon (Filho et al., 2016). Other MOA's are the enhancement of the plants' resistance to biotic and abiotic stress as well as inducing resistance (Filho et al., 2016). Arbuscular mycorrhiza (AM) primary functions are below ground, but it can also affect the above-ground pathogens, specifically by inducing the Salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) signaling pathways, affecting plant defense (Gallou et al., 2011).

In vitro trials by Gallou et al. (2011), revealed that arbuscular mycorrhiza potato plants had an effect on *P. infestans* infection. This was done in in vitro trials, on potato leaves, where the arbuscular mycorrhiza *Glomus sp.* was used. The impact of the AM was evaluated based on the leaf infection index and the AM root colonization. Furthermore, the expression of the 3 pathways (SA, JA, ET) was tested, to see the impact of arbuscular mycorrhizas resistance inducing properties. In all instances, there was a significantly lower infection index whit the arbuscular mycorrhiza, compared to the control. E.g. there was a significant reduction in AUDPC between the AM treated potato leaves compared to the untreated. 12.3 (±0.7) and 15.5 (±0.7), respectively. Further analyses also revealed that AM does induce the signaling pathway, also in the arbuscular mycorrhiza potato plants.

In a newer trial by Kabdwal et al. (2019), the effect of arbuscular mycorrhiza was examined against tomato late blight. In this field trial, arbuscular mycorrhiza was applied in the form of a commercial product, Jas mycorrhiza, and tested in combination with *Trichoderma (T. harzianum*), and *Pseudomonas* strain (*P. fluorescens*) and a fungicide (mancozeb). The effect was examined against the combined foliar blight of late and early blight. Arbuscular mycorrhiza (soil-applied) in combination with the 2 microbial strains, had a 61.09% reduction in foliar blight, compared to the control. AM in combination with mancozeb had the highest reduction at 78.43%, even higher than the two-time application of only mancozeb at 75.54%.
So, even though AM is not tested by itself, there is a potential in replacing a fungicide application with an AM soil application instead. Further confirming the possibilities in using arbuscular mycorrhiza plant inducing properties.

4.7.5 Rhodopseudomonas palustris

Rhodopseudomonas palustris is a photosynthetic bacteria, it being phototrophic enables it to use a unique metabolism during anaerobic conditions, by getting energy from light (Zhang et al., 2020). The bacteria are soil living and have a broad MOA with plant inducing resistance in addition to promoting growth and immune responses (Zhang et al., 2020).

In a new trial by Zhang et al. (2020), they examined the efficacy of *Rhodopseudomonas palustris* isolate GJ-22 in combination with the fungicides cymoxanil and mancozeb, against late blight in potatoes. The trials were done both under greenhouse and field conditions. In the greenhouse trial, the potato seedling was applied with the treatments, and after 24 hours, inoculated with the pathogen. To see some of the results from the greenhouse trial, refer to figure 5, illustrating the inhibition rates of the treatments, on late blight.

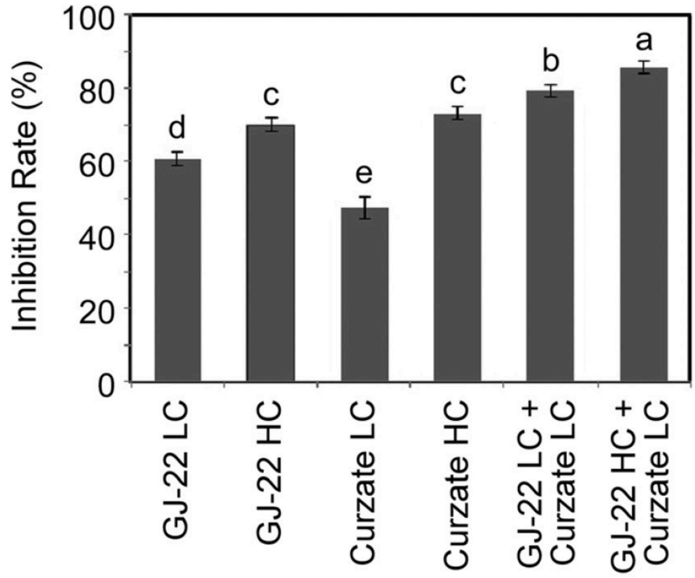


Figure 5: Inhibition rate of the treatments, compared to the control (mean of 3 experiments). LC = Low concentration, HC = High concentration (Zhang et al., 2020).

Based on figure 5, *R. palustris* GJ-22 at both low and high doses, have a significantly better inhibition rate (%) than the low dose of fungicide. The best effect is seen when combining a high dose of the biocontrol, with a low dose of fungicide, with approximately 85% inhibition. This is better than the both high dose of fungicide (approx. 72%) by itself and the bacteria treatment by themselves, indicating an additive effect.

In the field trial, the plants were treated 3 times with 7 to 10 days interval, followed by disease assessment, 15 days after the last treatment. All the treatments had a statistically significant reduction of the disease severity, with the best treatment (combination) reduced late blight by 85.3%. In general, the results from the field trials were in accordance with the greenhouse trials.

Not much literature is available on the biocontrol properties of this bacteria, but it seems that *R. palustris* has a good effect on late blight. Both by itself, and even better efficacy in combination with fungicides, where there is an additive effect. Making it a possible future tool, in the reduction of fungicide doses.

4.8 Examples - Plant strengtheners/biostimulants

4.8.1 Extract of Equisetum arvense/Horsetail

Equisetum arvense (Field Horsetail), is a perennial plant that has many beneficial properties, including fungicidal effects (Đurić et al., 2019). Extracts from Equisetum arvense are high in e.g.

tannins, organic acids, and resins in addition to flavonoids and alkaloids, of which some have antifungal properties and stimulating effect on the plants (Đurić et al., 2019).

The MOA of biostimulants is quite broad, which is also the chase with equisetum arvense. Eugene et al. (2020) did in vitro trials to test the direct and indirect effect of *E. arvense* (and 6 other medicinal plants) against late blight. By inoculating potato tuber discs with *P. infestans*, followed by the extract, the direct effect of the alternatives against *P. infestans* symptoms, could be evaluated. Here the degree of disease development was examined after 96, 120, and 144 hours after inoculation. The most promising results were obtained by the *E. arvense* extract, with almost complete inhibition of the disease at only approximately 2% of disease development, after 96 hours, compared to approximately 23% with the untreated control. This development only became more prominent with time, at approximately 5% (*E. arvense*) compared to approximately 40% with the control, after 120 hours and respectively approximately 10% compared to approximately 54% after 144 hours. Indicating a substantial effect against late blight, when using equisetum arvense as a control agent.

Another example of the *E. arvense* efficacy is presented by Nechwatal and Zellner (2015), who did potato detached leaf *in vitro* assay, potted plant assay, and small plot field trials. They used 24 different Cu-free alternatives including *E. arvense*, to test their effect against late blight. Here *E. arvense* was one of the more promising with a statistically significant effect against infection efficiency and lesion size (*in vitro*) compared to the untreated control. In addition to a statistically significant effect on infection rate (%) and infected leaves, in the pot trials, with 7.5 infected leaves compared to 17.2 in the control. In the field trials, *E. arvense* did reduce the leaf infection by 25-40% (both directly and indirectly) compared to the control.

So, *E. arvense* shows a promising efficacy with reductions of disease development at up to 40-45%, also under field conditions. Furthermore, even though equisetum arvense is regarded as a biostimulant, it also shows promising direct inhibitory effect against late blight disease spread.

4.8.2 Extract of Urtica dioica/Nettle

Urtica dioica is a perennial herb that has been used in folk medicine for many years. This is due to the extract's different properties, such as its high N amount and high amounts of oligoelements, that can have a growth-promoting effect on plants, as well as a repellant and fungicidal (and insecticidal) effects (Đurić et al., 2019).

To further evaluate the efficacy of *U. dioica*, refer to the trials by Nyankanga et al. (2012). Here, field trials with the susceptible potato cultivar Desiree, and more resistant Tigoni variety, was done with different treatments. Respectively Ridomil (mancozeb + metalaxyl), Dithane (mancozeb), phosphite, stinging nettle extract (*U. dioica*), control treatments, and combinations of phosphite and fungicides. With repeated application through the growing season. Plots treated with the nettle extract had a statistically significant different disease severity at 15.1% - 28.4% on Desiree (even lower with the tigoni variety) compared to 33.6-50% in the control treatment. *Urtica dioica* also did well compared to the mancozeb treatment at 12.8-27.6%, where there was no significant difference between the two, but it was still the treatment with the lowest efficacy (excluding the control). Moreover, in regard to lesion size and numbers, *U. dioica* did significantly reduce these symptoms, compared to the untreated plots, although it had in general the least effect compared to the other treatments.

Though based on 1 trial due to the lack of more available literature, all things considered, *U. dioica* seems to have a promising effect against late blight with a significant reduction in disease severity and spore development, though not on par with fungicides or even phosphite.

4.8.3 Lecithin

The description lecithin is used on any fatty substances (from plant/animal tissue) which contain phospholipids, glycolipids, triglycerides, glycerol in addition to choline, fatty acids, and phosphoric acid (M. Jolly, 2018). Besides its uses in food and feed application and industrial uses, it has shown to have plant protective/promoting properties (M. Jolly, 2018). One part of the MOA is as a physical barrier on the plant foliage, preventing fungal disease spread, however without killing the pathogen (M. Jolly, 2018).

In the study by Bohinc et al. (2015), they examined the field efficacy of four alternatives against late blight, on tomato. Respectively, metiram, arsaronaldehyde, garlic extract, and soybean lecithin. This was done in field trials, where the four treatments were repeated every 7-14 days. Based on the leaf infection area, the disease severity index was evaluated, and it was only plants treated with metiram, which was statistically significantly the least infected. Where there were no significant differences between the other treatments (including lecithin) and the control. Conclusively, lecithin showed no satisfactory effect against late blight, indicating that it is no good as an alternative.

Another trial was done by Dorn et al. (2007), who examined the control of late blight in organic potato production, with 53 “natural” alternatives. One of these alternatives was the commercial product Oekofluid P, which is composed of lecithin as well as stone-meal, plant extracts, sodium silicate, and phosphonic acid. Therefore, the effect of this product is not purely based on lecithin but also the other compounds, nonetheless the trials will still give an indication of the effect of lecithin and product where it is included. Small-plot potato field trials were established, and in one

of the trials, the treatment with Oekofluid P inhibited foliar blight with 63% second only to Kocide DF with 99%.
Contrary to the study by Bohinc et al. (2015), this study had a significant effect on the late blight (foliar blight), which could be due to a potential synergetic effect when in combination with the other compounds in Oekofluid P.

4.8.4 Endophytes

In this example, the focus will be partly on endophytes in general with further examination of the fungal endophyte *Phoma eupatorii*, and its efficacy against late blight.
Endophytes are microbes (typically fungi and bacteria) that live in the plant without causing any symptoms or harm to the host, they can possess many plant stimulating properties, and some have the potential to inhibit *P. infestans* (White et al., 2019).

The potential for an endophytic control of late blight was examined by de Vries et al. (2018), who tested 12 different fungal endophytes, *in vitro*, for their metabolites antifungal properties, against 9 different *P. infestans* isolates. Moreover, they further examined the 4 best in this regard, both on agar and in planta (tomatoes). The 4 most promising endophytes were, respectively, *Philocephala fortinii* (isolate 4197), *Phoma eupatorii* (isolate 8082), *Pyrenoochaeta cava* (isolate 9907), and *Monosporascus ibericus* (isolate 9913). On agar plate, the endophytes were able to inhibit as well as reduce the *P. infestans* growth. To test the efficacy *in planta*, tomato seeds were inoculated with both the endophytes and *P. infestans.* Here, *P. Fortinii* and *P. cava* killed the tomato seedling, while the 2 remaining endophytes did not. Especially, seedling inoculated with *P. Eupatorii* (and *P. infestans*) had a significant reduction in the relative necrotic area compared to the control, only inoculated with *P. infestans*, on all the isolates. This was not the case with *M. ibericus*.
There seems to be great potential with *P. eupatorii* as a control agent with a broad-spectrum of effect, against late blight. Even though the metabolites in itself acts like a BCA, the positive result in planta suggests a substantial effect by using the whole organism inside the host. Here all the positive properties of the metabolites can come into play, including the plant stimulating properties.

Another potential endophytic fungus is the *Fusarium oxysporum* (strain EF119), which showed promising results in *in vivo* trials by Kim et al. (2007). This endophyte inhibited the growth of *P. infestans*, the most. More specifically it suppressed the tomato late blight development by up to 90%.

4.8.5 Extract of Populus/populin

In this example, the focus will be on the extract from the black poplar (*Populus nigra*) and the active ingredient populin.
Populin is a glycoside that contains salicylates and flavonoids which is known for its antimicrobial and antioxidating properties, thereby reducing the free radicals and reducing potential stress (Benedec et al., 2014). Populin has earlier been used in the control of apple scab, with positive effects under field conditions, making it a possible solution in the control of late blight (Bálint et al., 2014).

In a study by Turoczi et al. (2020) they examined the effect of populin on 7 different strains of *P. infestans*. The black poplar extract was used in the recommended dose and tested both in a laboratory and field trial. The lab experiment evaluated the populin/extracts effect on the mycelium growth of the different strains, on agar plates. The full dose treatment showed a statistically significant reduction (compared to the control), on 6 of the 7 *P. infestans* strains. In the *in planta* field trials, the extract was applied by foliar spray, at two doses, through the whole growing season, on potato plots in Transylvania (2018). Moreover, there was a treatment with the fungicide Infinito 687 SC (fluopicolid + propamocarb) and Valis M (mancozeb + valifenalat) as well as an untreated control. Each time after application the symptoms were evaluated and disease severity estimated, after 24, 48, and 72 hours. An example of the result after 24 hours, can be seen in figure 6.

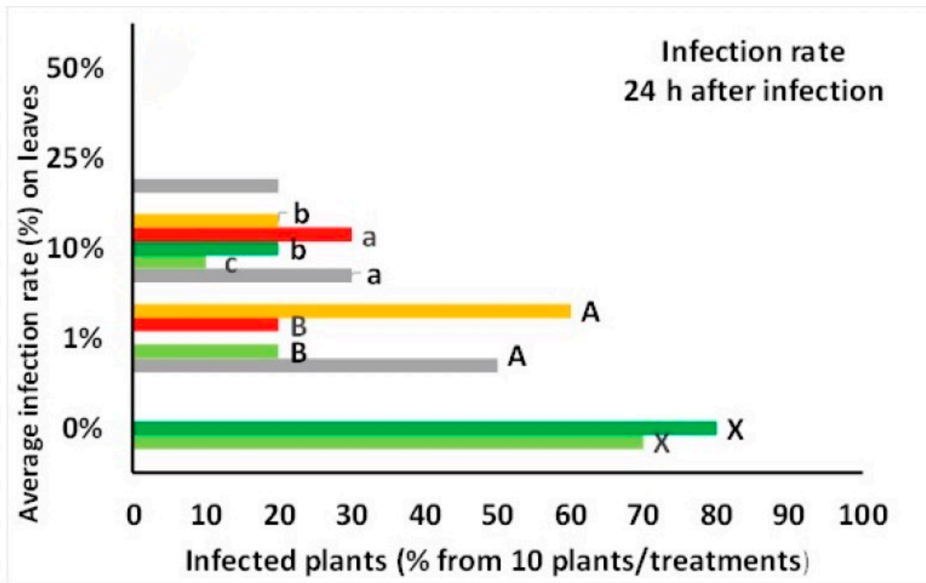


Figure 6: Infection rate 24 hours after infection. Grey = Control, light green = Populin5%, dark green = Populin10%, red = Infinito 687 SC, yellow = Valis M. The proportion (%) of plants with infections of 0, 1, 10, 25 and 50% (Turoczi et al., 2020).

In figure 6, the infection rate after 24 are detected at around 0% when using the populin, and only up to 10% infection rate was detected, also after 48 and 72 hours. In general, the results also showed a significantly better effect of populin than the fungicides, and only after 72 hours did Infinito 687 SC inhibit infection rate under 1%.

In earlier trials from 2014, by Bálint et al. (2014), the same efficacy tendency was seen. Here populin significantly reduced late blight growth rate, compared to the control, in lab trials as well as significantly reducing late blight severity under field conditions.

Poplar extract/populin has great potential in late blight control, and in the new study, it seems to be able to inhibit a wide span of *P. infestans* strains, with results rivaling the fungicides.

4.9 Examples - Other alternatives

4.9.1 Potassium phosphite/Resistim 0-7-11

Besides being of nutritional value, as mentioned earlier, the Phi based compounds are very effective against fungal diseases, especially oomycetes such as *Phytophthora infestans (Achary et al., 2017)*.

To ascertain the positive effect potassium phosphite have against late blight, field trials have been made in Sweden, by Liljeroth et al. (2016). This was done over several years, where the efficacy of potassium phosphite was tested on both table potato cultivars as well as different starch cultivars. The effect was evaluated (both foliar and tuber blight) either by itself or in combination with fungicides, on susceptible or partially resistant cultivars. An example of potassium phosphite efficacy can be seen in table 1.

Table 1: Field trials in Sweden 2012-2014, illustrating potato yields (ton/ha) and late blight severity when treated with potassium phosphite, fungicides, or a combination (Liljeroth et al., 2016).

Treatment	Susceptible cultivar		Partially resistant cultivars	
	Bintje 2012–2014		Ovatio 2012. Sava 2013. Perlo 2014	
	rAUDPC	Yield	rAUDPC	Yield*
Untreated Control	0.58a[1]	38.9d	0.45a[1]	54.6b
Fungicide 100% dose	0.22e	62.4a	0.068c	69.7a
Fungicide 50% dose	0.28c	57.7b	0.102c	67.6a
Phosphite 100% dose	0.45b	48.8c	0.201b	63.4a
Fungicide 50% + Phosphite 50% dose	0.24de	59.6ab	0.065c	68.9a
Fungicide 100% + Phosphite 100% dose. 2 week interval	0.27cd	59.7ab	0.091c	64.3a

When looking at table 1, the susceptible cultivar Bintje, the relative area under the disease progress curve (rAUDPC), compared to the untreated control, shows the best effect with the use of 100% of the recommended dose of fungicide. With a significantly better effect by using 100% and 50% fungicide dose compared to only a 100% phosphite dose (These tendencies are reflected in the yields). Potassium phosphite cannot by itself compete with a full fungicide dose or even half dose, but the combination (Fungicide 50% + Phosphite 50% dose) is not significantly different from the full dose fungicide strategy. This indicates the potential for a fungicide reduction when combining it with potassium phosphite, without compromising the effect and yield. The same is the case with the partially resistant cultivars, though with an even better effect of the fungicide-potassium phosphite combination.

The same tendency is present in field trials from 2015 and 2016 by Liljeroth et al. (2020), where the efficacy of phosphite, fungicide, and the combination, was tested on the cultivars Kuras and Sarion. In 2015 the rAUDPC of late blight and the potato yield, was not significantly different between 100% fungicide dose and 100% potassium phosphite dose and the combination, in both cultivars. In 2016, the rAUDPC result was more like as seen in table 1, where the 100% and 50% fungicide dose had a better effect than 100% potassium phosphite, but the best effect was seen with the 50/50% fungicide-phosphite combination. The field trials from 2015 indicate that the effect of the potassium phosphite compared to fungicides can differ based on the growing year. This can be due to the difference in tolerance and sensitivity, against phi-based compounds, between different *P. infestans* strains (Huang et al., 2018), as well as the potential difference in potassium phosphite effect, depending on the infection pressure (high infection pressure reduces effect) (Mulugeta et al., 2019).

All in all, a reasonable effect against late blight, seems to be obtainable by using potassium phosphite, which at times can rival the fungicide effect. Though, in most cases, potassium phosphite works best in combination with fungicides.

4.9.2 Sulfur/Kumulus S

Sulfur is one of the oldest fungicides used, and it is an attractive alternative, because of its low toxicity to animals and plants (Williams and Cooper, 2004). When applied by spraying, it works by emitting a harmful odor, as well as being harmful against organisms when they come into contact. Sulfur is systemic, which necessitates repeated application, to ensure a satisfactory effect (Williams and Cooper, 2004).

The efficacy of sulfur-based compounds on late blight is determined in the pot trials by Ryant et al. (2008). They applied the commercial product ORIN that contains elemental sulfur, by applying it to the potato foliage. In their case, the experiment showed no significant reduction in infection frequency compared to the control, and the highest frequency of infection was seen in the plants with the highest application of the sulfur-based compound. The authors Ryant et al. (2008) explain this, by the damage done by the oxidation of the sulfur on the leaves, which can damage the leaf cells as well as affecting processes such as photosynthesis.

Even though sulfur has been known to affect other plant pathogens (Ryant et al., 2008), it does not seem to be the case with late blight. Though this is only based on 1 analyzed trial, because of the absence of other relevant peer-revied literature.

4.9.3 Compost tea

Compost as a control agent is not as easy to define, because of the difference in compost origin and thus the microbial composition as well as the efficacy (Marín et al., 2013). Therefore, this literature review will focus on different trials and compost teas, from which a general conclusion will be drawn.

Compost teas can be defined as a watery extract of composted material, used in the fertilization of plants in addition to the control of a wide range of plant pathogens (Marín et al., 2013). Compost tea can be applied as a foliar spray, drench, or otherwise (Marín et al., 2013). The efficacy depends on the method of preparation (e.g. aerated or non-aerated compost), method of application, and the microbial community in the compost, which can have a wide range of MOA from antibiosis, competition, parasitism, and induced resistance (Marín et al., 2013).

An example of the effect of compost tea made from oak-bark, against late blight in tomatoes, was explored by Bahramisharif and Rose (2018), in tomato pot trials. The efficacy was partly evaluated based on the plant growth-promoting factors such as root and shoot length as well as fresh weight. Also, the disease suppressing effects based on necrotic lesions and defense-related compounds promoted by the control agent were evaluated. The trials also examined the effect of the compost in combination with e.g. BCA *Bacillus* and *Trichoderma spp.*
In the trials, the plant treated with only compost had the biggest growth compared to the untreated, compared to the other BCA tested. With 3.3-fold greater fresh biomass weight, 1.4-fold longer shoots, and 3.7-fold longer roots. Though, in combination with e.g. *Bacillus subtilis* the treatment had an even bigger effect on the growth of the plant. Furthermore, the compost as a standalone treatment had an average of 82% disease suppression against late blight, the second-best to the standalone *B. subtilis*. This effect was further improved when compost and *B. subtilis* were combined. This illustrates the potential positive efficacy of compost, against late blight, especially when combined with certain BCA’s.

The beforementioned trials are consistent with the result of a potato pot trial by Ghorbani et al. (2005). Here extracts from one-month-old cattle manure had a reduction in late blight disease development at 30-40% and house-hold waste compost showed a result between 30-45% reduction. Giving an idea of the variation between compost type.
The efficacy of compost is not always positive as proven by a potato field trial by Al-Mughrabi (2006), with an untreated, drench compost tea, foliar compost tea, foliar food, and combinations

of these. The compost tea was made from vermi-casting, thermal compost, and static wood chip as well as other additives like kelp and alfalfa, etc. Here the trials showed, in all cases, an increase in foliar late blight severity (45 days after planting) compared to the untreated. The foliar compost tea increased the disease severity most with approximately 8% and the drench compost increased it by approximately 1.5%. This indicates, that the efficacy of compost tea can vary a lot, and at times even have a negative effect on late blight control.

4.9.4 Potassium bicarbonate/Armicarb85 SP

Potassium bicarbonate ($KHCO_3$), an inorganic salt that is a commonly used control agent, against a wide range of plant pathogens, especially foliar diseases (Deliopoulos et al., 2010). The compound is safe for humans and the environment, and are easily applied by foliar spray, making it a suitable alternative against late blight (Deliopoulos et al., 2010). Potassium bicarbonate functions both by contact and eradicant and can suppress the existing fungal infection as well as work preventatively. This is done by inhibiting the fungal spore formation and germination, by disrupting the K^+ balance, resulting in fungal cell breakdown, dehydration, and also an increased leaf area pH, creating a more hostile habitat for the pathogen (Deliopoulos et al., 2010).

The effect of potassium bicarbonate against late blight in potatoes is tested by Abd-El-Kareem and Fatten (2012), in both laboratory and field trials. The trials tested the late blight severity with either potassium bicarbonate, sodium bicarbonate, citral, and fungicide (Ridomil) or in combination with each other. This was done repeatedly, by foliar application. *In vitro*, all the treatments inhibited the linear growth of *P. infestans* significantly, where potassium bicarbonate inhibited the growth the most (excluding the fungicide), with 93.3% compared to the untreated and completely with a full dose. In the field trials, all treatment had a significant reduction and potassium bicarbonate alone had the highest effect at 50% reduction (compared to sodium bicarbonate at 40.6%) the first year and 47.1% disease reduction the second year (compared to sodium bicarbonate at 44.1%). An even better effect was obtainable when combining potassium bicarbonate with citral, with an 84.4% and 82.4% reduction, even better than the single fungicide treatment, with 68.8% and 67.6% reduction.

This efficacy was further investigated by La Torre et al. (2019), who did tomato pot trials and *in vitro*, to test the effect of certain alternatives against to control late blight, including potassium bicarbonate as Amicarb85. In *in vitro* trials, the potassium bicarbonate completely inhibited the growth of *P. infestans* but did not show a significant reduction in sporangia germination compared to the untreated. In the pot trials, potassium bicarbonate was statistically significantly different from the control, with an approximately 60% decrease in disease severity compared to the control, and was one of the alternatives with the best effect.

This confirms the results seen in the beforementioned trial, and it seems that potassium bicarbonate can have a solid effect on late blight severity, especially if in combination with other alternatives.

4.9.5 Sodium Bicarbonate/Baking soda

As one of the two inorganic salts in the bicarbonate group, together with potassium bicarbonate, sodium bicarbonate has more or less the same properties. Both in the case of MOA and application methods etc. (Deliopoulos et al., 2010).

As examined by Abd-El-Kareem and Fatten (2012), sodium bicarbonate was one of the compounds used together with potassium bicarbonate, in both *in vitro* and field trials. In vitro, sodium bicarbonate inhibited the linear growth of *P. infestans* with 86.7% and completely at full dose. As mentioned earlier, in the field trials sodium bicarbonate did reduce the disease severity with respectively 40.6% and 44.1%, by itself, and 68.8% and 64.7% in combination with citral. All in all, sodium bicarbonate has a decent effect against late blight (based on these trials), especially in combination with citral (Aldehydes). However, when compared to potassium bicarbonate, it seems to be the alternative with the least efficacy. No other relevant literature was found in this analysis.

4.10 Challenges

Certain types of challenges present themself when using most types of the beforementioned alternatives, though with minor variations between the classification groups. It is these challenges that will be specified in this part, where the specific effect of some product, by using trial data, will be evaluated in the next main chapter, of the thesis. Also, some advantages of using the alternatives will be briefly explained. This brief introduction is due to the fact, that the main advantages are centered around the environmental benefits with the resulting legislations to reduce the traditional fungicides (European Commission, 2020), which have already been explained.

It is not only synthetically fungicide, that can have toxic effects on the environment and present a risk in the consumption of produce (Köhl et al., 2019). So, it is important to know the MOA of the different alternatives, to clarify the potential risk they propose, both environmental and against humans. Therefore, risk assessment on par with the traditional fungicides, is essential, especially because of the potential production of toxins or microbial metabolites, that some of the antagonistic organisms can produce (Köhl et al., 2019).

One of the most significant challenges, that affect almost all the alternatives, is the consistency of the compounds, in disease control (O'Brien, 2017). This can be due to, that the compound can be a living organism, so there will often be a difference in effect, based on the crop genotype and physiology. With some cultivars being more or less susceptible to the BCA, thus a differentiation in the effect consistency (O'Brien, 2017). Some of this uncertainty in effect consistency can be decreased by the mixing of more BCA strains, thereby broadening the MOA and reducing the compound's sensitivity to different conditions (O'Brien, 2017). Though this is not always the case, sometimes an antagonistic effect can occur between the isolates in the mixture, reducing the control effect, even further (O'Brien, 2017). The uncertainty in effect, when using the alternatives, will be further illustrated in the data assessment part of the essay, where the data will speak for itself.

Furthermore, the alternatives are often very dependent on the environmental conditions surrounding them, both abiotic and biotic (solar radiation, pH, N application, management practice, etc.) (Köhl et al., 2019). Because the factors that affect the microbiomes will also affect the microbes living there, thus the MBCA's. E.g. the hyperparasite BCA's activity, heavily relies on the environmental conditions, because their antagonistic effect is dependent on active growth (Köhl et al., 2019).
Also, in the case of the BCA's with antimicrobial metabolites, there is potential for the metabolites to interfere with antibiotic treatments in humans or animals (Köhl et al., 2019). This necessitates a proper risk assessment before a commercial release, of the product.

Some minor challenges are the storage and application of the BCA. In the case of BCA's with living organisms, storage with the right conditions (colder), is important to ensure the survivability and effect of the compound (O'Brien, 2017). Moreover, the application is dependent on the weather conditions, because extreme conditions during application, could kill the compound or make it less efficient (Köhl et al., 2019).

Moreover, certain complications can arise, when introducing new alternative products to a country, in the classification of the product and the registrations to use it. This type of challenge can especially be a problem with plant strengtheners/biostimulants (Yakhin et al., 2017). Here, legally these compounds can only contain traces of natural plant hormones, and the main mechanism of action, has to be due to another biological aspect, or it would have to be defined as plant growth regulators (Yakhin et al., 2017). Also, it is not uncommon to include some nutrients in the biostimulants, partly to get the compound regulatory approved as a fertilizer (Yakhin et al., 2017). These uncertainties complicate the possibility to clearly define this group as well as getting new compounds introduced on the market (European Commission, 2020).

Some of the advantages with especially the biological control compounds are the very low risk of non-target effect because the compounds are very specific to the pathogen (O'Brien, 2017). Also, with e.g. the hyperparasite compounds, the CWDE degrades easily in nature, reducing the toxicity risk, significantly (Köhl et al., 2019).
Furthermore, as stated by Köhl et al. (2019), pathogen resistance has yet to be seen, against a BCA based on a hyperparasite, making it a reliable control alternative.

5 Trial data

The data used in this part of the assignment, are from trials (experiment 1 and 2) made at Aarhus University, department of Agroecology, Flakkebjerg, supplied by Isaac Kwesi Abuley, from the growing season of 2020. Disclaimer, in the growing season of 2020 at the trial location in Flakkebjerg, it was not very favorable for the development and infection of *P. infestans*, because of extended periods of time with high temperature and low precipitation. Thereby, resulting in a later infection start than usual (late august), giving the alternatives a brief period to show their efficacy, affecting the results.

5.1 Materials and Methods - Experiment 1

5.1.1 Experimental design

In experiment 1, the efficacy of 11 different alternative treatments, was tested in a potato field trial with the cultivar Kuras, against late blight disease. The products/treatments was as follows - 6 kg/ha Kumulus S, 5 kg/ha Armicarb 85 SP, 0.5 kg/ha Serifel, 300 L/ha Extract of *Equisetum arvense*, 300 L/ha Extract of *Urtica spp.*, 0.8 kg/ha Lecithin, 0.3 kg/ha ChiProPlant, 600 L/ha Compost tea, 3 L/ha Resistim 0-7-11, 4 L/ha Serenade and 4 L/ha Fytosol.

The active ingredients of these, have been explained in the surveyed literature. The treatment dose, was repeated with a 7 days interval or a 3 to 4 days interval, in high risk periods, through the growing season. The treatments were done 12 times, from the first spraying in the 22nd of June to the last spraying the 7th of September, 2020.
The design setup in experiment 1, was a randomized complete block design, with 11 alternative treatments and 1 untreated control, which was repeated 4 times in a total of 48 randomized trial plots. The trials were done on sandy-clay (JB-5) soil type.

5.1.2 Foliar blight assessment (Disease severity %)

The symptom assessment of the foliar blight, of which the data analysis is based upon, is the same in both experiment 1 and 2.

The symptom assessment was done in % leaf area covered, with e.g. 1.0% corresponding to a general light infection with 10 leaves affected per plant or 20 lesions per plant. Furthermore, 25% severity would be when the plot still looks green but every plant is infected, and 50% is when every plant is infected and half of the leaf area are necrotic, equivalent to a green plot with visible brown spots. 95% severity would be when almost all the leaves on the plant is destroyed but the stems remain green, and 100% are when all the plant biomass are dead or dying.

The symptoms assessment was performed 8 times, from the first evaluation the 29th of July to the last assessment the 17th of September, 2020. To see an example of an untreated plot and 2 plots treated with Resistim and Kumulus S from experiment 1, refer to picture 4.

Picture 4: The foliar blight severity on different plots in experiment 1, 18.09.2020 - A) Untreated control plot, B) Plot treated with Resistim/Phosphite, C) Plot treated with Kumulus S (photo credit: Tobias Hove Jensen, Flakkebjerg)

Picture 4 shows the efficacy of two plots treated with Resistim and Kumulus S, B and C respectively, compared to the untreated plot A. The pictures were taken late in the season, the 18th of September, one day after the last "official" disease severity assessment.

5.2 Materials and Methods - Experiment 2

5.2.1 Experimental design

In experiment 2, the efficacy of the commercial products Resistim and AgriCHOS (product based on chitosan), by itself and in combination with fungicides, at different dosages, against late blight, was explored. This was done by comparing the effect to the singular effect of the fungicide and the untreated control. A detailed overview of the treatments and the dosages, can be seen in table 2.

Table 2: Overview of the different treatment done in experiment 2, the applied dosage and the application interval.

Treatment	Compound	Dose L/ha	Application description
1	Untreated		
2	Ranman Top	0.25	12 applications - 7 days interval.
3	Ranman Top	0.5	12 applications - 7 days interval.
4	AgriCHOS	2.0	12 applications - 7 days interval.
5	Resistim	3.0	12 applications - 7 days interval.
6	Ranman Top AgriCHOS	0.5 2.0	Ranman Top applied the first 6 times - 7 days interval. AgriCHOS applied remaining 6 times - 7 days interval.
7	Ranman Top AgriCHOS	0.25 1.0	Ranman Top applied 12 times - 7 days interval. AgriCHOS applied 12 times - 3-4 days later than fungicide.
8	Ranman Top Resistim	0.5 3.0	Ranman Top applied the first 6 times - 7 days interval. Resistim applied remaining 6 times - 7 days interval.
9	Ranman Top Resistim	0.25 1.5	Ranman Top applied 12 times - 7 days interval. Resistim applied 12 times - 3-4 days later than fungicide.
10	AgriCHOS Ranman Top	2.0 0.5	AgriCHOS applied the first 6 times - 7 days interval. Ranman Top applied remaining 6 times - 7 days interval.
11	Resistim Ranman Top	3.0 0.5	Resistim applied the first 6 times - 7 days interval. Ranman Top applied remaining 6 times - 7 days interval.
12	Resistim Proxanil Ranman Top	3.0 2.5 0.5	Resistim applied the first 6 times - 7 days interval. Ranman Top applied remaining 6 times - 7 days interval. (Proxanil in combination with Ranman Top the 2 first applications).

These 12 treatments were established in a potato field trial with the cultivar Kuras, in a randomized complete block design with 4 repetitions each, thereby 48 trial plots in total. The treatments were applied 12 times from the first spraying the 22nd of June to the last application the 7th of September, with treatment 7 and 9 having a follow-up treatment 3-4 days after.
The trials were done on sandy-clay (JB-5) soil type.

5.2.2 Foliar blight assessment (Disease severity %)

The foliar blight was assessed 8 times from the 29th of July to the 17th of September, 2020, and the disease severity was based on the same method as in experiment 1.
To see an example of an untreated plot, and treatments with a full dose Ranman Top by itself and in combination with AgriCHOS and Resistim, refer to picture 5.

Picture 5: The foliar blight severity on different plots in experiment 2, 18.09.2020 - A) Untreated plot, B) Ranman Top at full dosage, C) Ranman Top in combination with AgriCHOS at full dosage, D) Ranman Top in combination with Resistim at full dosage (photo credit: Tobias Hove Jensen, Flakkebjerg).

Picture 5 illustrates the efficacy of 3 different treatments, both a fungicide by itself (B) and in combination with 2 alternatives (C and D), compared to an untreated plot (A).

5.2.3 Yield and starch yield assessment

In both experiment 1 and 2, the tuber yield and the starch yield were evaluated after harvest. The tuber yield was calculated in hectares, based on the plot size at 15,75 m^2 and the starch are measured in % starch per 5 kg of tubers. Due to waterlogging in certain plots, these plots had to be excluded from the evaluations. Only the starch yield results, are evaluated in this thesis.

5.3 Statistical analyses

All the statistical analysis, was done in the R language and environment for statistical computing (R Core Team, 2020). It will be the descriptive statistic that will be in focus, with the graphical illustration of the disease progress curve and % control, being shown. The area under disease progress curve (AUDPC) was calculated with the disease assessment data using equation 1, where y_i is the disease severity at the i^{th} assesment and t_i is the date/time at the i^{th} assessment, and n is the total number of assessments. Late blight control (%control) was calculated using equation 2 where AUDPCt and AUDPCu are the AUDPC for the treatment and untreated, respectively.

$$AUDPC = \sum_{i=1}^{n} \frac{(y_i + y_{i+1})}{2} \times (t_{i+1} - t_i) \text{ Equation 1}$$

$$\% \, Control = 1 - \frac{AUDPCt}{AUDPCu} \cdot 100 \text{ Equation 2}$$

The data from experiment 1 and 2, was initially fitted to the Gaussian linear model, which operates under the assumptions of normally distributed errors and homogeneity in the variance. However, a test of the assumptions showed significant departure from these assumptions, in experiment 1 the data did not show homogeneity of variance, and in experiment 2 both the Q-Qplot, Shapiro Wilks, homogeneity of variance and pairwise comparison tests, did not support the model. Thus, a model that accommodates heterogeneity (generalized least squares (GLS)) of variance was used to fit the data, by it accommodating for heterogeneity in variance allowing for variation between treatments. The same applies for the yield data, where the GLS model fitted the data better. The GlS model and variance structure were fitted with the “gls” and “varIdent” function from the “nlme” package (PINHEIRO, 2020), respectively.
The fitted GLS model was in the form of:

$$E(Y_{tr}) = T_t$$

The GLS model states that the expected value of AUDPC/Starch yield ($E(Ytr)$) for the tth treatment and rth replicate/block (r) depends on the tth treatment (T).

5.4 Results

5.4.1 Weather data

Weather data was was recorded during the experiments with a weather station (Ranch Systems) placed about 100 m from the experiment site. The weather station recorded temperature (°C) and relative humidity (RH, %) and rainfall (mm) at 15-minute interval.

The weather conditions recorded during the experiment is shown in Figure 7.

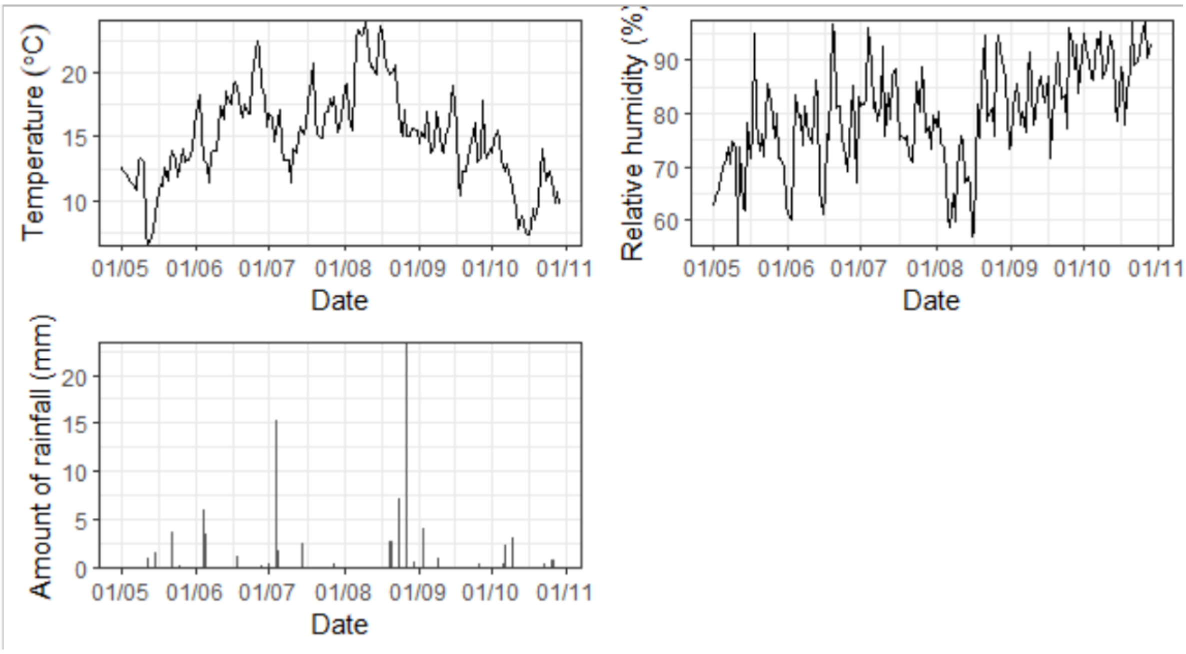


Figure 7: Temperature, rainfall and relative humidity recorded during the experiment with the Ranch system weather station. The weather station was placed at about 100m away from the experiments.

First attack of late blight was recorded on 5 August on all the plots. However, the disease development remained low until the end of August were the disease began to rise rapidly. As seen in figure 7, during these days, the weather was characterised by high relative humidity, rainfall and temperatures of about 15 °C. Generally, the disease developed successfully with final severity ranging from 95-100%.

5.4.2 Experiment 1

To get an overview of the disease development from the foliar blight assessments in experiment 1, refer to figure 8, illustrating the disease progress curve, through the trial period.

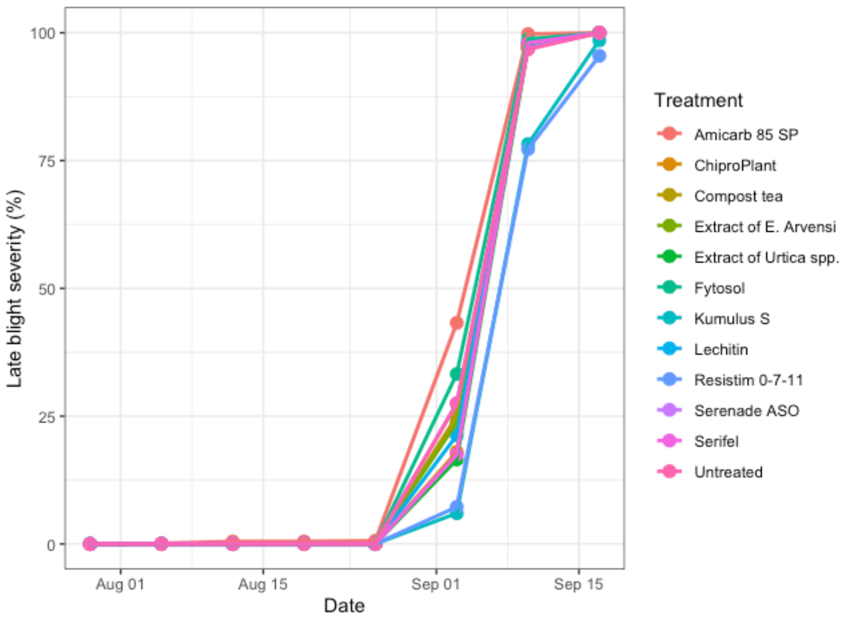


Figure 8: The disease progress curve, through the growing season, on potato plots treated with different alternatives to fungicides.

Figure 8 clearly shows the, more or less, even and non-significant disease development before late august, across all the treatments. The infection first really becomes severe after this point, with an almost immediate increase in severity towards the end of august. Though, compounds such as Resistim and Kumulus S, seems to inhibit the infection pressure with approximately a week, compared to the other treatments as well as the untreated.

Plots treated with Amicarb 85 SP, Fytosol and Serenade, shows a faster infection than the untreated. In the end of the trial period, the treatments ended with relatively the same late blight severity at 100%, as the late blight disease resulted in the complete foliar destruction of the potato plants.

The same tendency is present in figure 9, displaying the AUDPC and late blight control (%) of all the treatments done in experiment 1.

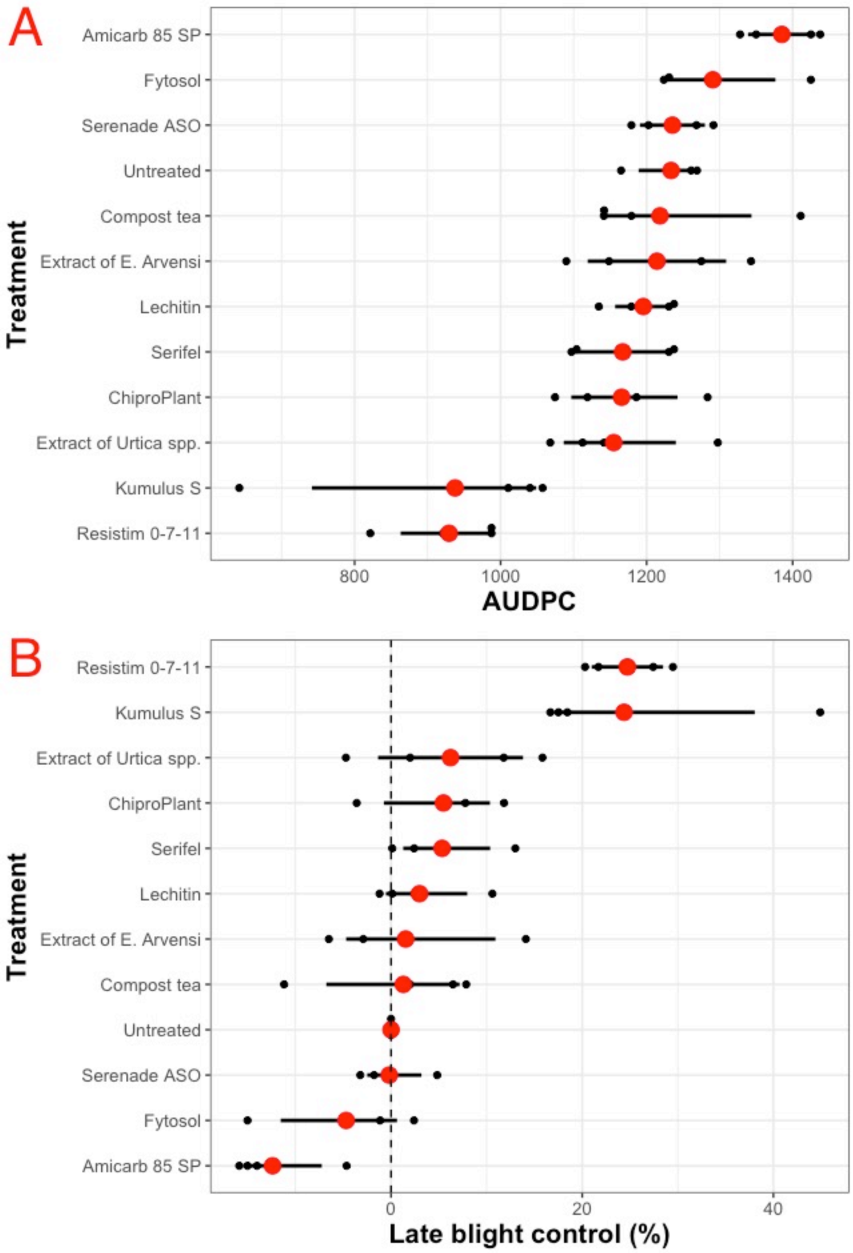


Figure 9: The AUDPC (A) and late blight control (%)(B), of the different treatments in experiment 1, with the dotted line at 0, being the untreated (the red dot is the mean and the bare is the 95% confident interval).

In figure 9 the superior effect of Kumulus S and especially Resistim, compared to the other treatments, becomes apparent. In figure 9 A, the lowest AUDPC units are between 900 and 1000 for Resistim and Kumulus S, compared to the untreated at around 1250 and Amicarb 85 SP at just below 1400 AUDPC. In figure 9 B this translates to around 25% better late blight control for Resistim and Kumulus S, compared to the untreated control. The other treatments do not differ much from the untreated, as seen by the dotted line in figure 9 B. With some having a bit better (mediocre) late blight control, such as Extract of Urtica spp, ChiproPlant and Serifel, at approximately 8, 6 and 5% better than the untreated control, respectively. With Amicarb 85 SP, Fytosol and Serenade ASO, having a lower late blight control, compared to the untreated.

To further validate the results, a pairwise comparison have been made to determine if there is a significant difference between the treatments or not. This can be found in figure 10.

contrast	estimate	SE	df	t.ratio	p.value
Amicarb 85 SP - ChiproPlant	219.44	52.9	10.02	4.144	0.0485
Amicarb 85 SP - Compost tea	166.82	70.3	8.19	2.374	0.4991
Amicarb 85 SP - Extract of E. Arvensi	171.06	63.8	8.68	2.680	0.3597
Amicarb 85 SP - Extract of Urtica spp.	230.28	56.8	9.28	4.054	0.0607
Amicarb 85 SP - Fytosol	94.49	54.0	9.73	1.748	0.8129
Amicarb 85 SP - Kumulus S	447.63	102.7	7.17	4.361	0.0591
Amicarb 85 SP - Lechitin	189.81	36.3	11.60	5.225	0.0076
Amicarb 85 SP - (Resistim 0-7-11)	455.81	47.7	10.59	9.553	0.0001
Amicarb 85 SP - Serenade ASO	149.83	38.0	11.51	3.943	0.0548
Amicarb 85 SP - Serifel	217.93	47.2	10.65	4.618	0.0224
Amicarb 85 SP - Untreated	151.68	36.0	7.43	4.208	0.0667
ChiproPlant - Compost tea	-52.61	79.2	10.48	-0.665	0.9998
ChiproPlant - Extract of E. Arvensi	-48.38	73.5	11.06	-0.658	0.9998
ChiproPlant - Extract of Urtica spp.	10.84	67.5	11.41	0.161	1.0000
ChiproPlant - Fytosol	-124.95	65.2	11.64	-1.917	0.7323
ChiproPlant - Kumulus S	228.20	108.9	8.52	2.095	0.6383
ChiproPlant - Lechitin	-29.63	51.5	9.25	-0.576	0.9999
ChiproPlant - (Resistim 0-7-11)	236.38	60.0	11.39	3.937	0.0560
ChiproPlant - Serenade ASO	-69.61	52.6	9.65	-1.322	0.9558
ChiproPlant - Serifel	-1.51	59.6	11.32	-0.025	1.0000
ChiproPlant - Untreated	-67.76	51.3	7.57	-1.322	0.9510
Compost tea - Extract of E. Arvensi	4.24	86.8	11.22	0.049	1.0000
Compost tea - Extract of Urtica spp.	63.45	81.8	10.72	0.776	0.9993
Compost tea - Fytosol	-72.34	79.9	10.53	-0.905	0.9972
Compost tea - Kumulus S	280.81	118.3	10.06	2.373	0.4895
Compost tea - Lechitin	22.98	69.2	7.77	0.332	1.0000
Compost tea - (Resistim 0-7-11)	288.99	75.8	9.65	3.814	0.0802
Compost tea - Serenade ASO	-16.99	70.1	8.02	-0.243	1.0000
Compost tea - Serifel	51.10	75.4	9.56	0.677	0.9998
Compost tea - Untreated	-15.15	69.0	6.98	-0.219	1.0000
Extract of E. Arvensi - Extract of Urtica spp.	59.22	76.3	11.15	0.776	0.9993
Extract of E. Arvensi - Fytosol	-76.57	74.3	11.06	-1.031	0.9926
Extract of E. Arvensi - Kumulus S	276.57	114.6	9.53	2.413	0.4722
Extract of E. Arvensi - Lechitin	18.75	62.6	8.17	0.300	1.0000
Extract of E. Arvensi - (Resistim 0-7-11)	284.75	69.8	10.26	4.079	0.0516
Extract of E. Arvensi - Serenade ASO	-21.23	63.6	8.46	-0.334	1.0000
Extract of E. Arvensi - Serifel	46.87	69.5	10.17	0.675	0.9998
Extract of E. Arvensi - Untreated	-19.38	62.4	7.15	-0.310	1.0000
Extract of Urtica spp. - Fytosol	-135.79	68.3	11.33	-1.987	0.6947
Extract of Urtica spp. - Kumulus S	217.35	110.9	8.84	1.961	0.7076
Extract of Urtica spp. - Lechitin	-40.47	55.4	8.66	-0.730	0.9995
Extract of Urtica spp. - (Resistim 0-7-11)	225.53	63.5	10.80	3.554	0.1044
Extract of Urtica spp. - Serenade ASO	-80.45	56.5	8.97	-1.423	0.9304
Extract of Urtica spp. - Serifel	-12.35	63.1	10.72	-0.196	1.0000
Extract of Urtica spp. - Untreated	-78.60	55.2	7.20	-1.423	0.9250
Fytosol - Kumulus S	353.15	109.5	8.61	3.226	0.1894
Fytosol - Lechitin	95.32	52.6	9.06	1.813	0.7813
Fytosol - (Resistim 0-7-11)	361.33	61.0	11.18	5.923	0.0031
Fytosol - Serenade ASO	55.34	53.7	9.38	1.030	0.9918
Fytosol - Serifel	123.44	60.6	11.10	2.037	0.6672
Fytosol - Untreated	57.19	52.4	7.40	1.092	0.9852
Kumulus S - Lechitin	-257.83	101.9	6.97	-2.530	0.4387
Kumulus S - (Resistim 0-7-11)	8.18	106.5	7.98	0.077	1.0000
Kumulus S - Serenade ASO	-297.80	102.5	7.09	-2.905	0.2988
Kumulus S - Serifel	-229.71	106.2	7.92	-2.162	0.6051
Kumulus S - Untreated	-295.96	101.8	6.67	-2.908	0.3049
Lechitin - (Resistim 0-7-11)	266.01	46.0	9.83	5.777	0.0056
Lechitin - Serenade ASO	-39.98	35.9	11.58	-1.114	0.9871
Lechitin - Serifel	28.12	45.5	9.89	0.618	0.9999
Lechitin - Untreated	-38.13	33.8	6.85	-1.128	0.9806
(Resistim 0-7-11) - Serenade ASO	-305.98	47.4	10.08	-6.458	0.0022
(Resistim 0-7-11) - Serifel	-237.89	55.0	11.28	-4.323	0.0315
(Resistim 0-7-11) - Untreated	-304.13	45.8	7.36	-6.637	0.0055
Serenade ASO - Serifel	68.10	46.8	10.12	1.454	0.9248
Serenade ASO - Untreated	1.85	35.6	6.61	0.052	1.0000
Serifel - Untreated	-66.25	45.3	7.32	-1.463	0.9139

Figure 10: Pairwise comparison of the different alternatives to fungicides treatments, with corresponding p-values, indicating if there is significant difference between the treatments (p-value below 0.05 indicates significance).

Here, the focus will be on the calculated p-values, which, if under 0.05 indicates significant difference between treatments. Based on this calculation Resistim is the only treatment with a positive significant difference from the untreated control with a p-value at 0.0055. This was not the case for Kumulus S with a p-value at 0.3049, and the other treatments, which means that their results are only tendencies (figure 10).

5.4.3 Starch yield - Experiment 1

To see the starch yield data and effect size, refer to figure 11.

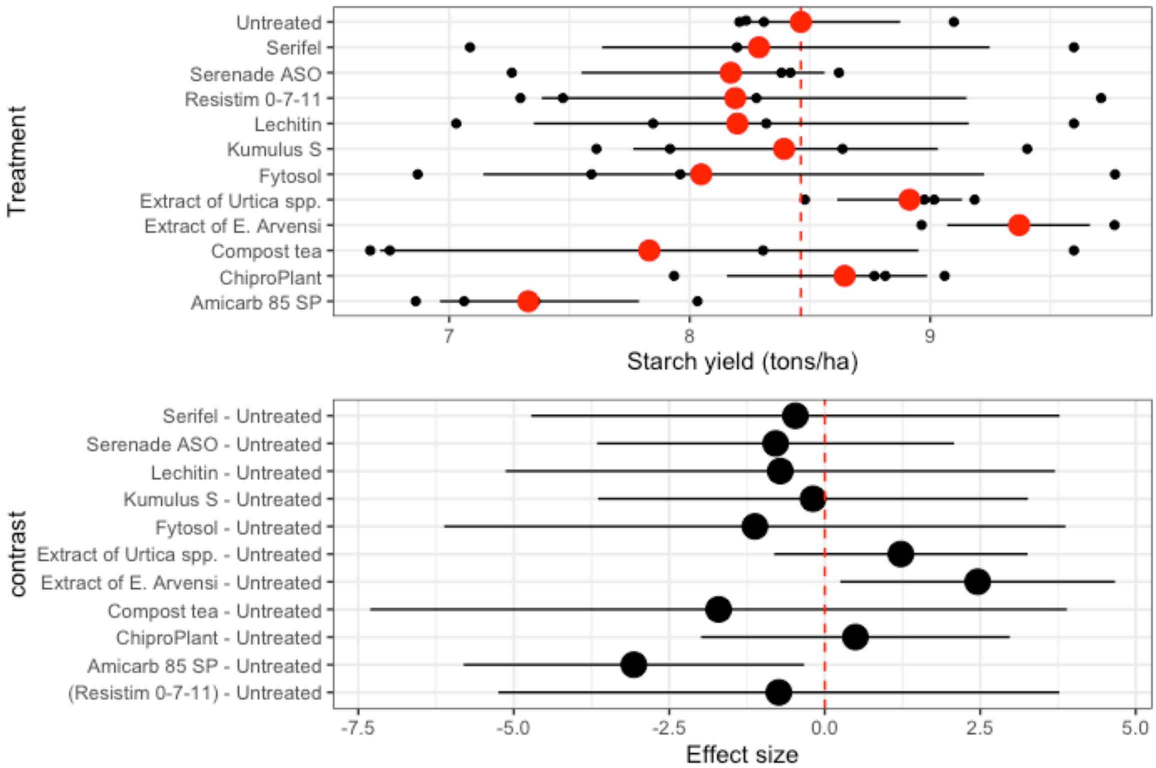


Figure 11: The starch yield in tons/ha of the different alternatives, as well as the effect size. The red dotted line is the untreated and the red/black dots are the mean and the horizontal lines are the confidents interval. The effect size is the relationship strength between the alternatives and the untreated, where a significant difference are illustrated by the horizontal lines not overlapping with the red dotted line.

When looking at the starch yield in figure 11, only the plots treated with extract of *Urtica Spp.* and *E. Arvensi* have a relative higher starch yield than the untreated with *E. Arvensi* having the best results (over 9 tons/ha). Furthermore, ChiproPlant also shows a bit higher starch yield compared to the untreated. Otherwise, all the other alternatives have a negative starch yield, lower than the untreated, with Amicarb 85 SP showing the worst results. When translating this to effect size, only *E. Arvensi* are “positive” significantly different from the untreated and Amicarb 85 SP are “negatively” significantly different from the untreated.

5.4.4 Experiment 2

To get an overview of the development of the disease, deduced from the foliar blight assessments in experiment 2, refer to figure 12, illustrating the disease progress curve, through the trial period.

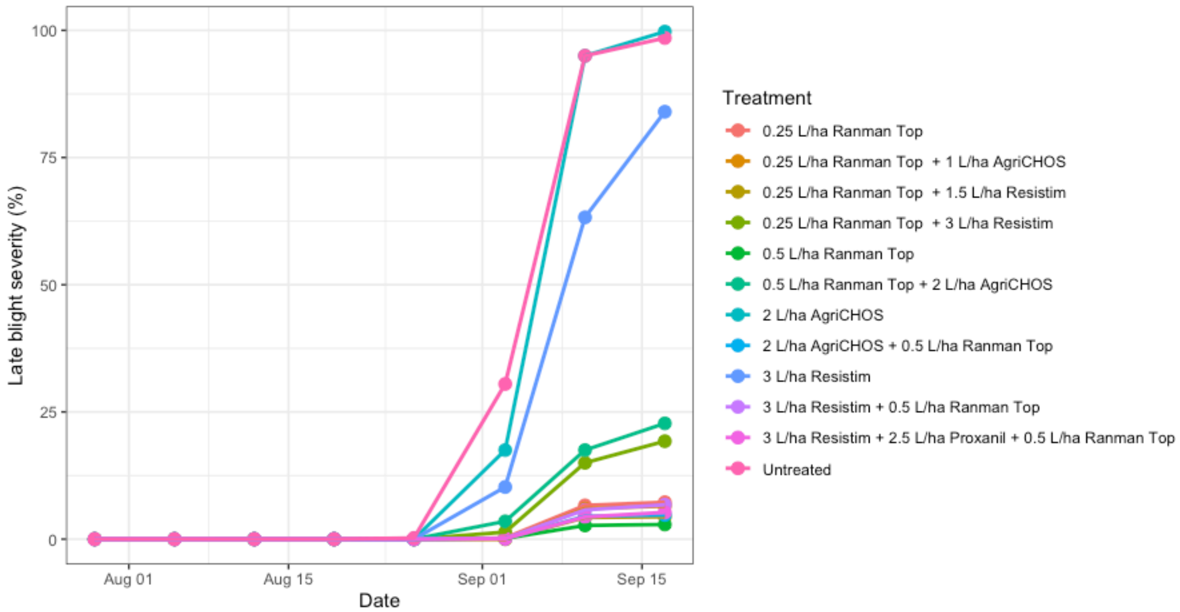


Figure 12: The disease progress curve (late blight) for the different treatment strategies in experiment 2, through the trial period.

As with the disease progress curve in figure 8, the infection progress in figure 12 also initiates in late august, with a sudden rise in infection and disease development. Here, the untreated and the pure AgriCHOS treatment, did not differ much, though with a slight inhibition of the initial disease development in plots treated with AgriCHOS, however they both ended at around 100% severity at the end of the trial period (figure 12). Followed by the better performing pure Resistim treatment, surpassed by the alternative + fungicide combinations, with the lowest late blight severity being achieved with the full dose of only Ranman Top (figure 12).

This difference in control efficacy is best illustrated in figure 13 with the AUDPC and late blight control (%) of the different treatments.

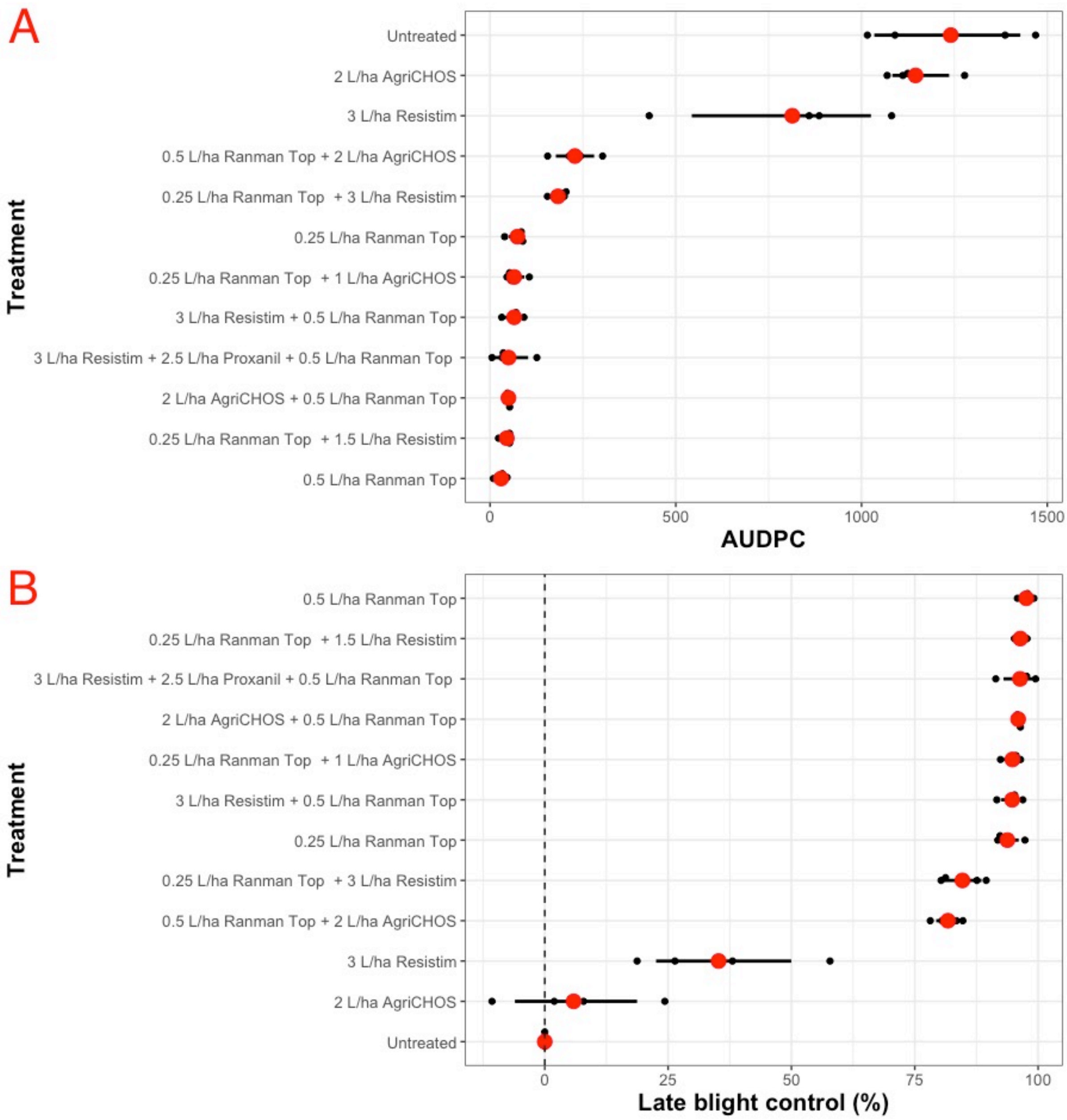


Figure 13: The AUDPC (A) and the late blight control (%) (B), of the different treatments in experiment 2, with the dotted line at 0, being the untreated (the red dot is the mean and the bare is the 95% confident interval).

In figure 13, the untreated have an AUDPC at around 1250 followed by AgriCHOS at approximately 1150 and Resistim at around 800 AUDPC, which gives around 8 and 36% (respectively) better late blight control than the untreated (figure 13).

The remaining treatments are more clustered together, with minimal variation in results, with the full dose Ranman Top treatment performing best with just over 0 AUDPC or almost 100% control, followed by the half dose Ranman Top in combination with half the dose Resistim.

This is again followed by the full dosage of Resistim, Proxanil and Ranman Top combined, followed by the full dose Ranman Top in combination with full dose AgriCHOS.

To evaluate the possible significant difference between the treatments and the control, refer to figure 14, showing a Pairwise comparison calculation of the data in experiment 2.

contrast	estimate	SE	df	t.ratio	p.value
(0.25 L/ha Ranman Top) - (0.25 L/ha Ranman Top + 1 L/ha AgriCHOS)	8.64e+00	17.94	11.36	0.481	1.0000
(0.25 L/ha Ranman Top) - (0.25 L/ha Ranman Top + 1.5 L/ha Resistim)	2.85e+01	13.69	10.24	2.083	0.6429
(0.25 L/ha Ranman Top) - (0.25 L/ha Ranman Top + 3 L/ha Resistim)	-1.09e+02	16.48	11.63	-6.619	0.0010
(0.25 L/ha Ranman Top) - (0.5 L/ha Ranman Top)	4.37e+01	13.84	10.42	3.161	0.1857
(0.25 L/ha Ranman Top) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	2.41e+01	11.64	6.69	2.069	0.6541
(0.25 L/ha Ranman Top) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	-1.55e+02	32.92	4.72	-4.702	0.0792
(0.25 L/ha Ranman Top) - (2 L/ha AgriCHOS)	-1.07e+03	46.98	7.13	-22.818	<.0001
(0.25 L/ha Ranman Top) - (3 L/ha Resistim)	-7.40e+02	138.05	6.47	-5.361	0.0257
(0.25 L/ha Ranman Top) - (3 L/ha Resistim + 0.5 L/ha Ranman Top)	8.67e+00	16.89	11.57	0.513	1.0000
(0.25 L/ha Ranman Top) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	2.38e+01	28.71	8.58	0.830	0.9984
(0.25 L/ha Ranman Top) - Untreated	-1.17e+03	111.07	6.51	-10.503	0.0006
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - (0.25 L/ha Ranman Top + 1.5 L/ha Resistim)	1.99e+01	15.66	9.36	1.269	0.9652
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - (0.25 L/ha Ranman Top + 3 L/ha Resistim)	-1.18e+02	18.15	11.44	-6.487	0.0013
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - (0.5 L/ha Ranman Top)	3.51e+01	15.79	9.53	2.224	0.5687
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	1.54e+01	13.90	6.62	1.111	0.9820
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	-1.63e+02	33.78	5.04	-4.838	0.0645
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - (2 L/ha AgriCHOS)	-1.08e+03	47.59	7.44	-22.707	<.0001
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - (3 L/ha Resistim)	-7.49e+02	138.26	6.50	-5.415	0.0241
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - (3 L/ha Resistim + 0.5 L/ha Ranman Top)	2.78e-02	18.52	11.53	0.001	1.0000
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	1.52e+01	29.70	9.33	0.511	1.0000
(0.25 L/ha Ranman Top + 1 L/ha AgriCHOS) - Untreated	-1.18e+03	111.33	6.57	-10.556	0.0005
(0.25 L/ha Ranman Top + 1.5 L/ha Resistim) - (0.25 L/ha Ranman Top + 3 L/ha Resistim)	-1.38e+02	13.96	10.08	-9.857	0.0001
(0.25 L/ha Ranman Top + 1.5 L/ha Resistim) - (0.5 L/ha Ranman Top)	1.52e+01	10.71	11.62	1.422	0.9363
(0.25 L/ha Ranman Top + 1.5 L/ha Resistim) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	-4.43e+00	7.66	7.08	-0.579	0.9999
(0.25 L/ha Ranman Top + 1.5 L/ha Resistim) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	-1.83e+02	31.73	4.27	-5.777	0.0447
(0.25 L/ha Ranman Top + 1.5 L/ha Resistim) - (2 L/ha AgriCHOS)	-1.10e+03	46.15	6.71	-23.844	<.0001
(0.25 L/ha Ranman Top + 1.5 L/ha Resistim) - (3 L/ha Resistim)	-7.69e+02	137.77	6.42	-5.579	0.0214
(0.25 L/ha Ranman Top + 1.5 L/ha Resistim) - (3 L/ha Resistim + 0.5 L/ha Ranman Top)	-1.98e+01	14.44	9.83	-1.374	0.9448
(0.25 L/ha Ranman Top + 1.5 L/ha Resistim) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	-4.70e+00	27.34	7.43	-0.172	1.0000
(0.25 L/ha Ranman Top + 1.5 L/ha Resistim) - Untreated	-1.20e+03	110.73	6.44	-10.793	0.0005
(0.25 L/ha Ranman Top + 3 L/ha Resistim) - (0.5 L/ha Ranman Top)	1.53e+02	14.11	10.26	10.835	<.0001
(0.25 L/ha Ranman Top + 3 L/ha Resistim) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	1.33e+02	11.96	6.67	11.139	0.0003
(0.25 L/ha Ranman Top + 3 L/ha Resistim) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	-4.57e+01	33.03	4.76	-1.383	0.9242
(0.25 L/ha Ranman Top + 3 L/ha Resistim) - (2 L/ha AgriCHOS)	-9.63e+02	47.06	7.17	-20.461	<.0001
(0.25 L/ha Ranman Top + 3 L/ha Resistim) - (3 L/ha Resistim)	-6.31e+02	138.08	6.47	-4.569	0.0553
(0.25 L/ha Ranman Top + 3 L/ha Resistim) - (3 L/ha Resistim + 0.5 L/ha Ranman Top)	1.18e+02	17.12	11.59	6.881	0.0007
(0.25 L/ha Ranman Top + 3 L/ha Resistim) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	1.33e+02	28.84	8.68	4.609	0.0326
(0.25 L/ha Ranman Top + 3 L/ha Resistim) - Untreated	-1.06e+03	111.11	6.52	-9.518	0.0010
(0.5 L/ha Ranman Top) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	-1.97e+01	7.92	7.03	-2.482	0.4585
(0.5 L/ha Ranman Top) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	-1.99e+02	31.80	4.29	-6.244	0.0333
(0.5 L/ha Ranman Top) - (2 L/ha AgriCHOS)	-1.12e+03	46.20	6.73	-24.151	<.0001
(0.5 L/ha Ranman Top) - (3 L/ha Resistim)	-7.84e+02	137.79	6.42	-5.688	0.0193
(0.5 L/ha Ranman Top) - (3 L/ha Resistim + 0.5 L/ha Ranman Top)	-3.51e+01	14.59	10.01	-2.405	0.4737
(0.5 L/ha Ranman Top) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	-1.99e+01	27.42	7.49	-0.727	0.9994
(0.5 L/ha Ranman Top) - Untreated	-1.21e+03	110.75	6.44	-10.929	0.0005
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - (0.5 L/ha Ranman Top + 2 L/ha AgriCHOS)	-1.79e+02	30.90	3.95	-5.789	0.0517
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - (2 L/ha AgriCHOS)	-1.10e+03	45.59	6.43	-24.043	<.0001
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - (3 L/ha Resistim)	-7.64e+02	137.58	6.39	-5.554	0.0221
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - (3 L/ha Resistim + 0.5 L/ha Ranman Top)	-1.54e+01	12.52	6.63	-1.232	0.9656
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	-2.65e-01	26.37	6.58	-0.010	1.0000
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - Untreated	-1.19e+03	110.49	6.39	-10.776	0.0005
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - (2 L/ha AgriCHOS)	-9.17e+02	55.01	7.96	-16.672	<.0001
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - (3 L/ha Resistim)	-5.85e+02	140.99	6.74	-4.151	0.0804
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - (3 L/ha Resistim + 0.5 L/ha Ranman Top)	1.63e+02	33.24	4.83	4.918	0.0648
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	1.79e+02	40.54	6.95	4.406	0.0588
(0.5 L/ha Ranman Top + 2 L/ha AgriCHOS) - Untreated	-1.01e+03	114.70	6.92	-8.821	0.0012
(2 L/ha AgriCHOS) - (3 L/ha Resistim)	3.32e+02	144.91	7.61	2.290	0.5427
(2 L/ha AgriCHOS) - (3 L/ha Resistim + 0.5 L/ha Ranman Top)	1.08e+03	47.20	7.24	22.893	<.0001
(2 L/ha AgriCHOS) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	1.10e+03	52.60	9.66	20.831	<.0001
(2 L/ha AgriCHOS) - Untreated	-9.47e+01	119.50	8.23	-0.792	0.9989
(3 L/ha Resistim) - (3 L/ha Resistim + 0.5 L/ha Ranman Top)	7.49e+02	138.13	6.48	5.421	0.0242
(3 L/ha Resistim) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	7.64e+02	140.06	6.80	5.454	0.0210
(3 L/ha Resistim) - Untreated	-4.27e+02	176.44	11.17	-2.418	0.4620
(3 L/ha Resistim + 0.5 L/ha Ranman Top) - (3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top)	1.52e+01	29.08	8.87	0.521	1.0000
(3 L/ha Resistim + 0.5 L/ha Ranman Top) - Untreated	-1.18e+03	111.17	6.53	-10.572	0.0005
(3 L/ha Resistim + 2.5 L/ha Proxanil + 0.5 L/ha Ranman Top) - Untreated	-1.19e+03	113.57	7.03	-10.482	0.0004

Figure 14: Pairwise comparison of the different alternatives in combination with fungicides, with corresponding p-values, indicating if there is significant difference between the treatments (p-value below 0.05 indicates significance).

Based on the Pairwise comparison (figure 14), all the treatments, except for pure Resistim (p-value at 0.4620) and AgriCHOS (p-value at 0.9989), showed a positive significant difference from the untreated control. All the other treatment is also significant different from the treatments with only AgriCHOS and Resistim, except for only Resistim versus 0.5 L/ha Ranman Top + 3 L/ha Resistim (p-value at 0.553), and full dose Ranman Top + full dose AgriCHOS (p-value at 0.0804). Other interesting significant differences, are that there is no significant difference between only AgriCHOS and Resistim (p-value at 0.5427). Also, even though the half dose Ranman Top + Resistim, had a better late blight control, than the full dose combination treatment, this is not significant with a p-value at 0.9448.

5.4.5 Starch yield - Experiment 2

To see the starch yield and effect size from experiment 2, refer to figure 15.

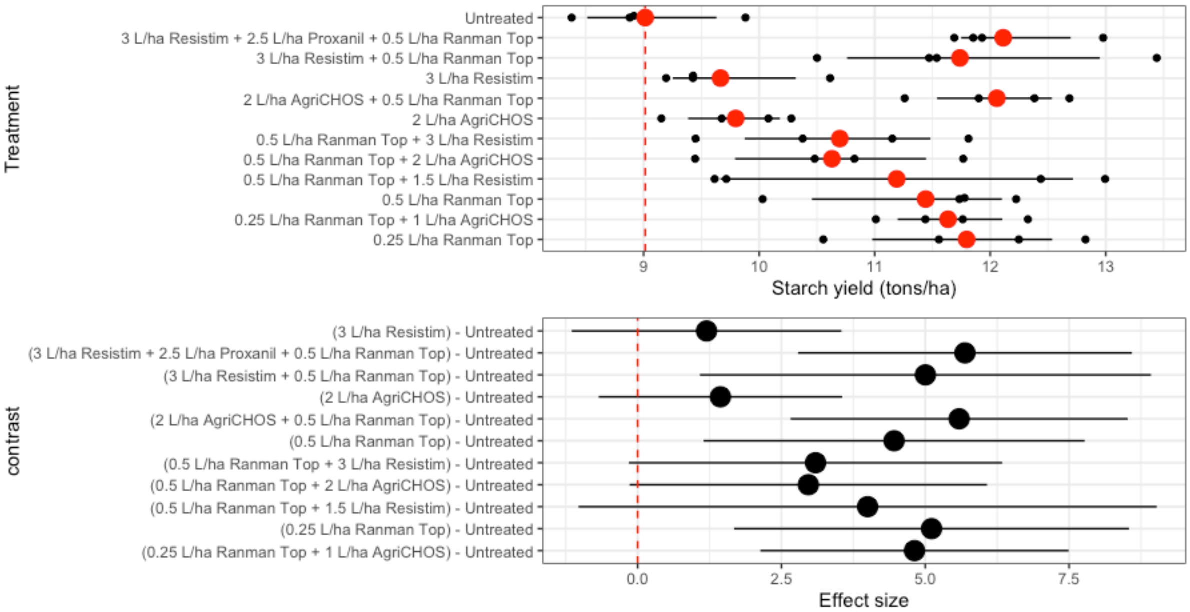


Figure 15: The starch yield in tons/ha of the different alternatives, as well as the effect size. The red dotted line is the untreated and the red/black dots are the mean and the horizontal lines are the confidents interval. The effect size is the relationship strength between the alternatives and the untreated, where a significant difference are illustrated by the horizontal lines not overlapping with the red dotted line.

When looking at figure 15, all the treatments/strategies results in a higher starch yield (tons/ha) than the untreated control at 9 tons/ha. The two lowest results are the Resistim and AgriCHOS treatments (around 9.8 tons/ha) by themselves with the treatments with the highest starch yield being Resistim + Proxanil + Ranman Top and AgriCHOS + Ranman Top Full dosage (around 12.1 tons/ha). When looking at the effect size, this translates to a positive significant difference between the treatments, Ranman Top Half dosage, Ranman Top Full, Resistim + Ranman Top Full, Resistim + Proxanil + Ranman Top, Ranman Top + AgriCHOS Half and AgriCHOS + Ranman Top Full, compared to the untreated. All the other treatments are not significantly different from the untreated control (figure 15).

6 Simulations

With the BlightManager decision support system (DSS) from Aarhus University, simulations were made based on different scenarios (such as organic or conventional potato production), to evaluate how best alternatives can be integrated into late blight control with or without fungicide treatments. This part of the thesis will be mostly speculative, due to the lack of concrete data available to support some of the assumptions.

6.1 Case scenarios

The case scenarios act as an overview and guideline for the making of the simulations/scenarios. Not all the mentioned actions in the scenarios, will be further expanded upon in the BlightManager DSS, because of limitations of the program to express these. This especially applies for scenario 3.

Simulation 1 focusses on resistant cultivars (Organic) and Simulation 2 focusses on reduced fungicide dosage (Conventional), for an overview refer to table 3.

Table 3: Scenarios based on an organic potato production, with resistant cultivars and the efficacy of alternatives to traditional fungicides, in mind. Furthermore, a conventional potato production with focus on the use of traditional fungicides in combination with alternatives to traditional fungicides.

Scenario 1	**Scenario 2**	**Scenario 3**
Simulation 1: - Resistant cultivars Simulation 2: - Conventional cultivar Other factors that will be discussed: - Certified seed - Early maturing cultivars - Early planting - Pre-sprouting	Simulation 1: - Alternatives to traditional fungicides - Application time Simulation 2: - Traditional fungicides - Alternatives to traditional fungicides - Application time Other factors that will be discussed: - Sanitary measures	Other factors that will be discussed: - Precision farming

6.2 Materials and Methods

6.2.1 Application, dosage and interval

The default dosage In the BlightManager DSS, are an estimated dosage based on available traditional fungicides, varying depending on the dose model (cultivar) chosen in the DSS. More specific, it indicated if there should be used 50, 75 or 100% dose of full dose Ranman Top (cyazofamid) (0,5 L/ha) or Revus (mandipropamid) (0,6 L/ha) (Hansen et al., 2019). Here, the experiment 1 and 2 data, will primary be used to estimate the dosage and days between application (Max day protection) of the alternatives, so it can be edited in the DSS default dosage, as required.

6.2.2 Disease surveillance network data

Data from the danish late blight registration network, have been made available for use, by Aarhus University research center of Foulum, provided by Jens Grønbech Hansen. This is a database where users (e.g. agricultural consultants) can register any case of late blight symptoms, with year, location, crop and cultivar etc., allowing for an overview of the spread of *P. infestans*. This can be used in determining the beginning of attack and spread in a certain location a certain year, which is essential in the creation of the simulations in the DSS (Hansen et al., 2019).
This data will be the foundation for when the different phases (except phase 4), in the DSS, will start. Phase 1 is when there are no observed sightings of late blight within the country, phase 2 is late blight attack in the country, phase 3 is attack in the region (<50 km away) and phase 4 is attack in the field (Hansen et al., 2019) or in the case of these simulations, when the resistance of the different cultivars breaks. The recommended dosage of the control product, will differ based on the current phase as well as the infection pressure. Also, because this information affects the application dosage used, it will be part of the calculation of the treatment frequency index (Hansen et al., 2019).

6.2.3 Late blight Infection risk and infection pressure

The infection pressure (actually sporulation pressure), used in the model, depends on different parameters such as the location and weather conditions. E.g. will 2020 and 2019 at Slagelse (representative for Flakkebjerg) be in focus in the simulations, because 2019 was wetter than 2020, comparatively. The difference in the years will have had an effect on the infection patterns (low and high infection pressure) of *P. infestans* (Hansen et al., 2019).
The infection risk model predicts when infection is likely to occur based on the temperature and leaf wetness or RH%. A red bar is shown in the infection risk sub-model when infection is likely to occur (Hansen et al., 2019).

Further determination of spray dosage used in the different phases, depends on the weather data in the certain year and location, as the DSS will use temperature, relative humidity and precipitation to predict the favorability of weather for late blight attack (Hansen et al., 2019). When these parameters have reached a specific threshold a specific duration of time (Default setting in DSS), determine infection pressure, infection risk and protection period. This will then affect what dosage is recommended, based on an infection pressure at 0 (no risk), 1-20 (low), 21-40 (moderate), 41-60 (high) and >60 (very high infection pressure) (Hansen et al., 2019).

6.2.4 Cultivar data from trap nurseries

To validate the speculations regarding the efficacy of the cultivars used in the simulations, data from trap nurseries with late blight severity data on a wide range of potato cultivars, have been made available by Aarhus Unversity Department of Agroecology Flakkebjerg, supplied by Isaac Kwesi Abuley. In the simulations, three specific cultivars have been chosen, a susceptible cultivar Bintje, a mediocre susceptible - commonly used cultivar Kuras and a resistant cultivar Nofy. Furthermore, the location of Flakkebjerg have been selected, to somewhat match the data from experiment 1 and 2. The different cultivars are expressed in the DSS in the form of dose models that, among other, differs in the recommended dosage (default dosage calculated in the DSS) based on the assessed resistance of the cultivar (Hansen et al., 2019). E.g. Bintje would necessitate a higher fungicide dosage in more phases, than would be the case with the resistant cultivar Nofy. Furthermore, because the trap nursery data, more precisely, represents the start of the epidemic development at the location of Flakkebjerg for the different cultivars, thus the precise time that the different cultivar resistances breaks, it will be the initiation of phase 4.

With the available cultivar data, the author has made some descriptive statistical analysis in the form of a disease progress curve, meant to illustrate the differences in late blight resistant between the 3 cultivars. This can be seen in figure 16.

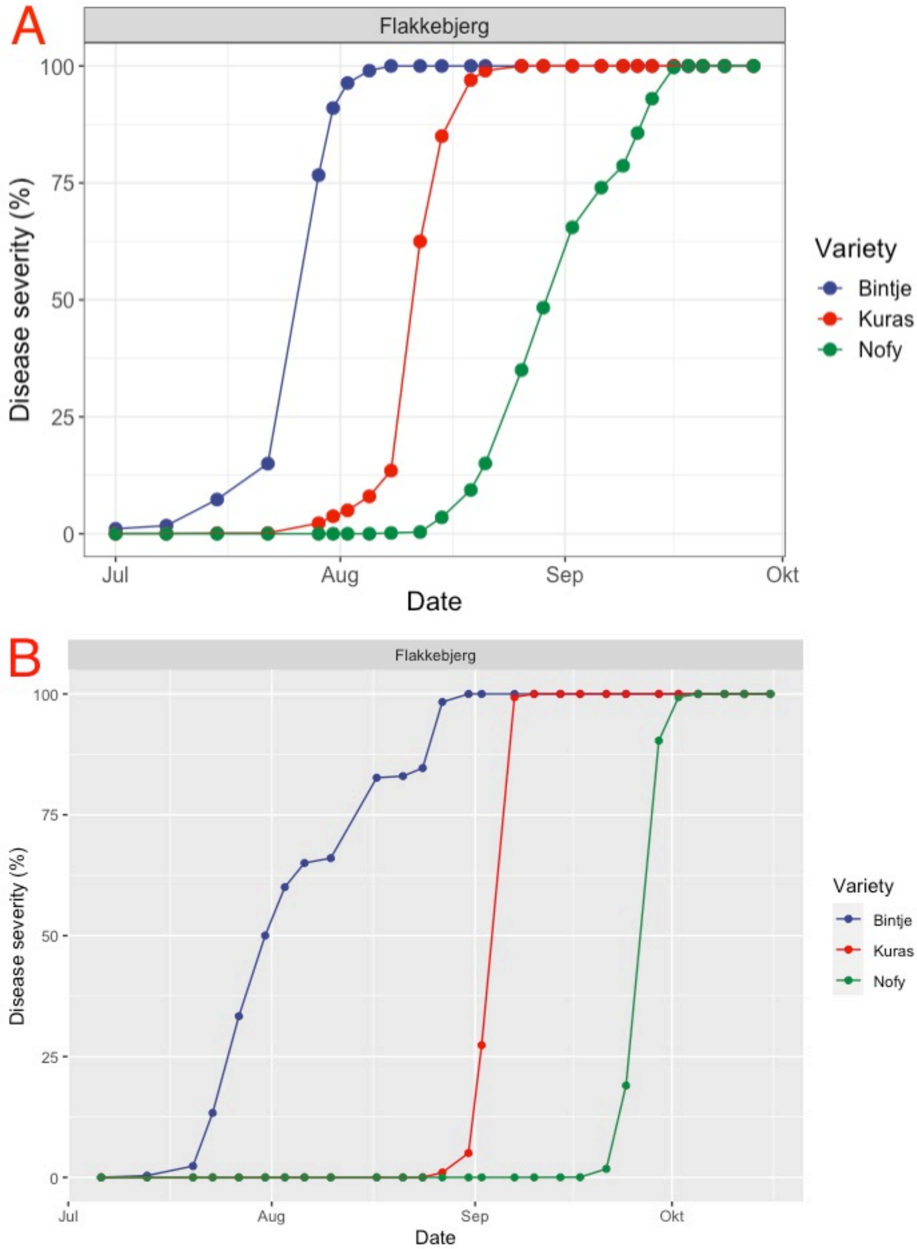


Figure 16: The disease progress curve of late blight, on 3 cultivars with different susceptibility to the disease, at Flakkebjerg 2019 (A) and 2020 (B).

In figure 16 the disease severity (%) of late blight on Bintje, Kuras and Nofy, through the growing season of 2019 and 2020, are illustrated. Most conspicuous are the earlier initiation of infection seen on the susceptible cultivar Bintje, compared to the two other cultivars. Already becoming symptomatic at the start of July, where Kuras shows infection in the end of July in 2019 and in late august in 2020, and Nofy around mid-August in 2019 and mid-September in 2020. Even with the big difference in late blight resistance, it seems that once the resistance is first broken, the disease severity increases rather rapidly, with all 3 cultivars ending at 100% severity. However, it seems that Nofy is able to slow down the disease severity, even after the resistance have been broken (at least In 2019), compared to the other cultivars, with a less steep increase seen in figure 16. All in all, Kuras delays the 100% disease severity with around 2 weeks compared to Bintje, and Nofy delays it around 3 weeks compared to Kuras and 5 weeks compared to Bintje, in 2019, and the difference are even more significant in 2020 (figure 16). The extra time before complete foliar destruction, can be valuable especially during tuber filling and starch production.

6.2.5 Other factors

The start date and stop date is chosen to specify the duration of the simulations. The stop date selected is the 30th September based on the assumption that cultivars for starch production, needs a longer growing period for the starch filling. Crop emergence and start date will be set at 20th of May, as a general mean.

6.3 Results

6.3.1 Simulation 1 and Simulation 2

The following years, location and weather, will be used in both simulation 1 and 2. The scenarios will be in 2019 where the infection pressure was higher, and 2020 where it was lower, comparable, at the location of Flakkebjerg (Slagelse). To see the weather patterns and thus the infection pressure and risk, in 2019 and 2020 in the location of Flakkebjerg, refer to figure 17, showing the late blight infection risk and infection pressure calculated in the DSS.

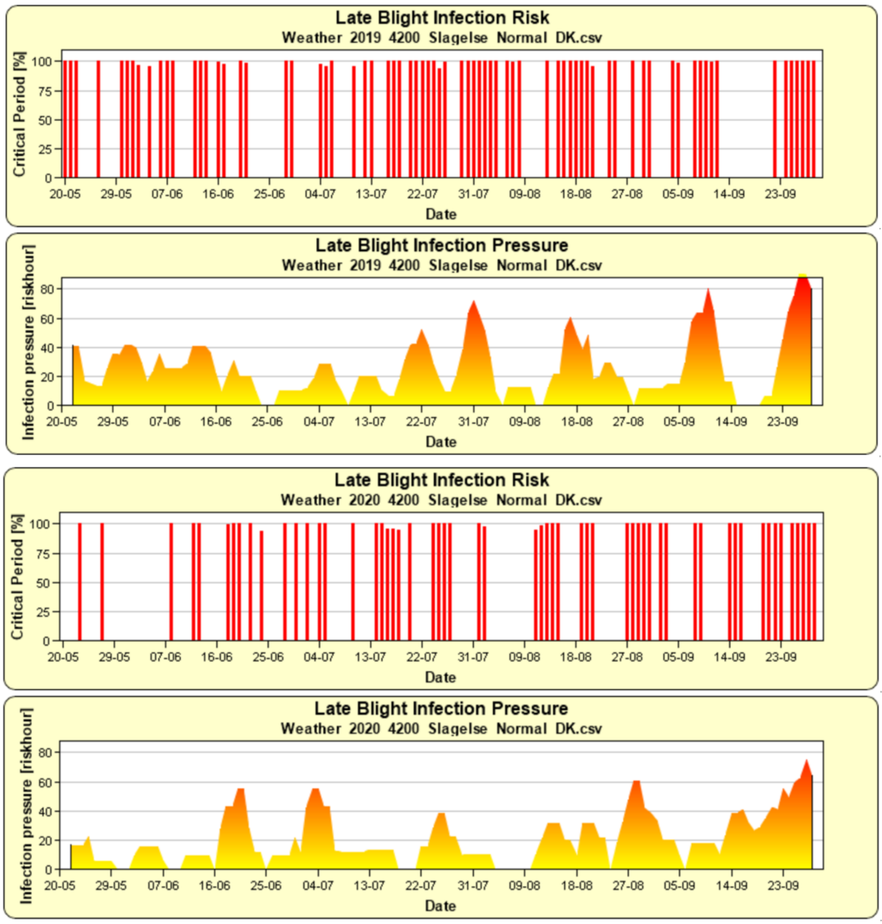


Figure 17: The late blight infection risk and pressure for 2019 and 2020, at the location of Slagelse (representative for Flakkebjerg). The top half of figure 15 are 2019 and the bottom half are 2020.

Figure 17 shows how the weather affected the late blight infection pressure, both in 2019 and 2020. With more days with risk of infection as well as a higher infection pressure in general, in 2019 compared to 2020. This difference becomes especially apparent when looking at the infection risk which have more critical periods in 2019.

Based on the data from the late blight disease surveillance network, in 2019 late blight was first observed 12.06.2019, initiating phase 2. Late blight was then found in the region 14.06.2019 (phase 3) and based on an approximation from the results in figure 13, phase 4 was initiated around the 01.07.2019 for Bintje, 22.07.2019 for Kuras and the 12.08.2019 for Nofy.
In 2020 late blight was first registered 12.06.2020 in the country, then observed in the region at 09.07.2020 and broke the different cultivar resistances at Flakkebjerg the 13.07.2020 for Bintje, 21.08.2020 for Kuras and 17.09.2020 for Nofy.

The following part illustrates scenario 1 in simulation 1 and 2 (table 3). This is due to the fact that the two case-scenarios are more or less identical in scenario 1, where the focus is primarily on the resistant cultivar Nofy or the cultivar Kuras. To illustrate the resistant cultivar Nofy, the dose model, Model B+ 2020 (fewer fungicide application and lower dosage), are chosen, and for Kuras

Model A 2019 are used and for Bintje a custom model is used. To see the difference in recommended dosage, treatment frequency index and late blight protection period, between the 3 cultivars, in 2019, refer to figure 18.

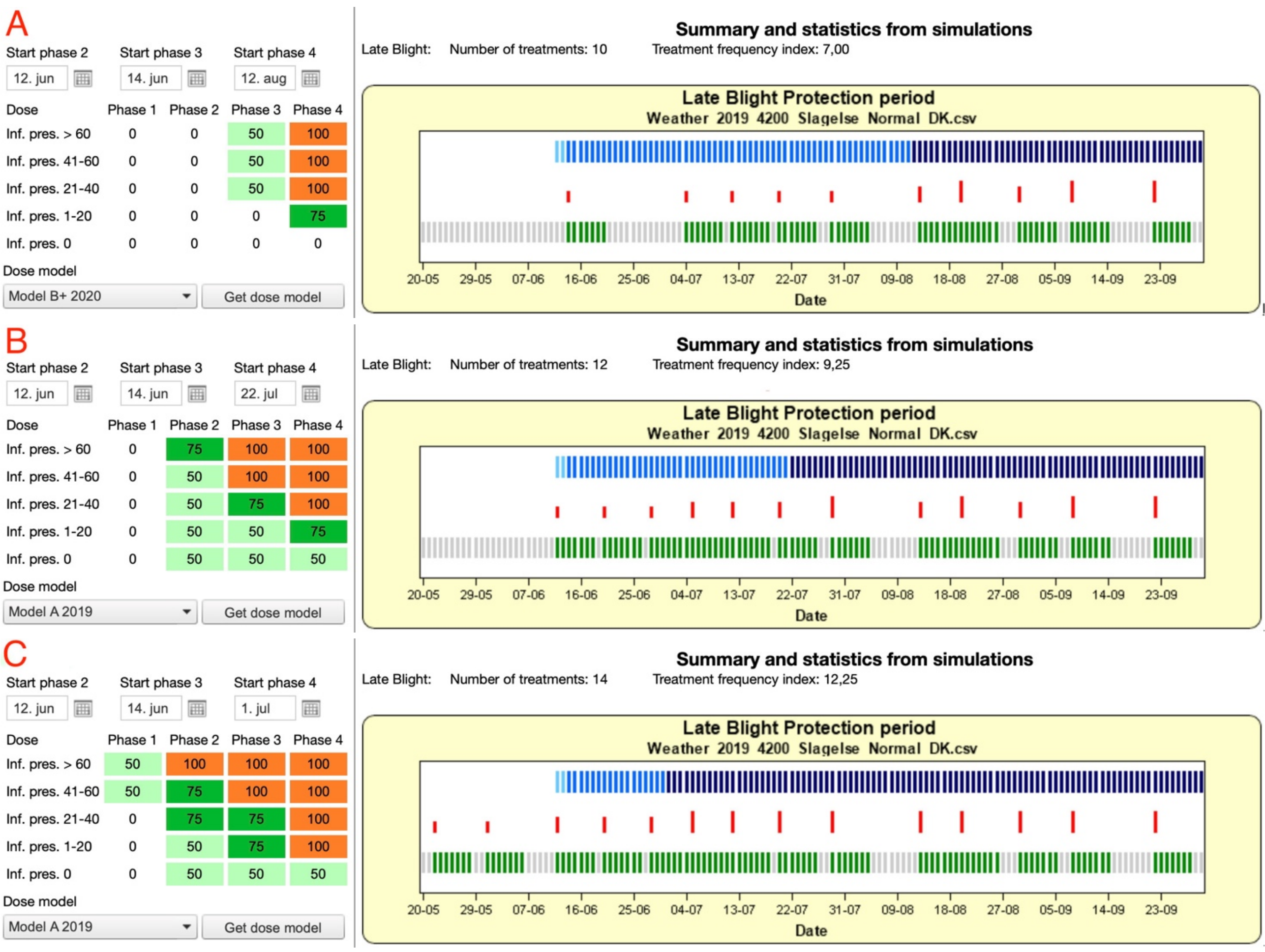


Figure 18: The recommended dosage at the different phases, and the effect on the treatment frequency, by A) Nofy, B) Kuras and C) Bintje, 2019. Vertical red line, signifies dosage (%) of treatments applied relative to a full dose (%). BlightManager DSS.

Figure 18 illustrates the difference between the recommended dose (with 7 days application interval) and treatment strategy, based on the cultivar. The recommended dosage varies significantly, and is made based on the information found in figure 16, that e.g. shows that Bintje are earlier infected by the disease, therefore it makes sense to recommend spraying already in phase 1. When comparing Nofy (A) and Kuras (B), there is a difference in number of treatments and treatment frequency index. Respectively, at 10 treatments and 7.00 frequency for Nofy and 12 treatments and 9.25 frequency index for Kuras. When looking at Bintje (C) it needs 14 treatments at a 12.25 frequency, which is especially due to the application in phase 1 and the higher dosage in phase 2. This is different from both Nofy and Kuras.

To see the difference in 2020, refer to figure 19.

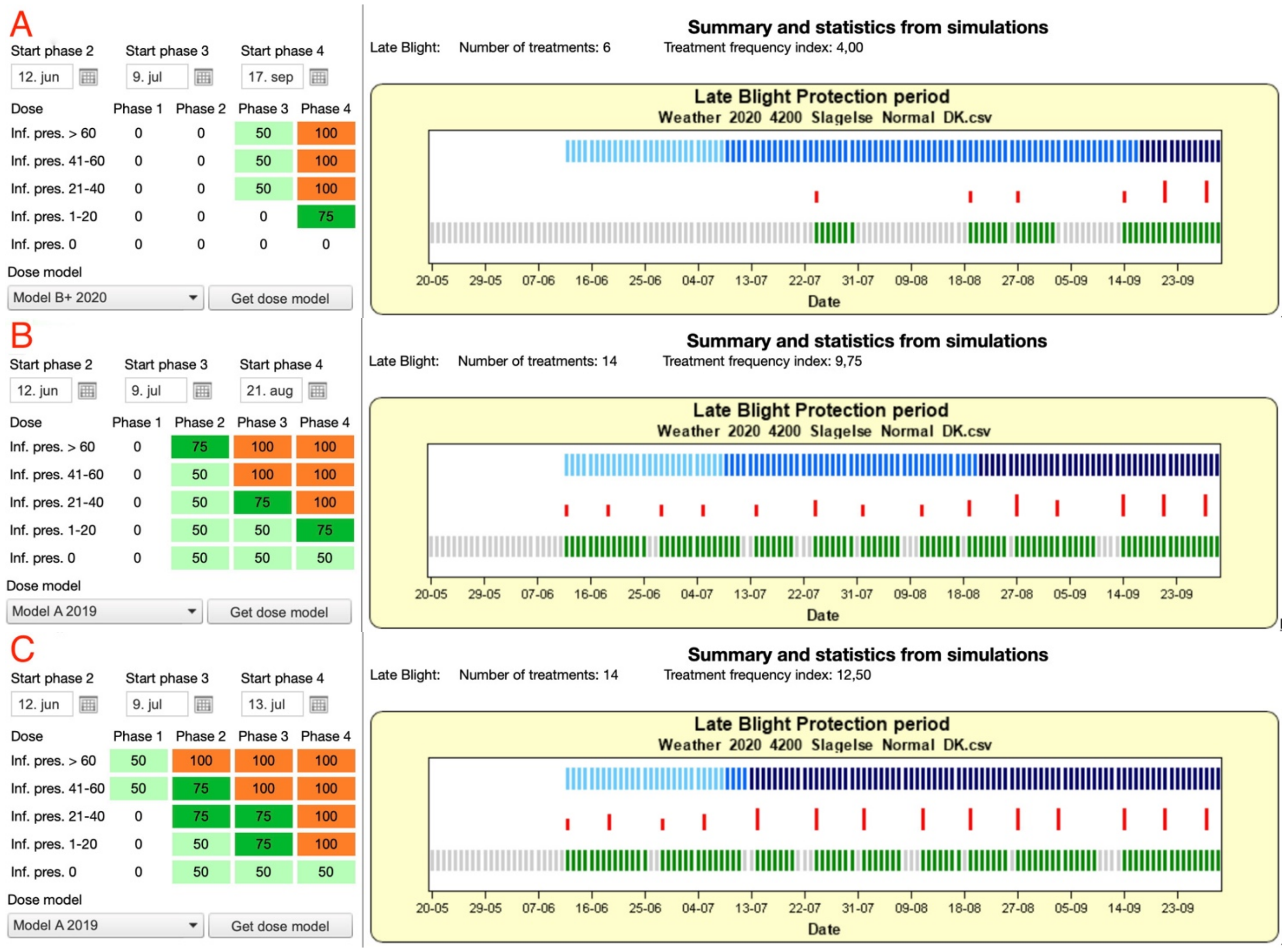


Figure 19: The recommended dosage at the different phases, and the effect on the treatment frequency, by A) Nofy, B) Kuras and C) Bintje 2020. Vertical red line, signifies treatments. BlightManager DSS.

In figure 19, the same cultivars (dose models) as figure 18, are used, but with the weather and infection patterns from 2020. There is a bigger difference between Nofy (A) and Kuras (B), because of the longer time interval between the phases due to the difference in infection pressure. Here, Nofy have 6 treatments and a frequency at 4.00 compared to Kuras with 14 treatments and 9.75 frequency, due to Nofy not getting treated in phase 2 and at lower dosage in the other phases. Bintje gets 14 treatments and a frequency at 12.50, the same number of treatments as Kuras but with a higher frequency, due to the general higher dosage.

If comparing between 2019 (figure 18) and 2020 (figure 19), even though, the infection is higher in 2019, e.g. Kuras is still treated more in 2020, especially because of the difference between the duration of phase 2. Making it hard to directivity compare. All in all, it illustrates the importance of using a resistant cultivar compared to a susceptible variety.

6.3.2 Simulation 1

The next part will focus on scenario 2 in simulation 1, which will build upon scenario 1 in simulation 1. By simulating the possible efficacy certain alternatives will have on the recommended dosage and or timing/application interval (max day protection), seen in figure 18 and 19. Because simulation 1 is an organic production, the following part will center on the use of the alternatives by themselves, with the resistant cultivar Nofy, and the simulations have been divided based on "high efficacy alternatives" and "low efficacy alternatives".

Due to the superiority of Resistim and Kumulus S in experiment 1, a simulation will be made, based on the information and results from these 2 compounds (high-efficacy alternatives). Furthermore, another simulation will be made, representing the less effective alternatives, with a late blight control ranging from 5 to maybe 15%, compared to the untreated. The simulation with high efficacy alternatives, will have 5 days interval between application (max day protection) and the simulation with low efficacy alternatives, will have 3 days interval between application.

To see the simulation based on e.g. Resistim and Kumulus S, with Nofy in 2019 and 2020, refer to figure 20.

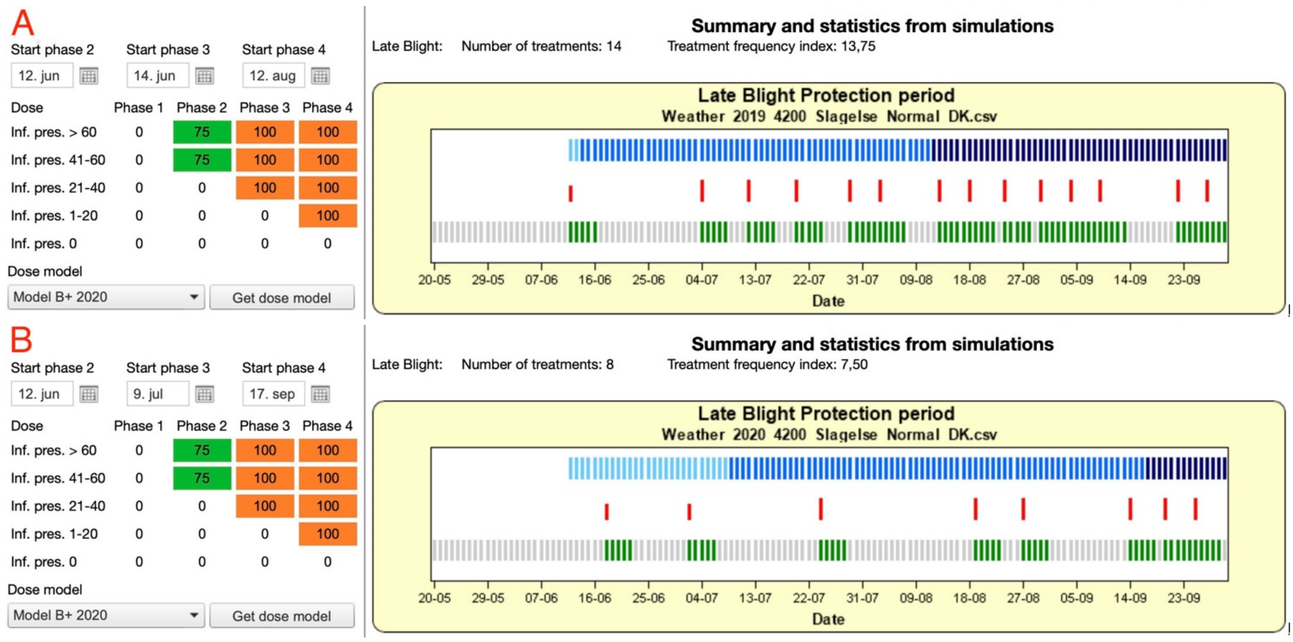


Figure 20: The recommended dosage of alternatives (high efficacy alternatives), in an organic production with the cultivar Nofy, in 2019 A) and 2020 B). 5 days between applications. BlighManager DSS.

When comparing to figure 18 and 19 A, in figure 20, the dosage has been changed to 100%/full available dose in most cases. This is done to ensure that the alternatives control late blight as much as possible, especially knowing the general lack of control persistency of alternatives (O'Brien, 2017). Even though an efficacy on par with traditional fungicides, cannot possible be achieved with the full dosage/allowed dosage of the alternatives as seen in experiment 2. However, in phase 2, a 75% dosage is recommended at the 2 highest infection pressures, which may be even more relevant in 2020 where the duration of phase 2 are longer. The reason for the lower dosage as well as no further dosage recommendations in all the phases, are because of the cultivar's resistance. As seen in figure 16, Nofy actually inhibit the infection for a big part of the growing season, and too much spraying, would be a waste. Another addition in figure 20 are the application interval, which have been changed from the "normal" 7 days for the fungicides, to 5 days. All in all, as seen in figure 20, in 2019 (A) this resulted in 14 treatments and a treatment frequency at 13.75 and in 2020 (B) 8 treatments and a treatment frequency index at 7.50.

To see a simulation based on alternatives with less efficacy, in 2019 (A) and 2020 (B), refer to figure 21.

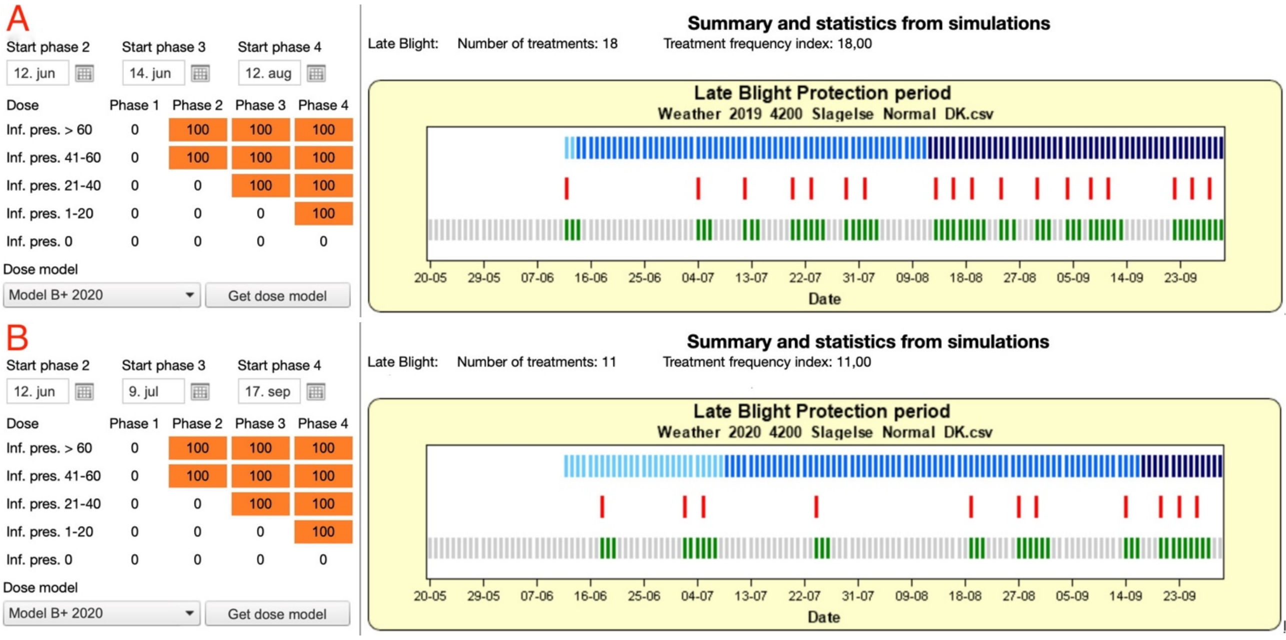


Figure 21: The recommended dosage of alternatives (Low late blight efficacy), in an organic production with the cultivar Nofy, in 2019 A) and 2020 B). 3 days between application. BlighManager DSS.

In figure 21, the same reasoning applies as in figure 20. However, due to the even lower effect against late blight, a full dose is also recommended in phase 2. The most significant change is the application interval, at 3 days, in this simulation. This also increases the number of treatments, significantly. With 18 treatments (18.00 frequency) in 2019 (A) and 11 number of treatments (11.00 frequency) in 2020 (B).

In the case of both figure 20 and 21, if Nofy was replaced with Kuras or Bintje, it would be essential to spray more in phase 2, at full dose, and phase 1 (especially with Bintje). Since, the infection would start earlier due to the lack of cultivar resistance as seen in figure 16. To put it into perspective if Nofy was replaced with Kuras in the BlightManager DSS, under the same criteria's, the number of treatments would reach around 17 treatments at a 17.00 frequency index in 2019 and 16 treatments at 15.00 treatments frequency index in 2020, with the high efficacy simulation.

The numbers would be even higher with the simulation based on low efficacy alternatives to traditional fungicides (23 treatments and 23.00 index in 2019 and 22 treatments at 21.00 index 2020). The number of treatments is even higher with the very susceptible cultivar Bintje.

6.3.3 Simulation 2

This part will build upon scenario 1 in simulation 2, with scenario 2. This case-scenario are the conventional production, with the cultivar Kuras (and compared to Nofy and Bintje), where alternatives are used in the combination with fungicides. The focus will be on a simulation with the reduction in the recommended fungicide, by supplementing with alternatives. This will be calculated, by comparing to the "normal" recommended fungicide application seen in figure 18 and 19, depending on the different cultivars.

The foundation for this simulation is based on different assumptions and criteria. In phase 1, no spraying of fungicide or alternative is necessary because late blight is not observed in the country at that stage, except for Bintje. In phase 2 and 3 the alternatives will replace the fungicides in the low infection pressure periods (1-20), in the DSS, due to the assumption that the alternatives have a higher late blight effect, under lower infection pressures. In phase 4, only fungicides are used, and no alternatives. The alternatives will at all times be applied at full dosage, to optimize the potential late blight effect.

With focus on 2019, When looking at the infection pressure in figure 17, and the time of treatment (red vertical bars) in figure 18, the infection pressure during treatment can be determined. In the case of Kuras (figure 18 and 19 (B)), 3 treatments are done with an infection pressure under 20 before phase 4, and can be replaced with an alternative treatment. Therefore the 12 treatments at 9.25 treatment frequency index, can be reduced to 9 fungicide treatments with a treatment frequency index at 6.75. This can be converted to a 16.21% reduction in fungicides, compared to the standard fungicide recommendations for Kuras. In the case of Nofy, due to the previously decided criteria and that no fungicide is applied before phase 4, in the 1-20 infection pressure range, it is not comparable. Bintje, had 14 treatments at an index at 12.25, where 2 can be replaced. Resulting in 12 treatments at a treatment frequency index at 10.75, which is a reduction in fungicide at 12.24%.

In 2020, based on the infection pressure in figure 17 and the treatments in figure 18 B, Kuras had 14 treatments at an index at 9.75, of which 5 50% dose treatments can be replaced with alternatives. Therefore, it can be corrected to 9 fungicide treatments at a treatment frequency index at 7.25, a reduction in fungicides of 25.64%. Due to the beforementioned reason, Nofy are not included. Bintje had 14 treatments at an index at 12.50, here 2 treatments can be replaced with alternatives, thus 12 treatments with a treatment frequency index at 11.50, so an 8% reduction.

To further put the potential fungicide reduction into perspective, another criteria was added, which allows for the replacing of fungicides with alternatives, at moderate infection pressure (21-40). This simulation would be applicable to the alternatives with a higher efficacy against late blight, to be able to control the disease at moderate levels.

In Kuras 2019 (Figure 18 B), 5 of the treatments were either under low or moderate infection pressures, so reduced to 7 treatments at a 6.5-treatment frequency index. Corresponding to a 29.72% fungicide reduction. Nofy are reduced to 7 treatments at a 5.5 index, so a 21.42% fungicide reduction. Bintje are reduced to 11 fungicide treatments with a treatment frequency index at 10,00, being a 18.36% reduction in fungicides.

Kuras in 2020 (Figure 19 B), resulted in 7 treatments instead of 14 with a 5.75 treatment frequency index instead of 9.75, corresponding to a 41.02% fungicide reduction. In Nofy the treatments were reduced to 3 with a treatment frequency index at 2.5, translated to a 37.5% fungicide reduction. In the cultivar Bintje, the treatments were reduced to 12 with a corresponding 11.50 treatment frequency index, being an 8% fungicide reduction.

7 Discussion:

7.1 Efficacy of the alternatives to traditional fungicides against late blight in potato plants

The literature survey and the results from experiment 1 and 2 presented in this thesis, focused on finding potential promising alternatives to fungicides in the control potato late blight, a disease whose control relies on a repeated fungicide application (Haverkort et al., 2008). Furthermore, by evaluating the efficacy of alternative products as a standalone treatment (experiment 1) and in combination with fungicides or other alternatives (experiment 2). The potential and efficacy of surveyed literature, will be compared and discussed through the discussion.

7.1.1 Standalone control efficacy against late blight of alternatives to fungicides

The weather in 2020 at Flakkebjerg during the trials was relatively dry, thus inhibiting the sporulation potential of *P. infestans (Narouei-Khandan et al., 2020),* thereby increasing the potential efficacy of the alternatives (O'Brien, 2017). Though the lower infection pressure in 2020, are not easily seen in the results from experiment 1, with varying effect of all the alternatives to fungicides, with most displaying under 10% better control (except Resistim and Kumulus S) compared to the untreated control plots. However, when comparing to the trial results from Flakkebjerg in 2019 (Hansen and Abuley, 2020), where the same 12 alternatives as in experiment 1, was tested during a season with a higher infection pressure of *P. infestans,* with in general an even lower efficacy of the compounds. Here most of the tested alternatives to fungicides, had a lower control percent than the untreated control plots, with only Resistim, Kumulus S, ChiProPlant and extract of Urtica yielding a higher control (Hansen and Abuley, 2020). So the relative low control efficacy found in experiment 1, actually performed better than the year before, with higher infection pressure (Hansen and Abuley, 2020). Suggesting that the general low efficacy shown in experiment 1, might be the best case scenario, and that alternatives by themselves might be insufficient (Hansen and Abuley, 2020), and highlights the importance of incorporating resistant cultivars (Cooke et al., 2011) or using reduced fungicide dosages in combination with the alternatives (Zhang et al., 2020).

The alternative Resistim had the highest percent control of late blight in experiment 1, with around a 25% higher control than the untreated control, which is still not an overwhelming effect (Liljeroth et al., 2020), compared also to the approximately 40% in 2019 (Hansen and Abuley, 2020). Though, the beforementioned inhibition during higher infection pressures, does not seem to be the case for Resistim, which performed better under the higher infection pressure in the 2019 Flakkebjerg trials (Hansen and Abuley, 2020), compared to 2020. Corresponding to the difference in Phosphite efficacy between years, seen in the trials by Liljeroth et al. (2020). Signifying that this compound is not restricted by the infection pressure, in the same degree as the other alternatives (Hansen and Abuley, 2020), so the restriction in efficacy may be due to other parameters, like the difference in tolerance and sensitivity against phi-based products, between different *P. infestans* genotypes (Huang et al., 2018).

Another alternative control method of *P. infestans* that as a single product showed potential, in experiment 1, was Kumulus S, with a late blight control (%) on par with Resistim. Besides the fact that there are a very limited available peer-reviewed literature on this compound, also this relatively high effect seen in experiment 1, are not supported by the analyzed literature, with no significant effect against late blight, found by Ryant et al. (2008). Furthermore, the efficacy (% control) achieved by Kumulus S in the Flakkebjerg trial 2019 (Hansen and Abuley, 2020), resulted in approximately a 3 % control compared to the untreated. Suggesting that the effect of the compound is not reliable, and also that it is highly inhibited by a higher infection pressure.

Serifel (*Bacillus amyloliquefaciens*) was the product, based on microorganisms, that had the best control effect in experiment 1, in correspondence to the moderate efficacy result seen in trials by Caulier et al. (2018). Furthermore, the Serifel results from experiment 1 were also more effective than Serenade ASO (*B. subtilis*), which had a lower control efficacy compared to the untreated control plots. However, this was not supported by Caulier et al. (2018) who found that *B. subtilis* had a better control efficacy than the untreated control, and this was further supported by Kumbar et al. (2019) that demonstrated a significant effect of *B. subtilis* against *P. infestans*, and also controlled late blight better than *B. amyloliquefaciens*. Likewise, in the 2019 Flakkebjerg trials (Hansen and Abuley, 2020), Serenade ASO had higher controlling probertites against *P. infestans* than Serifel, though both still had a lower control efficacy than the untreated control plots. This indicates that the effect of Serifel as well as Serenade ASO are inconsistent, especially in the case of Serenade ASO, and this may call for more field experiments with different potato cultivars under different climatic conditions before a potential biocontrol strategy against *P. infestans* based on these products may be developed.

Moreover, the alternative compost tea is built up on many different microorganisms with varying MOA and production techniques (Marín et al., 2013), and the compounds analyzed in the literature will most likely vary in some regards, from the compost tea used in experiment 1, because of it being "homemade" at Flakkebjerg. Nonetheless in the analyzed literature the compost tea had a satisfactory efficacy against late blight, both alone and even better in combination with *B. subtilis* (Bahramisharif and Rose, 2018). This was not reflected in the experiment 1 results, with barely a better control efficacy than the untreated control plots, and with a lower control efficacy in the 2019 Flakkebjerg trials (Hansen and Abuley, 2020). This suggest that either the alternative performs differently under field conditions, or that the difference in both the microbial communities, MOA as well as the substrate, production and application (Marín et al., 2013), between the compost tea in experiment 1 and the literature, makes the difference in efficacy.

One of the alternatives with the lowest efficacy against foliar blight, in experiment 1, are FytoSol (only surpassed by Amicarb 85 SP), which was also the case in the 2019 Flakkebjerg trial (Hansen and Abuley, 2020). So, there were not much difference in efficacy between a high infection and low infection year. In the analyzed literature the COS-OGA compound in FytoSol, performed really well in e.g. a potted potato trial (Aubel et al., 2018), and showed in general a much higher control efficacy compared to the untreated control. The inconsistencies between the data, indicate that the COS-OGA compound might not function properly under field conditions compared to e.g. greenhouse conditions (Clinckemaillie et al., 2017), where its efficacy is limited by the different abiotic and biotic factors. Furthermore, COS-OGA is a Chitosan based product (van Aubel et al., 2016), like the alternative ChiProPlant (Chitosan hydrochloride), which performed better in both experiment 1 and the 2019 Flakkebjerg trial (Hansen and Abuley, 2020). This suggest that the efficacy of Chitosan-based products is not equally dependable and can vary, depending on its composition.

As mentioned earlier, Amicarb 85 SP (potassium bicarbonate) was by far the alternative with the least control efficacy, with a lower control efficacy compared to the untreated control plots, in both experiment 1 and the 2019 Flakkebjerg trial (Hansen and Abuley, 2020). This is contradicted by the overwhelming positive inhibitory effect against *P. infestans in vitro* as well as the significant reduction in late blight severity, under field trial, found by Abd-El-Kareem and Fatten (2012), and a significant disease reduction (compared to the untreated control) in a tomato pot trial by La Torre et al. (2019). The inconsistencies between the data and literature are comprehensive. This can be partly due to the possibility of a phytotoxic effect on the plant, when using this salt (Deliopoulos et al., 2010), which was further proved by Wenneker (2016), who frequently found severe phytotoxic effect on gooseberry, when using Potassium bicarbonate.

It seems that the significance of the Resistim and Kumulus S foliar blight control achieved in experiment 1, is not reflected in the starch yield, where the plots treated with Resistim and Kumulus S, the tubers had a lower starch content than the tubers from the untreated control plots. This suggest that the better control of the foliar blight and thus the extension of the tuber filling period, might not have had a noticeable effect (Liljeroth et al., 2020). Though, this is contradicted in the field trials by Liljeroth et al. (2020), where the full dosage of phosphite, produced tubers with a starch yield at 12.5 tons/ha significantly better than the 9.9 tons/ha in the untreated control tubers.
The contrasting starch yield to the foliar blight efficacy in experiment 1, might be due to the possible harmful properties by the oxidation of the sulfur on the plant leaves (Ryant et al., 2008), possibly stressing the plant, thus inhibiting the starch filling. This seems plausible due to the fact that they are the same classification (in the thesis) as Armicarb85 SP, and share certain MOA and mechanisms (Borza et al., 2017). Otherwise, it might suggest that it is not the alternatives insignificant foliar blight control efficacy, that are the main tuber filling/starch promoting factor,

but instead something else as seen with the growth promoting properties of the plant strengtheners extract of *Equisetum arvense* and *Urtica spp (Đurić et al., 2019).*

Extract of *E. arvense* performed marginally better in the analyzed literature, e.g. in the trials by Eugene et al. (2020) with disease reduction reaching up to 40%, than was achieved in the foliar blight results in experiment 1. Furthermore, extract of *Urtica spp.* did under field conditions, significantly reduce the disease severity compared to the untreated control, in the trials by Nyankanga et al. (2012), which was not reflected in the foliar blight results in experiment 1. Though, an interesting discrepancy are the starch yield results of extract of *E. arvense* and *Urtica spp.*, from experiment 1, that do not reflect the foliar blight results. Here, *E. arvense* was the only alternative with a significant higher starch yield, than the untreated, followed by *Urtica spp.* This might be because of the growth promoting physiologically active substances (e.g. amino acids, humic acids, polysaccharides and hormones) present in these plant strengtheners (Đurić et al., 2019), that are known to have a promoting effect on the yield (Tandon and Dubey, 2015). This imply that the starch yield is in the case of experiment 1, is not primarily controlled by the foliar blight control, but instead the growth promoting factors seen in e.g. extract of *E. arvense* and *Urtica spp.* This corresponds to them both being classified as plant strengtheners in the thesis.

Moreover, in experiment 1, plots treated with Amicarb 85 SP resulted in tubers with the lowest starch yield, significantly worse than the untreated control plots. Which is most likely because of the beforementioned phytotoxic effect caused by this alternative, on the plant, and the inhibition of the potato plants growth (Deliopoulos et al., 2010).

All in all, the lack of control efficacy suggests that the use of alternatives to fungicides as a suitable replacement, are unlikely.

7.1.2 Alternative management strategies in combination with traditional fungicides against late blight

As was the case in experiment 1, the alternatives by themselves, here Resistim and AgriCHOS, was not significantly different from the untreated in experiment 2. Resistim did perform better than AgriCHOS, but still not near the other strategies. AgriCHOS is based on Chitosan, resembling the alternative ChiProPlant (Malerba and Cerana, 2016), and due to the lack of available literature about AgriCHOS, its effect in experiment 2, will be compared to the literature about Chitosan hydrochloride/ChiProPlant. Chitosan hydrochloride did perform rather well in the trials by La Torre et al. (2019), with significant difference from the untreated control, in *in vitro*, and mediocre results under greenhouse conditions. This does not match the results in the foliar blight control efficacy, in experiment 2, where the chitosan-based product only had a little higher late blight control efficacy than the untreated control. This different might be due to the fact that AgriCHOS was tested under field conditions, and would most likely perform worse, than is the case, under a more controlled environment (La Torre et al., 2019). All in all, together with the Resistim results, it suggests that the alternatives by themselves cannot compete with the other strategies/combinations in experiment 2, and need to be in combination with fungicides, to even come near to the same efficacy (at least under field conditions). Though, other trials have shown results where alternatives was on par with the compared fungicide e.g. in the trial by Shanthiyaa et al. (2013), where the BCA *Chaetomium spp*. in a potato field trial had around the same late blight infection level as a potato field with the fungicide treatment (metalaxyl+mancoceb).

Another observation worth discussing in experiment 2, was that Ranman Top in combination with Resistim, at half dosage, had almost the same efficacy to control late blight in potato plants as the full dosage of Ranman Top, by itself. This was further confirmed in trials by Liljeroth et al. (2020) where the combination of phosphite combined with reduced dosage of fungicides, performed around the same (or better) than the full dosage fungicide by itself. The same tendency was seen in a trial by Zhang et al. (2020), where *R. palustris*, in combination with a lower concentration of fungicide, performed better. This supports the theory that the fungicide dosage can be reduced without affecting the control efficacy, when combined with certain BCA's and other alternatives to fungicides.

In experiment 2, the fact that the full dosage of Ranman Top performed better by itself, than in combination with the full dosage of alternatives, suggest that the alternatives in general does not give an additive effect to the full dosage treatment. Instead, the alternatives need to be combined with a reduced fungicide dosage, to take effect.

The starch yield data illustrated in figure 14, do not show much of a pattern, other than the fact that all the strategies resulted in a better starch yield compared to the untreated control. Additionally, potatoes from fields treated with Resistim and AgriCHOS had the lowest starch yield compared to potatoes grown with the other strategies, which suggest that, unlike the starch yield in experiment 1, in experiment 2 the foliar blight control efficacy clearly have an effect on the corresponding starch yield, most likely because of the bigger difference in late blight control efficacy in experiment 2, between the 2 alternatives Resistim and Kumulus S, and the combination strategies. This is further supported by Liljeroth et al. (2020), where the starch yield increased when phosphite was in combination with fungicides, compared to applied by itself.

All things considered, the increase in disease control when the alternatives to fungicides are integrated with fungicides suggest the best usage of such product is in combination with fungicides.

7.2 Evaluation of effectiveness of using simulation models in management strategies against potato late blight to reduce use of fungicides

The objective of the simulations was to simulate the use of alternative (to fungicides) potato late blight compounds in an organic and conventional production, with cultivars of different late blight susceptibility, to survey the possible efficacy by themselves, as well as in combination with fungicide, to assess the feasible fungicide reduction. As shown from the result in this thesis, the efficacy of alternatives to fungicides as a standalone in an organic production is difficult to estimate in a simulation, because the model does not allow for estimation of the potential effects of the simulation on actual disease control. It is therefore important to empathize that the simulations are only limited to the timing/application of the alternative (to traditional fungicides) compounds, and nothing about the potato yield, can be concluded based on this.

7.2.1 The potential of using other products than traditional fungicides in combination with tolerant potato cultivars against late blight

The literature survey and experimental results, did not focus on the use of resistant cultivars, alone or in combination with alternatives to traditional fungicides. Instead, this was one of the main areas in the simulations (simulation 1 and 2 scenario 1), where a resistant cultivar (Nofy) was compared to a “normal” cultivar (Kuras) and a highly susceptible cultivar, and the results (number of treatments and treatments frequency index), differed accordingly from low to high respectively, in 2019 and 2020.

It is worth discussing the representability of the results, because they depend on the constructed dose model and could vary with even small changes to the dose model (Hansen et al., 2019). In case of the dose model used for Nofy, it was already constructed and available in the BlightManager DSS, and the fact that fungicides are recommended in phase 3, even though the resistance first breaks in phase 4, are controversial and seems a wasted application. Although, when taking into consideration, *P. infestans* big genetic diversity and tendency to break the host resistance (Cooke et al., 2011), the importance of introducing fungicides/or other alternatives, to prolong the resistance (Agrios, 2005), becomes apparent. Suggesting that the fungicide treatments should not be reserved for phase 4 only, but are essential in phase 3 as well (Hansen et al., 2019). Furthermore, the dose model for the susceptible cultivar Bintje was constructed, most noticeable as to recommend application at high infection pressure in phase 1. This decision might seem redundant due to the fact that phase 1 are when no disease symptoms have been registered in the country, and any application would be unnecessary (Hansen et al., 2019). On contrary the big difference in disease severity between Bintje (absolutely no resistance) and the other cultivars in the data from the trap-nurseries, necessitating a “better safe than sorry” application in this phase (Nielsen et al., 2015). This was further recommended in a dose-model by Nielsen et al. (2015),

where the model represented a “safe” recommended dosage (based on different trials), in which half dosage application was recommended at high infection risk, in phase 1.
This suggest that the application in phase 1 might not be strictly a necessity, but could be the sensible choice under high infection conditions, especially with a susceptible cultivar as Bintje, with such a high risk of infection as seen by the trap nursery data.

In simulation 1, scenario 2 (Organic production), the alternatives to fungicides are simulated in combination with the different cultivars, by editing in the dose model and max day interval (application interval)(Hansen et al., 2019). An interesting decision worth discussion are the shorter application interval chosen for the alternatives, compared to the standard 7-day interval (for fungicides) (Hansen et al., 2019) in the DSS. This was due to the general lower efficacy of alternatives as seen in experiment 1, and the lack of control consistency of the alternatives (Köhl et al., 2019), thus leading to a more frequent application. Furthermore, the efficacy consistency, and the results from e.g. the phosphite trials by (Liljeroth et al., 2020), supported the need for 100% dosage to achieve max effect.
With the cultivar Nofy, this resulted in the simulated 18-times application in 2019 and 11 treatments in 2020 for the low-efficacy alternatives (3 days interval), and 14 in 2019 and 8 treatments in 2020, for the high-efficacy alternatives.
Moreover, when using a less resistant cultivar Kuras (trap nursery data), the low-efficacy alternatives simulation results in 22 treatments, and if using Bintje this number increases even more, which is both not economically/energy viable, and generally not the practice (Haverkort et al., 2008). This illustrates the importance of using resistant cultivars together with the alternatives to fungicides, and suggest that the alternatives with low efficacy, by themselves, even with a resistant cultivar cannot realistically provide the necessary protection, as suggested by the DSS.

7.2.2 Discussion of the efficacy and MOA of alternative potato late blight management strategies

The simulation results are also highly dependable on factors such as the alternative compounds efficacy which can vary substantially (Köhl et al., 2019). This have led to the distinguishing of the alternative control compounds in simulation 1 and 2 scenario 2, as high-efficacy and low-efficacy alternatives to fungicides. The distinguishing of the efficacy does affect the number of treatments and dosage substantially in the simulations (Hansen et al., 2019), and are subject for discussion.

The classification of high-efficacy versus low-efficacy alternatives are necessary because of the big difference between their efficacy (O’Brien, 2017). This difference becomes apparent when looking at Resistim, in experiment 1 and 2, it had around 25% and 36% late blight control, respectively, compared to the untreated control. Also, Liljeroth et al. (2016), showed that Phosphite by itself is as standalone treatment is not more than 20% better than the untreated control. Based on the assumption that the fungicide has around a 98% late blight control by itself (at least in experiment

2), therefore suggesting that Resistim is around 65 - 70% less effective than fungicides. Kumulus S have around 24% late blight control compared to the untreated control in experiment 1, so again far from the same efficacy as fungicides.
Extract of Urtica, with around 8% control (experiment 1) and in the literature survey, in the trial by Nyankanga et al. (2012), it had approximately half the disease severity that the control had. *Trichoderma spp*. from the literature survey, in the trial by Yao et al. (2016), it had a disease index on par with the used fungicide and almost half the disease index compared to the untreated. Realistically, this is difficult to translate to direct control effect and would most likely be much less effective than a full dose fungicide (Köhl et al., 2019). *Pseudomonas spp*. also showed potential in the trial by Caulier et al. (2018), with a starting effect at 64% protection index compared to control, later fading to 5%, further proving the lack of effect consistency (O'Brien, 2017). Extract of populus/populin, showed promise and had a significant reduction of late blight compared to the control, with a maximum at 10% disease severity found the days after application compared to a higher infection in the untreated control, ranging at around 25% infection rate. Sodium Bicarbonate did in the trial by Abd-El-Kareem and Fatten (2012), reduce disease severity, on average over 2 years, with around 42% compared to the control. Again, based on this compound's close resemblance in results and MOA, to the active substance in Amicarb 85 SP (Deliopoulos et al., 2010), (seen in the trial), its effect is questionable. Especially due to the negative results of Amicarb 85 SP in experiment 1, suggesting that Sodium Bicarbonate would realistically control late blight much less than a fungicide.
Therefore, with the effect of the alternatives being put into perspective and taking into consideration that the alternatives to fungicides with lower control efficacy than the untreated control in experiment 1, have not been compared, suggest that the distinguishing is valid. Consequently, more clear efficacy and consistency between the alternatives are needed, which is usually is not the case (O'Brien, 2017), therefore necessitating for the differentiation of high-efficacy alternatives like Resistim and maybe Kumulus S and the low-efficacy comprising the remaining.

One of the reasons for the difference in efficacy can partly be due to the MOA, and the alternatives to traditional fungicides are classified in the thesis, based on their primary MOA. Resistim and Kumulus S, are the compounds with the highest late blight control efficacy in experiment 1, and both being classified as "Other" being an inorganic product with a direct MOA (Borza et al., 2017, Williams and Cooper, 2004). Suggesting that there is a connection between their MOA and efficacy, e.g. facilitated by the nutritional "MOA" (besides the direct MOA) in Resistim and Kumulus S, that acts preventative against late blight, by making the plant more robust and prepared (Borza et al., 2017). Contrasting this, is Armicarb 85 SP from the same classification, having the lowest late blight control efficacy in experiment 1, properly due to the phytotoxic effect of the compound (Deliopoulos et al., 2010). Suggesting that the efficacy of this type of compounds can attain high control efficacies, if not inhibited by a phytotoxic effect.

7.2.3 The potential for reducing the use of traditional fungicides against potato late blight

The general uncertainties surrounding the simulations due to it being primarily speculations and the limitations of the BlightManager DSS (Hansen et al., 2019), are worth discussing.
As stated by Cooke et al. (2011), if followed correctly, a DSS can reduce fungicide with up to 62%, but the real situation in practice, the fungicide application are often done more preventatively and relying on repeated fungicide application (Haverkort et al., 2008), negating the DSS. Additionally, the high genetic diversity seen with *P. infestans* populations, together with the future environmental changes, increasingly makes it harder for the DSS to predict disease development correctly (Narouei-Khandan et al., 2020).
Moreover, also the difference in infection pressure between the 2 years used in the simulation, affected the simulation results, with 2019 in general needing more treatments than 2020. Suggesting that the usage of the DSS and efficacy of the alternatives, are highly dependable on the weather, due to its effect on *P. infestans* spread and development (Judelson and Blanco, 2005). Suggesting that many parameters are affecting the potential for fungicide reduction (Hansen et al., 2019).

In simulation 2 scenario 2 (Conventional potato production), alternatives replaced traditional fungicides at low infection pressures (except phase 4) and the highest fungicide reduction was achieved with Kuras, with a 40.01 reduction.
The timing of the application of the alternatives are important to achieve the best effect and reduction in traditional fungicides (Köhl et al., 2019), here in combination with the DSS (Cooke et al., 2011). Firstly, the phase dates in BlightManager DSS was based on dates from the disease surveillance network and trap nursery data, to make the phase initiation dates as close to the real situation as possible. The low efficacy of the alternative compounds, as seen in experiment 1, and the lack of consistency (O'Brien, 2017), excluded the use of the alternatives to fungicides in phase 4, because of late blight being present in the field (Hansen et al., 2019), and at high infection pressures. Suggesting the importance of using the alternatives before this phase, and when the *P. infestans* sporulation potential are low, to achieve the best effect (Hansen et al., 2019).

The case scenarios used in the simulations was primarily limited to the cultivars and the usage of the alternatives or traditional fungicides. It is plausible that the simulations could have yielded more significant results if sanitary measures etc, could have clearly been utilized and illustrated with BlightManager (Hansen et al., 2019). Suggesting that if the other factors in scenario 1 as well as the sanitary measures in scenario 2, are able to reduce up to 39% of the early late blight infection (Cooke et al., 2011), the lower infection pressure could extend the window of when to use the alternatives to traditional fungicides (Köhl et al., 2019). This effect could be even more significant if scenario 3, some form of precision farming, could be implemented, as shown by KESSEL et al. (2019), that with a day to day DSS can further optimize application timing and choice of compound. E.g. by using satellite data to establish plant health/Normalized difference vegetation index (NDVI) and the leaf area index of the fields, to further optimizing the precision of the application timing and thus the late blight control (KESSEL et al., 2019).

7.2.4 Summarizing:

The fact that none of the alternatives suppressed late blight below the total defoliation (100% severity) (experiment 1), strongly suggest that the alternatives on their own are inferior replacement to fungicides, which gives much higher efficacy (experiment 2). However, the better disease control when the alternatives were integrated in fungicide programs suggest the best utilization of such product is in combination with fungicides (experiment 2). This means that the best we could do with alternatives is by replacing some fungicide treatments with these alternatives and this reduce the number of fungicides usage. However, it is important that the alternatives are timed correctly or used at the right times to reap its disease control potential (Köhl et al., 2019). It is the proposition in this thesis that an appropriate time for the application of alternatives is during periods of low disease pressure.

8 Conclusion:

This thesis focus on finding promising alternatives to fungicides in the control of potato late blight, determining their control efficacy, alone or together with fungicides or resistant cultivars, as well as their potential for reducing fungicide dosage. The results from experiment 1 found that only one alternative (Resistim) had a significant control effect on late blight, in comparison with the untreated control, with most alternatives to fungicides performing a little better or worse than the untreated. Moreover, not all the experimental results matched the results from the literature survey, with certain inconsistencies in efficacy, where the literature in general showed a more promising effect of the alternatives. Besides the alternatives from the experiment 1 and 2, e.g. compounds such as Extract of Populus, the endophyte *Phoma eupatorii*, *Rhodopseudomonas palustris* and *Chaetomium globosum* showed future promise. The starch yield, was lower than the control, in the highest late blight controlling alternatives (Resistim and Kumulus S), and favored instead compounds with a growth stimulating MOA. Suggesting that the potential of the alternatives by themselves is doubtful to say the least.

The results from experiment 2 further validated that the alternatives to fungicides, applied by themselves (Resistim and AgriCHOS), was far less effective than fungicides, suggesting that they are not a suitable for replacement. However, a higher disease control was attainable when the alternatives were in combination with traditional fungicides, with one strategy (Ranman Top + Resistim), at reduced dosage, performed almost on par with the full dosage fungicide applied by itself. This suggests that the alternatives are best applied in combination with fungicides, replacing some treatments, thus reducing fungicide dosage.

In the simulation studies with the BlightManager DSS, it was demonstrated that the best efficacy of the alternatives and reduction in fungicide dosage are best attained in combination with resistant cultivars. Although, a substantial reduction in fungicides (up to 40%) was attainable in less resistant cultivars with the use of alternatives to fungicides, the timing of the application is important, and varies based on the application interval. This indicates that it is essential that the alternatives are used in combination with resistant fungicides and/or timed correctly, used at the right times to attain its highest disease control potential, especially during low disease pressure periods. Conclusively, the Blightmanager DSS simulations illustrated this in a satisfactory and practical manner.

9 Future perspectives:

9.1 The need for new alternative products

A big part of this thesis focused upon the efficacy and potential of the alternatives as a substitute for traditional fungicides or at least their potential in reducing fungicide dosage. Based on the experimental results, it was clear that the alternatives to fungicides was no way near able to reach the same efficacy as traditional fungicides, with many compounds struggling to even reach the control efficacy of the untreated control. Even though, this low control efficacy was contradicted by some results in the literature e.g. ((Zhang et al., 2020),(Aubel et al., 2018),(Shanthiyaa et al., 2013)) the same alternatives to fungicides from the experimental data, had performed even worse in 2019 with a general higher late blight infection pressure at Flakkebjerg (Hansen and Abuley, 2020). Begging the question if the current available alternative product, are even worth the economical expenditure, against such a potent disease as late blight? Moreover, the challenges besides the effect consistency, presented by these compounds, such as the effect of biotic and abiotic factors, affecting the application time and storage of the alternative compounds, further complicates the commercial usage. Therefore, it seems that new easily appliable compounds with significantly higher efficacy are needed, before this type of alternatives can become a truly impactful tool or substitute in the control of potato late blight.

Resistim based on potassium phosphite and Kumulus S based on sulfur, from same classification had the highest control efficacy in the results, considerably better than e.g. the best performing BCA based on a living organism, presenting the question if the inorganic compounds perform better than the BCA's and if so should the future research be focused there? Although, this could be a problem due to the toxicity and bioaccumulation present in this type of compounds. Therefore, the replacement of another inorganic control alternative cobber (or fungicides) as a control of pathogens in the potato production should not be replaced by alternatives that might themselves become illegal in the near future. So, a better understanding of what the potential side effects of the different types of alternatives to fungicides, could be, and which should be prioritized, are important.
Furthermore, there is potential in using endophytes as alternatives (to fungicides) against late blight, due to them being able to improve the persistence of the alternatives. This is because they reside in the plants and are not affected as much by the ambient weather conditions, as well as the fact that they are always in the plant and can therefore offer protection anytime the pathogen arrives. Thus, the endophytic alternatives show potential and future research into this type of organisms are needed

The implementation of the alternatives to fungicides, as accepted compounds in different countries can be a hassle, due to different legislations and classifications of the compounds. It is important to make this process easier, by developing a common classification of the different compounds, as well as optimizing the implementation product. Thereby, making It more appealing to both develop more alternative products as well as using them.

Furthermore, the use of the BlightManager DSS, have put into perspective the importance of the timing when controlling late blight, together with alternative compounds. Thereby underlining the importance of using DSS's, and the future potential of developing these systems. Therefore, further research of how precisions farming can be implemented into DSS's, are essential, also in the optimization of the alternative's efficacy and usefulness.
Although, this is only one tool, other important factors that can improve the timing and efficacy of the alternatives to fungicides, are desired. Also, the importance of using resistance cultivars to achieve best control in combination with the alternatives and reducing fungicide dosage, have been illustrated in the simulations. Therefore, the advantages of using resistant cultivars, such as Nofy, should be advertised further, as to grow in popularity among the farmers. When that is said, resistance cultivars can "pay for" their resistance by being less effective in other regards, such as starch yield. Therefore, another future focus area could be in the developing of cultivars with high late blight resistance, that still maintain sufficient yield/starch yield.

10 Bibliography

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