ZÜRICH UNIVERSITY OF APPLIED SCIENCES
SCHOOL OF LIFE SCIENCES AND FACILITY MANAGEMENT
INSTITUTE OF ENVIRONMENT AND NATURAL RESOURCES

Antagonistic activity of entomopathogenic bacteria against *Phytophthora infestans* in potato tubers

Master's Thesis

by
Marta Hernández-Pinzón Maldonado

Master's degree course 2019
Submision date: 07.01.2019
Degree programme: Natural Resources

Supervisors:
Dr. Esther Fischer
ZHAW Life Sciences and Facility Management, Institute of Environment and Natural Resources, Grüental 8820 Wädenswil, Switzerland.

Prof. Dr. Jürg Grunder
ZHAW Life Sciences and Facility Management, Institute of Environment and Natural Resources, Grüental 8820 Wädenswil, Switzerland.

ABSTRACT

Late blight disease of *Solanum tuberculosum L.* (potato) provoked by the fungus-like *Phytophthora infestans* is considered to be one of the biggest problems in agriculture worldwide. Prior studies have demonstrated the ability of several entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*, mutuallistically associated with soil dwelling nematodes, to suppress *Phytophthora infestans*. In the current study the main objective was to determine whether bacterial treatments, consisting on bacteria cells and its supernatants on LB medium, showed the ability to supress *Phytophthora infestans* development in potato tubers. Late blight inhibition power of entomopathogenic bacteria was evaluated by performing assays with tuber slices and whole tubers. For this purpose 24 entomopathogenic bacteria strains were tested *in vitro* on *Phytophthora infestans* inoculated potato slices and 6 strains were tested in whole tubers. Evaluation and quantification of suppresive effect on the growth of the pathogen was performed as well as observation of phytotoxicity signs. Disease severity was quantified by means of necrosis and sporangiophore coverage measurements after 10 to 12 days of inoculation. Distilled sterile water and copper-based fungicide were used as negative and positive controls respectively. Experimental results of this study demonstrated that 21 of the 24 tested strains had ihibitory effects on *P. infestans* development. Additionally, 12 of those strains showed to be as effective as copper-based fungicide (positive control) with 100% disease suppresion and no visible phytotoxicity symptoms. Moreover, the study also suggested that slices treated with bacteria are more likely to produce sprouts compared with the controls. This study provides new insights into the battle against the destructive oomicete *P. intestans* using a singular entomopathogenic bacterial collection.

TABLE OF CONTENT

LIST OF ABREVIATIONS

Cu	Positive control (Copper-based treatment)
B	Bacteria treatment
W	Negative control (sterile water treatment)
P. infestans	*Phytophthora infestans*
OD	Optical density

LIST OF FIGURES

1. INTRODUCTION

Late blight caused by the fungus-like microorganism *Phytophthora infestans* (*P. infestans*) is an aggressive, destructive and dreaded plant pathogen best known for causing the disease that triggered the devastating Irish potato famine in the 19th century. It is the plant pathogen that has most greatly impacted humanity to date (Goss et al., 2014). It has the capacity to attack potato aerial plant parts as well as tubers (Bode, 2009) and nowadays still endangers the global potato industry representing a high risk in potato cultivation (Judelson & Blanco, 2005). Indirectly, studies of late blight after the Irish famine were a stimulus to the origins of plant pathology as a science (Abad & Abad, 1997).

Chemical-based fungicides to combat the disease have been developed and are nowadays extensively used, although associated problems make them dangerous for human, animal and ecosystems health (Pirozzi et al., 2016). Development of pathogen resistance to those compounds is creating as well concern about the use of chemically based fungicides (Montes et al., 2016).

Entomopathogenic bacteria from the genera *Xenorhabdus* and *Photorhabdus* spp. belonging to the family *Enterobacteriaceae* mutualistically associated with insect-pathogenic nematode species (Thomas & Poinar, 1979), are able to produce several secondary metabolites, which are known to act against a variety of fungal diseases (Akhurst, 1982; Chen et al., 1994; J Li et al., 1997; Mcinerney et al., 1991; Ng & Webster, 1997b). These group of bacteria can be isolated from their hosts and artificially cultivated as independent organisms in a controlled environment. The possibility of mass-producing secondary metabolites could be explored as an alternative strategy of disease control in agriculture (Jang et al., 2012).

The present study contributes in this regard by assessing the inhibitory effect of entomopathogenic bacteria with the purpose of evaluating their inhibition potential on *P. infestans* growth on potato tubers. In this Thesis, results from the screening of 24 specific selected bacteria strains are reported for the first time. During experimental phase, phytotoxicity of the bacterial solutions has also been evaluated through visual observations.

2. LITERATURE REVIEW

2.1 Origin of the disease.

The origin of *P. infestans* is still a controversial open question. While some evidences support a South American origin of the disease, specifically located in the Andean region, others suggest its roots in central Mexico. Both areas are centres of great variety of potato plants (Goss et al., 2014). At the starting point of the studies it was assumed that *P. infestans* had its origin in the Andean region (Berkeley, 1846) but years later, a new hypothesis arrived suggesting that Mexico was the centre of origin (Reddick, 1939). This new theory prevailed until the early 21st century (Fry et al., 2015). But nowadays, these two hypotheses still compete. Some authors defend that *P. infestans* has an Andean origin supporting their hypothesis on mitochondrial and nuclear gene genealogies of isolates from several locations worldwide (Gómez-Alpizar et al., 2007; Martin et al., 2016). However, others still support the genesis of the disease in central Mexico based on studies in which a wider set of isolates and more close relatives of *P. infestans* have been included (Goss et al., 2014).

2.2 Historical impact. The Irish Potato Famine and other important pandemic events.

Late blight pathogen was introduced to Europe by mid 19th century. Since then it has expanded worldwide and today it is present in every location where potatoes are cultivated (Arora et al., 2014). During 19th and 20th centuries famines have occur as a consequence of late blight epidemics. The Irish Potato Famine in the 19th century was especially severe killing or forcing into emigration to more of one fourth of the inhabitants in Ireland, as potato was at the time the main resource of food. Since then, the whole potato production in Europe became vulnerable to this new disease (Savary et al., 2017). A second severe outbreak of the disease occurred in the late 1970s simultaneously with the introduction in Europe of a new mating type of the pathogen. This event produced a big transformation in the DNA of European pathogen populations, making the disease even more difficult to control (Goodwin et al., 1996) as well as turning the pathogen considerably more aggressive (Day & Shattock, 1997). Thanks to the better knowledge of the pathogen, prophylactic measures and the use of chemicals, an event of such magnitude has not occurred again to date (Savary et al., 2017). However, *P. infestans* continues being a significant danger to food security (Goss et al., 2014). In fact, besides these past events, nowadays the disease continuously appears in new locations and with a remarkable and serious severity. United States and Canada have suffered since the 1970s severe outbreaks of late blight. Potato crops in Tunisia, Chile, China, Oman and Nigeria have been also affected by extreme late blight epidemics in the first decade of the

21st century (Fry et al., 2015). In India, late blight has re-emerged and become again a very dangerous disease and application of fungicides has increased considerably (Fry et al., 2015; Lal et al., 2016). It is has been demonstrated that changes in the pathogen characteristics are the explanation for these new outbreaks rather weather conditions (Fry et al., 2015).

2.3 Economic impact.

Late blight represents the most expensive potato disease worldwide. Its annual costs has been conservatively estimated at approximately $6,000,000,000 for the global potato industry (Goss et al., 2014; Nowicki et al., 2012). The costs in which the control of the disease incurs are associated with the costs of the applied products (synthetic fungicides) plus the costs of machinery, energy and work. Furthermore, spraying machines locally damage crops reducing potato production in around 3%. In addition, potato late blight leads to costs due to economic losses, which can be attributable to lower yield, low quality and reduced storability among others (Haverkort et al., 2008; Nowicki et al., 2012).

In the EU the most important potato producers are Belgium, Germany, Spain, France, Italy, Netherlands, Poland and the United Kingdom. The costs on which those countries incur due to the disease are estimated in more than €1,000,000,000 annually, including both, disease control and damage.

Healthy potato crops would increase revenues and food security, which would have great positive repercussions specially on the population of developing countries where losses would be highly reduced (Haverkort et al., 2008).

2.4 Description of the oomycete *Phytophthora infestans*.

Taxonomy: *Phytophthora infestans* (Mont.) de Bary, Kingdom Chromista, Phylum oomycete, Order Peronosporales, Family Peronosporaceae (Birch & Whisson, 2001).

P. infestans belongs to the oomycete Phylum. Oomycetes were originally classified inside the Kingdom *Fungi* and only a few decades ago this group was reallocated in the Kingdom Straminipilia (Chromista or Heterokonta), together with brown algae and diatoms. Oomycetes are more related to then than to the true Fungi (Rossman, 2009). This initial classification was done by taxonomists because of the production of filamentous threads, but the relative recent development of effective genetic tools suggested that oomycetes belong to the Straminipilia Kingdom (Birch & Whisson, 2001). Some characteristics differenciate oomycetes from true fungi. Oomycetes are diploid, usually unpigmented, they form non-desiccated spores, unicellular multinucleate sporangia and they have biglagellated zoospores (Judelson & Blanco, 2005).

Regarding reproduction, before the 1980s, the whole population of *P. infestans* outside Mexico was presumed to be asexual with a single mating type A1 but it is now extensively recognized that new strains migrated into Europe in 1976. This event originated changes in the population along with the introduction of the A2 mating type. It is widely believed that the destructive capacity of the pathogen has increased in the past 20 years due to this episode (Cooke et al., 2011; Drenth et al., 1994). In 1981 isolates with the A2 mating type were as well reported in Switzerland (Hohl & Iselin, 1984).

The presence of both mating types allows for sexual recombination, which has led to dramatic increase in genetic diversity and creation of new and more virulent strains due to recombination during meiosis (Judelson, 1997; Nowicki et al., 2012). Variability has become more evident by frequent appearance of new pathogenic types in field with new and stronger virulence (Arora et al., 2014). This distinctive characteristic has made late blight management even more challenging (Judelson, 1997; Nowicki et al., 2012). The emergence of sexual reproduction has conduced to better adaptability plus the capacity to survive in adverse conditions. Additionally, when only A1 mating type is present, thus only asexual reproduction occurs, other ways for genetic changes are also possible leading to the appearance of pathogen variability e.g., mutation, mitotic recombination or parasexual recombination (Abu-El Samen et al., 2003b). These variations may affect the virulence phenotype as well as fungicide susceptibility. Genetic variations during asexual reproduction needs to be taken into consideration as well when predicting the appearance of new strains (Abu-El Samen et al., 2003a).

In asexual reproduction *P. infestans* produces considerable amount of sporangia in each plant lesion, and they use wind and rain to passively spread. Sporangia can sprout immediately with favourable weather conditions (more than 15°C) and rapidly develop mycelia growth and subsequent sporangia on leaf, stem and fruit tissues. At temperatures below 15°C, sporangia do not produce mycelia growth and directly form and release asexual spores (zoospores), which then germinate and cause new infections at an even higher speed. With favourable weather, the entire cycle can be repeated in about one week (Nowicki et al., 2012).

The introduction of mating type A2 gave the pathogen the opportunity of sexually reproduction when the hyphae of A1 and A2 interact. This interaction generate the male form called antheridia and the female equivalent called oogonium which after fertilization produce a diploid oospore (Drenth et al., 1994).

Asexual forms (sporangia, zoospores, mycelium) and sexual forms (oospores) are considerably different according to size, physiology and resistant capacity (Andrivon, 1995).

Oospores are large resistant spores tolerant to adverse conditions that enable the organism to survive between growing seasons on debris or soil without the need of a living host. This peculiarity does not exist with asexual spores (Nowicki et al., 2012). *P. infestans* can be detected in soil at any stage of its life cycle. This characteristic makes the pathogen outbreaks even more severe (Andrivon, 1995). Stages of the spores and life cycle of *P. infestans* are shown in Figs. 1 and 2.

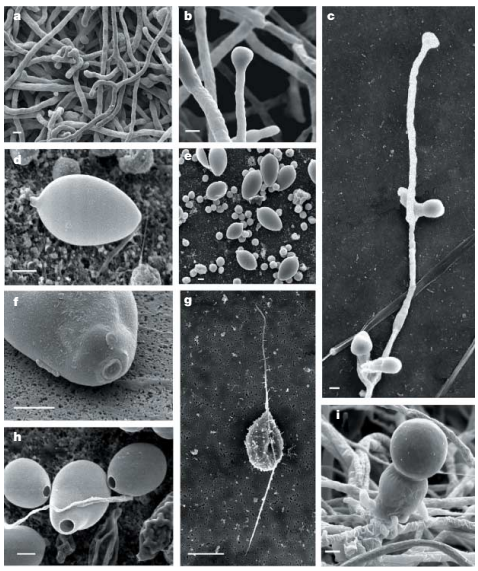


Figure 1: Typical forms of *P. infestans* a. Vegetative hyphae, b. Swollen tip of asexual sporangiophore, c. Sporangiophore with four asexual sporangia, d. Ungerminated sporangium, e. Sporangia and zoospores together, f. Operculm, g. Zoospora with its two flagella, h. Sporangia with open opercula, i. Oospore formed by association of A1and A2 hyphae (Source: 2019, Blanco F.).

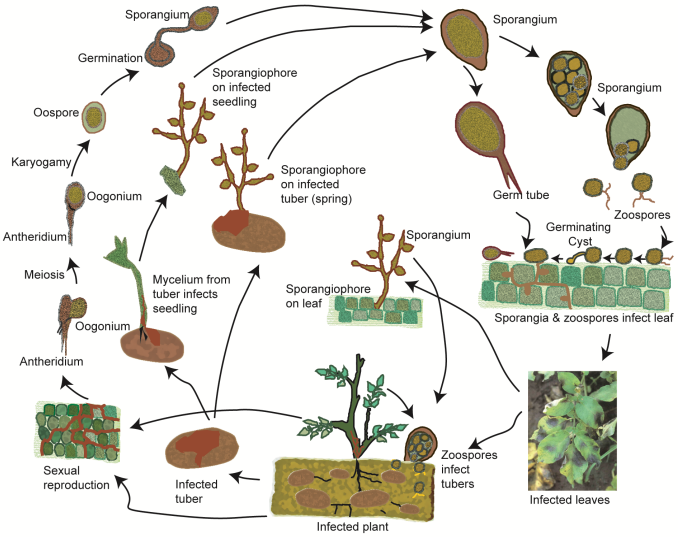


Figure 2: Disease cycle of potato late blight caused by *P. infestans* (Source: 2012, Vetukuri R.).

In laboratory, *Phytophthora* can be repeatedly grown as hyphae but in nature spore production is necessary. When infected plants are already lacking of health, spore generation is essential to allow traveling in order to colonize another host. Therefore, in one season various series of spore formation and plant colonization occur (Judelson & Blanco, 2005).

2.5 Exposure and containment of the causal organism.

The existing mycelium on the plant surface produces the biotrophic structures haustoria (hyphal tips), which penetrates the host plant cell wall and siphon nutrients from the space between the cell wall and plasma membrane. However these structures do not penetrate the cell membrane itself. Haustoria generates effector proteins that penetrate the cell membrane which, at this point, can either be recognized or not by the host plant. In order to be able to sink into the cell membrane, the effector proteins require the help of the so-called RXLR-EER

motif and a signal peptide. The signal peptide makes possible the secretion of the effector out of the hyphae and the RXLR-EER motif is required for translocation into the host cell. Effectors have to be produced in large amounts in order to succeed and be able to manipulate the own resistance mechanisms of the host cells (Haverkort et al., 2009).

2.6 Description of the disease: signs and symptoms in potato plants and tubers.

Infected foliage initially presents water-soaked yellowish lesions mostly at the leaf tip and borders. These firsts symptoms quickly develop into more notable brown-purplish black necrotic areas. Whitish areas of sporangia develop on abaxial surface of the leaves with more intensity at the margins of the necrotic areas (Fig. 3). These symptoms are later spread to the stems, which present dark brown lesions and end up collapsing. When the disease develops under favourable weather conditions the crop can be totally destroyed within 5 to 7 days (Nowicki et al., 2012). Potato tubers get the infection consecutively by rain borne sporangia from the infected aerial parts of the plant. In early stages, tubers present little reddish-brown to purple spots on the surface, which later penetrate the internal tissues (Fig. 4).

Figure 3: Lesions of late blight on potato leaves.

Figure 4: Lesions of late on potato tubers. The surface of the tuber is severely affected by *P. infestans* and the disease has already penetrated the internal tissues.

Affected tubers are very susceptible to get attacked by secondary infections such as bacterial infections called “wet rot” either in the fields or later in warehouses. In damp soils tubers putrefy quickly before harvest (Birch & Whisson, 2001; Nowicki et al., 2012).

The big difficulty in the detection of infected crops is that symptoms at early stages are not evident as not all plants show signs of infection at the same time. Additionally, the first lesions on leaf tips and plant stems, are perceivable only after 3 to 4 days of infection and at this stage the pathogen has the capacity to penetrate the plant tissue very quickly and the disease is uncontrollable (Nowicki et al., 2012).

2.7 Environmental conditions that favour the spread of the disease

The optimum temperature for fungus development is 16 to 24°C (Arora et al., 2014). Germination of asexual forms can occur through two different ways: direct and indirect germination. Temperatures higher than 14°C favours direct germination, consisting in the hyphal outgrowth through the sporangia wall whereas at cooler temperatures, indirect germination or formation of zoospores is more frequent (Judelson & Blanco, 2005; Maziero et al., 2009) . Either direct or indirect germination, sporangia need high and continuous humidity to survive. It is believed that zoosporogenesis is the most important strategy in epidemic events. However, the existence of two pathways increases the chances to infect plants with different environmental conditions. But these two ways force sporangia to stay operative, consuming energy and reserves, which make them more likely to die during dry and sunny periods. Under field conditions sporangia would only survive a few days. Thus these asexual forms are sometimes vulnerable and not effective depending on weather conditions. On the other hand, oospores (the sexual spores) present thick walls that make them very resistant to unfavourable environmental conditions. These structures are able to survive from one season to the next, being a significant source of inoculum and making management of the disease an arduous task (Judelson & Blanco, 2005). Oospores germination occurs at temperature range between 12 to 25°C only when submerged in water (Arora et al., 2014). Once the pathogen penetrates the plant, the progress of the pathogen is also influenced by temperature being the optimal 18 to 22°C. Relative humidity over 90% and low temperatures highly stimulate late blight epidemics on potato crops. The presence of water is essential for sporangia propagation and pathogen survival (Harrison, 1992).

There are many studies available on the literature confirming that late blight disease development is highly dependent on humidity. Experiments carried out by Cohen et al. (1996) on potato and tomato leaves concluded that wetness of the infected plant tissues is critical for oospore development. Other studies conducted in irrigated potatoes also required high continuous humidity and low temperatures for infection and disease development (Olanya et al., 2007). In the same direction, studies carried out by D. A. Johnson (2010) suggested that late blight infection is dependent on humidity. Mist periods favoured the spread of *P. infestans* from the seeds to the shoots when the plants sprouted. Humidity also promoted sporangia production, recurrence of sporulation cycles and infection of leaves. Symptoms of the disease were visible after short periods (within one to two days) after wet conditions started. This suggested as well that the pathogen was most probably latent on plant tissues and it germinated as a consequence of the exposure to moistness.

Irradiation is as well affecting the life cycle of late blight. Sporulation is hindered during sunny periods as well as oospores formation. It has been demonstrated in laboratory that dark conditions favours oospore production, while under continuous light oospores production is very rare. Regarding wind conditions, at high humidity, wind allows disease dissemination and new plants can be colonized, whereas, dry environment impedes late blight development due to the evaporation of water and desiccation of sporangia (Arora et al., 2014).

2.8 Disease management

The notable adaptation power of *P. infestans* to control strategies, attributable to its highly genetic variability, makes the management of late blight disease exceptionally difficult and challenging. Genome sequence analysis of this pathogen found out the presence of "fast-evolving effector genes" which most likely participate on its fast genetic evolution and ability of adaptation to new potato varieties and fungicides (Arora et al., 2014; Haas et al., 2009).

2.8.1 Use of fungicides

The most demanded potato varieties by consumers in Europe are not resistant to *P. infestans*, thus potato varieties cultivated are vulnerable to late blight. Applying chemically based fungicides is still essential for the control of the disease even though governments and end consumers are reluctant to its use. Some formulations commonly used in Europe against *P. infestans* are copper, dithiocarbamates, chlorothalonil, cyazofamid, fluazinam, zoxamide, mandipropamid, cymoxanil, mancozeb, dimethomorph, fenamidone, benalaxyl, metalaxyl-M, and sometimes a combination of two of these products is used (Cooke et al., 2011).

For actualized information regarding fungicide rating, the following website can be consulted: http://euroblight.net/control-strategies/late-blight-fungicide-table/.

Applying fungicides before infection starts is the most effective weapon combating late blight as it is believed that most of the fungicides work in a protective way and are not efficient once the pathogen has entered the plant tissues (Johnson et al., 2000). According to Stein and Kirk (2002), regardless of the fungicide composition or its mechanism of action, neutralizing the disease is not warranted when fungicides are used after leaves have 1% of late blight symptoms. They observed that application of fungicides 72 hours previously to inoculation gave the best results by containing late blight. The study revelled as well that applications 72 hours after inoculation could also control the pathogen in most of the assays.

Since the end of the 19th century Copper is commonly used to combat a broad spectrum of fungal diseases in agriculture. Cu-based compounds employed in crop protection are hydroxides, oxides, sulphates and oxychlorides among others (Pavlovic, 2011). Regarding

organic agriculture the use of copper as a fungicide is nowadays permitted. In fact, some authors believed that organic potato production can only be effectively protected from late blight with the use of copper-based compounds (Nechwatal & Zellner, 2015). On the other hand, a studies carried out with organic potato production in Germany suggested that crops can be successfully protected against late blight by "long term and preventive management strategies" thus, not big amounts of fungicides are needed. Moreover, results of this study suggested that it is often possible to manage late blight epidemic prevention with only half of the copper normally used in combination with disease predictive systems as BioPhyto-PRE, specially designed for bioagriculture. It is suggested as well that acceptable crop protection levels are even feasible when the risk of infection is high (Finckh et al., 2008).

2.8.2 Disadvantages of fungicides

- Resistance

Pathogen resistance to synthetic fungicides is among the biggest concerns in potato cultivation. Owing to its high variability potential, *P. infestans* is susceptible to develop resistance to those substances (Skelsey et al., 2009). Metalaxyl, a phenylamide fungicide introduced at the beginning of 1980s, showed high efficiency against susceptible strains of *P. infestans*. However, its excessive use led to very fast development of resistance in the British Islands and Europe (Goodwin et al., 1996). At this time, immune genotypes of *P. infestans* to metalaxyl were discovered and since then, these isolates have been globally reported (Maridueña-Zavala et al., 2017). Currently, immunity of some *P. infestans* isolates to Metalaxyl represent a crucial problem creating big concerns in food security worldwide (Montes et al., 2016).

- Environmental consequences

Controlling late blight requires enormous amount of chemicals, some of them with a negative environmental impact (Judelson & Blanco, 2005)

Besides the high economic costs derived from the application of fungicides, the components of these substances have as well environmental costs. There is environmental pollution from the fungicide mancozeb specially in groundwater in studies carried out by The Netherlands Committee for the Admission of Crop Protection Agents (CTB) (Haverkort et al., 2008). In a recent study carried out in Italian honeybees during 3 years of survey, data showed the presence of various fungicides in pollen. This study showed that 39% of the pollutant agents exceeded the EU safety and legal levels of pesticide in pollen with ten pesticides involved. Among those pesticides, mandipropamid and metalaxyl were present (Tosi et al., 2018) which are commonly used for the control of late blight in potato crops.

Due to the extensive and intensive long-term use, copper (Cu) has become as well an important pollutant agent, creating concern among the scientist community and population.
However copper is a natural compound in soils (2-60 mg/Kg), when copper compounds are artificially added to crops it tends to stay in the upper layers and becomes susceptible to be washed and disseminated increasing its toxic potential (Pavlovic, 2011). Approximately 95% of the Cu released in the environment reaches the soil and marine and freshwater exceeding natural levels (50-500 μgrams/L) becoming potentially toxic (keller 2017).
In western countries, downy mildew disease in vineyards has been intensively treated with Cu-based products leading to Cu accumulation in soils. At concentrations over safety limits Cu can produce phytotoxicity, which mostly occurs at pH under 6 (Brun et al., 1998). A pH below 5 notably favours solubility and thus plant bioavailability, thus considerably increase Cu toxicity (Adriano 1986).
Owing to bioaccumulation and production of reactive oxygen species (ROS), even low concentrations of Cu can produce metabolism disorders at different biological levels. Additionally, other effects of Cu are: damage of DNA plasmids, effects on embryo-hatching enzymes, membrane damage, lower electron transport activity and lower antioxidant capacity. Excess of Cu can also influence in the seed germination process lowering growth rates of crops. Moreover, Cu absorbs phosphorus, causing nutrient deficiency in plants lowering the nutritional value of the vegetables (Keller et al., 2017) It has been also observed that, due to higher dissolution, Cu presents more bioavailability in aquatic ecosystems than in terrestrial ecosystems, therefore toxicity is much higher for aquatic life at lower concentrations (Flemming & Trevors, 1989; Keller et al., 2017). Studies carried out by (Zhao et al, 2016a) showed that Cu, when applied in the soil, could be transported from roots to aerial plant tissues (stems, leaves and fruits). Moreover, direct applications of Cu compounds on the aerial parts of the plant led to a larger concentration in leaves and fruits.
Brun et al., 2003 observed on ruderal plant species that high levels of Cu in the soil caused reduction of plant survival, depletion of plant biomass, later flowering and fruiting and decrease of seed production.
Supposing that Cu concentration increases exceeding toxicity thresholds ecosystems may be experiment serious damage. Moreover, crops that have received Cu-based fungicides can still contain Cu in their tissues after harvest and biomagnification can occur, which could also be dangerous for humans and other species (Keller et al., 2017).

2.8.3 Available alternatives to fungicides

Regardless of necessary fungicide applications, a well-organized combination of good practices in agriculture is crucial for an efficient late blight control. The use of resistant potato varieties, healthy seed tubers, prevention of inoculum on potato debris, sterilization of compost, adequate irrigation systems as well as disease predictive systems are among the most used (Johnson et al., 2000).

- Regional disease predictive systems

Numerous farmers decide in advance that the use of chemically based fungicides in vast quantities and with often applications during potato season is indispensable for a correct late blight control. However, this way of crop management is environmentally not sustainable and is creating public concern. Negative impacts on health and pathogen resistance to synthetic fungicides are as well among the biggest preoccupations.

As previously described, late blight epidemics are highly correlated to specific weather conditions. This feature has been used to develop prediction models, which can help to make decisions in order to rationalize the use of fungicides. When weather conditions are incompatible with disease dissemination, fungicide applications can be avoided, thus reducing chemical inputs to the environment (Skelsey et al., 2009). They are used for the foresight and prevention of pandemics in agriculture and usually consist of logistic regression models based on documented regional weather data plus disease historical information. These algorithms can predict the risk of epidemics and the need of crop protection by analysing several factors (Gent et al., 2013). Some examples of these predictive systems are *VNIIFBlight*, designed to recognize weather situations that are known to favour the spread of the disease in Russia (Filippov et al., 2015). BLITE-DVR, developed to predict potato late blight in the Republic of Korea, uses non-parametric techniques, specifically support-vector regression (Gu et al., 2016). BlightPro DSS is a web-assisted predictive system that assesses potato and tomato late blight risks in USA and the system is able to interconnect different models into the same system using weather predictions, crop data and control strategies (link: http://blight.eas.cornell.edu/blight/) (Small et al., 2015). PhytoPre is an information and prognosis system with up-to-date information about the infestation situation throughout Switzerland. This system has also the version BIO-PhytoPRE, available for organic potato producers. It provides an app as well, which make it available via smartphone (link: http://www.phytopre.ch/)).

In a study carried out by (Taylor et al., 2003) several predictive systems were evaluated for more than six years in five separate spots presenting different disease risky conditions. The

evaluated systems were Smith Period, Negative Prognosis, Blitecast, Sparks and NegFry. Results of the study suggested that predictions were very irregular and imprecise between systems. None of the assessed systems were totally reliable on their purpose to alert about epidemics or fungicide optimization. Nevertheless Taylor et al., 2003 allude that the use of forecasting schemes could be very interesting for farmers and hence consumers as the use of fungicide would be optimized, thus forecasting systems must meet the future challenge of being useful tools. This study was done with systems which are now obsolete, though. New studies comparing up-to-date predictive systems would be necessary.

Objectives for the development of better models in the future are for example the need of "Linking epidemiological models to crop yield and ecosystem services" or designing "Realistic dispersal models, including meteorological and anthropomorphic drivers" among them. Linking all modelling disciplines is still a crucial challenge (Cunniffe et al., 2015). In fact, models that would be able to combine the entirety decisive factors and its interactions should give precise epidemics prediction, but on the other hand considerable efforts are needed to obtain complete data in order to achieve this goal (Hartill et al., 1990).

- Resistant potato varieties to late blight

Traditional breeding techniques still haven't been able to suppress resistance because of the great power of variability and adaptability of *P. infestans* (Haverkort et al., 2008). After new resistant potato varieties bred with wild species were cultivated for some years resistance would always come back (Haverkort et al., 2009). Many stakeholders in the agricultural field find the development of resistance through genetic engineering attractive because it has shown some improvement in resistance. For example overexpression of rice *oxalate oxidase 4* gene in potato plants improves resistance (Ghosh et al., 2016). It is as well believed that the *Rpi-blb2* gene gives the potato crops long term resistant in the Netherlands (Orbegozo et al., 2016).

Besides, the DuRPh program in the Netherlands claims to be the solution for durable resistance through cisgenic modification. Cisgenic technics apply genetic modification using only inherent resistant genes from the own plant or from crossable species of wild potato. It was believed that cisgenic modification would be more socially accepted as it claims not to use alien genes. Cisgenic techniques have proved that the introduction of certain resistant genes to a genome of a susceptible plant is able to transform it in a resistant one. However, there are several limitations to this method. The resistant genes are not operating in all potato varieties. In addiction, the technique itself is about 15 times more expensive than transgenesis.

Also, using cisgenic in old potato varieties has the disadvantage of providing possible quality weakness. Moreover, the genes used in DuRPh project are patented thus the patent owner determines the price and who can profit from them (Haverkort et al., 2016). Finally, comparing with conventional methods, cisgenic is not compatible with the principles and values of organic farming and social acceptance would be difficult to achieve (Haverkort et al., 2009).

2.9 Entomopathogenic Bacteria

Xenorhabdus and *Photorhabdus* spp. are two bacteria genera belonging to the phylum Proteobacteria, class Gammaproteobacteria, order Enterobacteriales, family *Enterobacteriaceae*. They are facultative anaerobic, motile, gram negative, nonsporulating rod-shaped bacteria. All species of *Xenorhabdus* and *Photorhabdus* are entomopathogenic except a few specimens (Forst et al., 1997).

The strains used for this Master's Thesis belong to the species: *Photorhabdus luminescens, Photorhabdus temperata, Xenorhabdus bovienii, Xenorhabdus budapestensis* and *Xenorhabdus kozodoii* (Table 1, Chapter 4).

The genus *Photorhabdus* spp. was first created by Thomas and Poinar (1979). *P. luminescens* and *P. temperata* were first proposed 20 years later by Fischer-Le Saux et al. (1999). *X bovienii* was in the past classified as a subspecies of *X. Nematophilus* but after DNA analysis was then contemplated as a separate species (Akhurst & Boemare, 1988). Finally, *X. budapestensis* was more recently described in 2005 (Lengyel et al., 2005).

Bacteria belonging to these two genera are mutualistically associated with soil dwelling nematodes that are pathogen of some insects. In particular, entomopathogenic nematodes of the family *Steinernematidae* establish mutualism with bacteria of the genera *Xenorhabdus* while nematodes from the family *Heterorhabditidae* are symbiotically associated with bacteria of the genera *Photorhabdus* (Poinar & Grewal, 2012).

The majority of *Xenorhabdus* and *Photorhabdus* species are known to have primary and secondary cell forms. Primary forms are those found in their natural habitat in symbiosis with the corresponding nematodes (Akhurst, 1982). This forms are able to produce antibiotics in the hemocoel of the colonized insect as well as *in vitro* cultures with aerobic conditions (but not in anaerobic conditions) (whole tubers) (Hu et al., 1998). This is a remarkable peculiarity of *Xenorhabdus* and *Photorhabdus* spp. with relevant biological implication (Li et al., 1999). On the other hand, when bacteria are cultivated *in vitro* for more than about 2 weeks the primary cells transforms to a secondary form. It is believed that low-osmolarity conditions may affect also to this transformation (Krasomil-Osterfeld, 1995). Secondary cells are not

found in the nematode-bacterium-insect life cycle and they usually lose the capacity of producing antibiotics (Akhurst, 1982; Forst & Nealson, 1996).

2.9.1 Life cycle

Bacterium and nematode go through symbiotic and pathogenic phases throughout its life cycle. In the symbiotic period, bacteria live inside the gut of the infective juvenile nematode while in the pathogenic stage bacteria and nematode undergo a co-working process bringing the target insects to death (Forst et al., 1997).

The insect's natural orifices (mouth, anus or spiracles) serve as gateway for the nematode to enter the host's body (Bode, 2009). When infective juveniles penetrate the body of a target insect and reach the insect's hemocoel, the entomopathogenic bacteria are liberated and proliferate causing the insect death (Goodrich-Blair & Clarke, 2007; Poinar & Grewal, 2012). The death of the host occurs only within 48 hours (Bode, 2009). Entomopathogenic bacteria use nematodes as vectors in order to enter the host as part of their complex symbiotic life cycle (Sicard et al., 2004). During reproduction, bacteria transform the insect cadaver into nutrients for the nematode (Goodrich-Blair & Clarke, 2007) and they excrete diverse broad-spectrum antibiotics. These compounds not only kill the host, additionally defeat other microorganisms that compete for those nutrients (Fang et al., 2014), preserve the insect from putrefaction and allow the nematode reproduction with almost no competition (Li et al., 1999). Studies conducted by Gouge and Snyder (2006) suggested that those competitors can be found in the soil (as saprophytic microorganisms), in the insect's hemocoel or in the nematode's cuticle. In order to succeed in this competition, *Xenorhabdus* and *Photorhabdus* generate a wide range of antibiotics compounds (Bode, 2009). At this stage the nematodes reproduce once to three times inside the hemocoel. The offspring, consisting of new infective juveniles (that have previously recovered its bacterial co-worker) is ready to look for others susceptible insects (Goodrich-Blair & Clarke, 2007).

2.9.2 Antibiotic substances from entomopathogenic bacteria

With approximately 1.4 million species characterized in the literature, insects are about 80% of all life currently described on planet Earth. They are the most dominant and diverse group of terrestrial animals and have an important ecological role and human life (Erwin, 1996). Moreover, this group of invertebrates have their origin approximately 400 million years ago in the Silurian period and are considered among some of the first terrestrial animals (Engel & Grimaldi, 2004). This enormous variety of species and the ancient origin of insects have given the chance for many organisms to create parasitic relationships with them. Throughout this

adaptation process, the dependent organisms have developed different protein-based toxins as well as secondary metabolites (Bode, 2009).

Stilbene and indole were the first two antibiotics compounds discovered from *Xenorhabdus* and *Photorhabdus* species Paul et al. (1981).

Xenorhabdus and *Photorhabdus* spp. are able to produce an extraordinary amount of different antibiotic groups. *Xenorhabdus* spp. produces xenorhabdins, xenorxides, xenocoumacins and indole derivatives (e.g. nematophin, genistein). *Photorhabdus* spp., with less diverse antibiotics than *Xenorhabdus* spp., produces stilbene derivatives (Hydroxystilbenes), anthraquinone derivatives. Bacteriocins (phage particles) are produced by both *Xenorhabdus* and *Photorhabdus* spp (Forst & Nealson, 1996; Jianxiong Li et al., 1999).

These compounds have demonstrated to have antagonistic effect against diverse microorganisms being able to neutralize several bacterial and fungal pathogens with great importance for agriculture. Therefore, they are potential resources for bio agrochemical development. Inhibition of RNA and subsequent inhibition of protein synthesis have been proposed as the mechanism of action of these compounds (Li et al., 1999).

Several of these antimicrobial substances produced by entomopathogenic bacteria have been isolated and identified (Chen et al., 1994; Gualtieri et al., 2009; Li et al., 1997; Li et al., 1995; Yang et al., 2011).

- Secondary Metabolites From *Photorhabdus*

Nowadays, Owing to DNA-sequencing, a considerable number of molecules implicated in the symbiotic and pathogenic interrelation between insect, nematode and bacterium have been identified and characterized. Genetic code sequencing of enzymes implicated in biosynthesis of secondary metabolites have been crucial to identify a large number of gene clusters that make this bacteria genus comparable to *Streptomyces*, a great secondary metabolite producer. The majority of this gene clusters encode nonribosomal peptide synthetases (NRPS) implicated in the production of peptides, lipopeptides or depsipeptides. Two additional gene clusters have also been shown to encode hybrids compounds of polyketide synthases and NRPS. *Photorhabdus* spp. produces as well stilbene derivates. Moreover, it is the only organism known to produce this compound outside the kingdom Plantae. Stilbenes have shown antagonistic effects towards several bacteria species and fungi and they have been found in all members of the genus *Photorhabdus* spp. (Bode, 2009).

In a recent study carried out by Shi et al. (2017) stilbene compounds from a *Photorhabdus temperata* strain were analysed for antifungal effect against diverse phytopathogenic fungi. Some of these stilbene compounds showed remarkable mycelium growth inhibition. Thus,

stilbenes can be considered potential antimycotic substances to be used in agriculture and crop protection.

- Secondary Metabolites from *Xenorhabdus*

Xenorhabdus spp. genome holds as well gene clusters encoding production of secondary metabolites. Most of them are low weight molecules as indole derivatives, and phenethylamides, among others. However, *Xenorhabdus* spp. strains are also able to synthetize bigger molecules as xenorhabdins, xenorxides and xenocoumacins, which are derivates for PKS/NRPS (Bode, 2009). Other metabolites produced by *Xenorhabdus* spp. are lysine-rich cyclolipopeptide (Gualtieri et al., 2009) and nematophin (Li et al., 1997). *Xenorhabdus* spp. by-products have shown to have inhibitions effects on fungi and bacteria, which compete for resources in the reproduction phase of the nematode. In particular, studies carried out by Li et al., (1995) showed that *Xenorhabdus bovienii* produce indoles derivatives with bioactivity against *P. infestans*. Additionally, studies carried out by (Ng & Webster, 1997b) showed that the indoles derivatives from *Xenorhabdus bovienii* tested *in vitro* assays had strong effect on growth inhibition of *P. infestans* on potato leaves with insignificant toxicity. This study concluded that metabolites produced by *Xenorhabdus bovienii* could represent a reliable solution for the control of fungal diseases in agriculture (Wang et al., 2011).

Xenorhabdus bovienii strain A2 is known to have antibiotic properties which particularly some of them have shown to have an antifungal effects (Ng & Webster, 1997b). Li et al (1995) could identify and separate two types of antibiotics from *Xenorhabdus bovienii* with powerful and selective antimytotic properties denominated indoles and dithiolopyrrolones. A study carried out by Ng and Webster (1997) on potato plants, using soluble organic metabolites from *Xenorhabdus bovienii* demonstrated potent inhibition of the development of oomycete *P. infestans*. No blight symptoms, mycelium or lesions on potato leaflets were observed on the inoculated areas. It is believed that the antibiotic activity comes from the combined action of indole derivates plus xenorhabdins. The experiments showed as well some level of phytotoxicity on potato leaves at all treatment concentrations after 24h application; however, these effects were imperceptible after 8 days.

The use of *Xenorhabdus* spp. in bioagriculture has presumably a great potential and future. The bacterium produces extracellular by-products with antibiotic and fungicide attributes that can be use to reduce the application of synthetic compounds on the fields (Fang et al., 2014). *Xenorhabdus* spp. can produce nonprotein-based substances e.g. indoles, xenorhabdins, xenocoumacin, nematophin, benzylideneacetone, xenortide, xenematide and cyclolinopectide

and protein based compounds as xenorhabdincin and chitinases. Some of the nonproteinaceous groups like nematophin and xenocoumacin are able to impede the development of *B. cinerea* and *P. infestans* (Fang et al., 2014). Xenocoumacin 1 is known as an antibacterial component from *Xenorhabdus nematophila* var. *pekinensis* but it has been demonstrated that this substance has powerful antifungal properties against *P. infestans*, showing 100 % mycelia inhibition in vitro plus suppression of sporangia production. Xenocoumacin 1 also showed remarkable *in planta* inhibition of *P. infestans*. Thus, Xenocoumacin 1 should be contemplated as a novel biofungicide for late blight in potato cultivation (Yang et al., 2011). Nematophin, a novel biotoxin produced by a few strains of *Xenorhabdus nematophilus*, has been tested against several microorganisms with positive results showing as well strong in vitro bioactivity against *P. infestans* among other plant pathogens (Li et al., 1997).

Xenorhabdus spp. has a broad activity spectrum towards pathogens of agronomic importance such as bacteria, fungi and insects, among others. However, culture conditions have a crucial role for the production of these compounds. Either yield or metabolic characteristics of the bacteria are very sensitive to small alterations in the composition of the growing medium. By changing nutrients content or cultivation parameters the production of secondary metabolites can be certainly improved. These findings are determinant for large scale production and subsequent use for biocontrol of plant diseases in agriculture (Wang et al. 2011).

3 AIMS OF THE STUDY:

The aim of this Thesis was to evaluate the suppressive effects of 24 selected entomopathogenic bacteria strains from the genera *Xehorhabdus* and *Photorhabdus* on the fungus-like pathogen *Phytophthora infestans* inoculated on potato tubers under *in vitro* conditions with the purpose of setting up a base for further *in vivo* and field research in a framework where developing safe commercial formulations and treatments are needed and with the purpose of responding to the demands of the agroindustry.

Objectives of this study were to find among the selected 24 strains, effective isolates with inhibitory effect on the growth of *P. infestans*. Perform basic and preliminary phytotoxicity tests (by simple visual and touch observations/approach) and establish groundwork for further research was also a target. A long-term objective would be characterization of the specific most active secondary metabolites and mechanisms of action in further studies.

In order to meet the objectives the following Hypothesis were formulated:

1. The selected strains from the genera *Xenorhabdus* and *Photorhabdus* suppress the development of *P. infestans* in inoculated potato tuber slices.
2. The Antimycotic effect is strain specific (different strains of the same species have different antimycotic effects).
3. Strains suppressing disease do not produce phytotoxicity.
4. Bacteria strains show as well antimycotic effect in a whole tuber system.
5. There are significant differences on the inhibitory behaviour between strains

For the purpose to fulfil all questions mentioned, *in vitro* experimental procedures were designed using either tuber slices or whole tubers. These methods are detailed explained in the following chapter.

4. MATERIALS AND METHODS

During 3 months experiments with tubers of cv. Bintje were conducted at ZHAW University in Wädenswil. The entire process was carried out at Campus Grüental in the Biosafety laboratories for Phytomedicine (GA 128 and GA 113.1) belonging to the Institute for Environment and Natural Resources. Tubers slices and whole tubers were inoculated with *P. infestans* and treated with entomopathogenic bacterial solutions with the purpose to assess any growth inhibition of the oomycete.

3.1 Tuber stock

Solanum tuberculosum L. tubers cv. Bintje, were selected from the "Netherlands Catalogue of Potato Varieties 2011". Bintje variety was chosen based on certain suitable characteristics for the purpose of the study: High susceptibility to Late Blight (4.5 of scale from 3 to 10), high to good yield (7.5 on a scale from 4 to 9), good dry matter and good consumer quality. Susceptibility of Binje variety was contrasted with scientific studies (Flier et al., 2001).

Bintje potato seeds were planted at the beginning of June, 2018 in the facilities meant for this purpose at ZHAW University, Campus Grüental in Wädenswil (Fig. 5). Seed potatoes were planted in two different environments, free land and greenhouse to increase the probability of growth success (Figs. 6 and 7, respectively), according to the standards of agriculture. Plants were visually inspected every two weeks.

Figure 5: Solanum tuberculosum L. cv. Bintje plants. 8 days after being planted.

Figure 6: *Solanum tuberculosum L.* cv. Bintje plants in open field. 3 months after sowing.

Figure 7: *Solanum tuberculosum L.* cv. Bintje plants in greenhouse.2 months after sowing.

Healthy potato tubers were harvested mid of September 2018 by hand in order to minimize wounding and were either used directly or preserved in open grid boxes at 6°C until use for up to 56 days.

Undamaged and healthy tubers obtained from either the field or from 6°C room were gently and thoroughly rinsed under running tap water to remove adhered soil. Surface was disinfected in 5% sodium hypochlorite for 20 minutes, then immersed in 70% Ethanol for 1 minute and finally briefly immersed in 90% Ethanol (Fig. 8). After disinfection process, tubers where left to dry in a laminar flow hood (S@afeflow 1.8) until they were totally dry (Fig. 9). After disinfection tubers were either immediately used for the experiments or kept at 5°C for maximum two days before used in sterile containers.

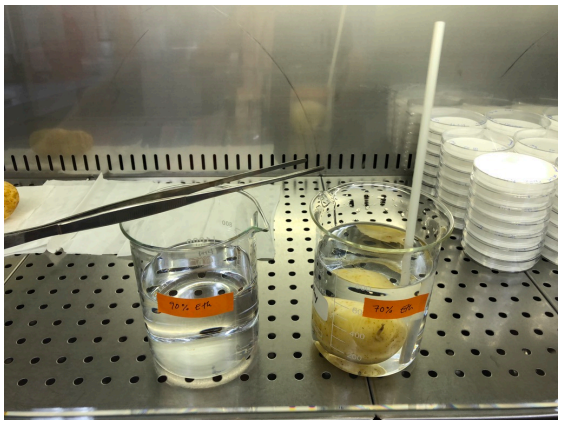


Figure 8: Part of the disinfection process. 70% and 90 % Ethanol in 1L beakers.

Figure 9: Potato tubers drying in the laminar flow hood on absorbent paper.

3.2 Preparation of media

Different growing media were needed for the purpose of this study:

- V8-agar: *P. infestans* growth in Petri dishes
- PDA-agar: *P. infestans* new isolates growth in Petri dishes

- Standard-I-agar: bacteria growth in Petri dishes
- LB-agar: bacteria growth in Petri dishes
- LB-broth: bacteria growth in Centrifuge tubes

Media were prepared according to standardized formula (Annex X) in 1L Schott glass bottles and sterilized in autoclave Systec VX-95 at XXX°C for XX minutes using the program intended for this purpose: 8-liquids.
After sterilization, Schott bottle content was homogenized on a magnetic stirrer (either IKA® RH basic 2 or IKAMAG® RET) and in case of agar media, immerse in an laboratory water bath at approximately 50°C until they could be safely touch by hands (Figs.).
Once the temperature was adequate, the bottles were transferred inside the laminar flow hood where agar was poured into 9 cm diameter Petri dishes. Plates were then left to solidify and dry inside the laminar flow hood to eliminate humidity (Fig.). When totally dry, they were piled up, labelled with a colour identification code and stored in a refrigerator at 5°C until use (Fig.). Storage was not longer than two months to guarantee its quality. Broth media were left to cool down at room temperature (23°C) and kept in these conditions until use.

3.3 *Phytophthora infestans* source of inoculum

P. infestans isolate (identification), obtained by Dr. Esther Fischer from tomato plants in the facilities of Campus Grüental in 2014 was used throughout all experimental phase. In order to allow *Phytophthora* to produce a maximal amount of spores and thus, work with high pathogen efficacy, tests were always made with approximately 21-day-old cultures. The oomycete was regularly inoculated in sterile Petri dishes containing V8-agar medium so that 21-day-old cultures were always available. V8-agar containing the inoculum was perforated with a sterilized cork borer and the resulting plugs were transferred with an inoculation needle to the centre of a new V8 Petri dish (Fig. 10).
This process was carried out inside the laminar flow hood and material was sterilized using a LABFLAME burner (Fig.). The cork borer and inoculation needle were as well sterilized in the autoclave after use to avoid any contamination. Petri dishes were wrapped with paraffin film (PARAFILM®M) and incubated in a climatic chamber at 18°C for 21 days (BINDER).

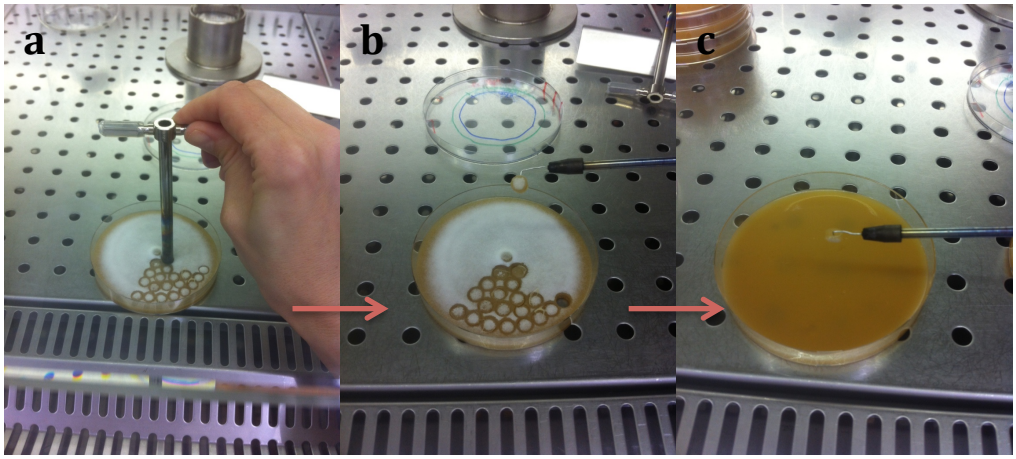


Figure 10: *P. infestans* inoculation process. a. Agar plugs cut with cork borer from V8 agar plate with *P. infestans*. b. Taking an agar plug with inoculation needle. c. Finally, inoculation of the *oomycete* in a new V8 plate.

Inoculum preparation:

21-day-old cultures of *P. infestans* were used as a source of inoculum. In case of whole tuber assay, preparation of inoculum was done as follows. 20ml sterile water was very carefully poured on an open Petri dish containing the oomycete culture being especially cautious so the spores would not fly out of the plate. After a few minutes, with the sterile end of a microscope slide (Thermo Fisher), sporangia and mycelium were gently scraped from the surface of the agar (Fig. 11) and harvested on a sterile 250ml Schott glass bottle using a sterile glass funnel with glass wool to filter (Fig. 12). The solution was kept then at 5°C for two hours to stimulate zoospore release. Using a 0.1000mm deep hemacytometer, sporangia per ml were counted after 2 hour. Spore concentration used for inoculation was between 8000 and 10000 spores per millilitre.

For tuber slices assay agar plugs directly from V8 Petri dishes were taken and placed on the centre of the potato slice.

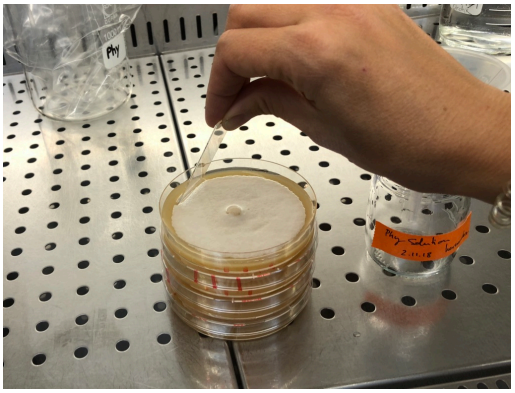


Figure 11: Scrapping *P. infestans* culture with microscope slice.

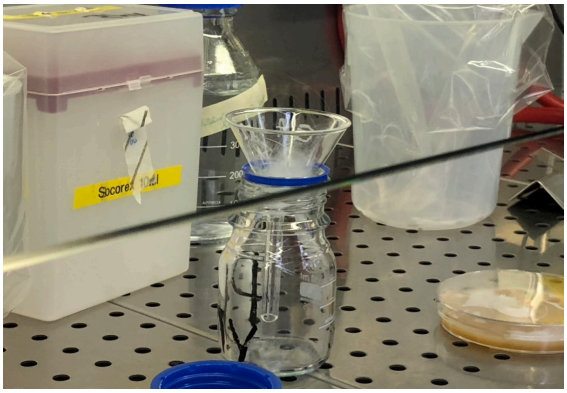


Figure 12: Glass funnel containing glass wool (red arrow) used as a filter for the scrapped oomycete culture and the 250 ml schott glass bottle in which the solution was collected.

3.4 Entomopathogenic bacteria

For the purpose of this Thesis, 24 entomopathogenic bacteria strains were selected (Table 1). They belong to a exclusive collection from Institute of Environment and Natural Resources from ZHAW. These strains were isolated from entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* collected in different locations throughout Switzerland within the framework of the project X led by Dr. Jürg Grunder in collaboration with the European Cooperation in Science and Technology (COST).

Table 1

List of entomopathogenic bacteria strains *tested on slices assay*

Number	16S sp. determination	Nematode species
3	*Photorhabdus luminescens*	*H. megidis*
4	*Xenorhabdus bovienii*	*S. affine*
7	*Photorhabdus temperata*	*H. megidis*
10	*Photorhabdus temperata*	*H. megidis*
16	*Xenorhabdus budapestensis*	*S. bicornutum*
18	*Xenorhabdus budapestensis*	*S. bicornutum*
22	*Xenorhabdus budapestensis* DSM 16342T	*S. bicornutum*
31	*Xenorhabdus bovienii*	*S. feltiae*
34	*Xenorhabdus bovienii*	*S. feltiae*
45	*Xenorhabdus bovienii* strain FR44	*S. intermedium*
49	*Xenorhabdus bovienii* strain FR44	*S. affine gruppe*
59	*Xenorhabdus bovienii* strain CS03	*S. feltiae*
63	*Xenorhabdus bovienii* strain FR44	*S. kraussei*
83	*Xenorhabdus bovienii* strain FR44	*S. feltiae*
102	*Xenorhabdus bovienii* strain FR44	*S. kraussei*
103	*Xenorhabdus bovienii* strain FR44	*S. kraussei*
107	*Xenorhabdus bovienii* strain FR44	*S. kraussei*
113	*Xenorhabdus bovienii* strain CS03	*S. intermedium*
114	*Xenorhabdus bovienii* strain FR44	*S. intermedium*
117	*Xenorhabdus bovienii* strain CS03	*S. intermedium*
118	*Xenorhabdus bovienii* strain CS03	*S. intermedium*
119	*Xenorhabdus bovienii* strain CS03	*S. affine*
120	*Xenorhabdus bovienii* strain FR44	*S. intermedium*
121	*Xenorhabdus bovienii* strain CS03	*S. intermedium*

This collection was genetically characterized by Dr. Beat Ruffner at the Swiss Federal Institute of Technology (ETH). These specific 24 strains were selected based on previous studies carried out at ZHAW, which demonstrated their fungicide activity against *P. infestans* in tomato plants (Walch, 2016). However, they had never been tested in *Solanum tuberculosum L.* tubers.

Bacteria-based treatment preparation:

Bacterial treatments consisted in a mixture of supernatants and bacteria cells immerse on LB broth medium. Previously, entomopathogenic bacteria were preserved at -86°C in 2ml tubes containing 50% glycerol and 50% SIB. Prior to the experiments bacteria were thawed at room temperature (23°C), shaken with the help of a shaker IKA® Vortex Genius 3 and aseptically inoculated in Petri dishes with either Standard-I-agar or LB-agar media using sterile disposable loops for 3 days. Single colonies growing on the agar were inoculated in 50mL centrifuge tubes (only one single colony per tube) containing 40ml LB sterile broth medium. Tubes were then agitated on the Vortex shaker (Fig.14) in order to dissolve the bacteria pellet and improve bacterial contact with the medium, which facilitates growth. Immediately after, the tubes were placed on a Universal Shaker SM 30A (Edmund Bühler GmbH) using a rack placed at 45°(Fig. 13). Conditions for bacterial optimal growth were: room temperature (23°C) and 125 rpm for 48h.

After incubation time of 48h optical density (OD) was measured with a UV-1600PC spectrophotometer (VWR) at 600nm. An OD of 0.625 ($5x10^8$ cells/ml) in the bacterial solution was the target. In case OD was under 0.625 then concentration of the solution was required. Tubes were then centrifuged for 15 minutes at 3200 rpm on a Heraeus Multifuge 1S centrifuge (Thermo Electron Corporation). In order to concentrate the solution and obtain the desired OD, a determinate volume of supernatant was poured out from the tube. The rest of the tube content was resuspended with the Vortex shaker (Fig. 14). OD was measured again with the spectrophotometer. This process was repeated until the desired OD = 0.625 ± 0.015 was obtained. The bacterial treatments used for the experiments consisted in 48h bacteria cultures plus its supernatants.

The above-described process was carried out with strict aseptic practises, working on the Laminar flow hood to avoid contaminations and guaranteeing that the treatments were axenic.

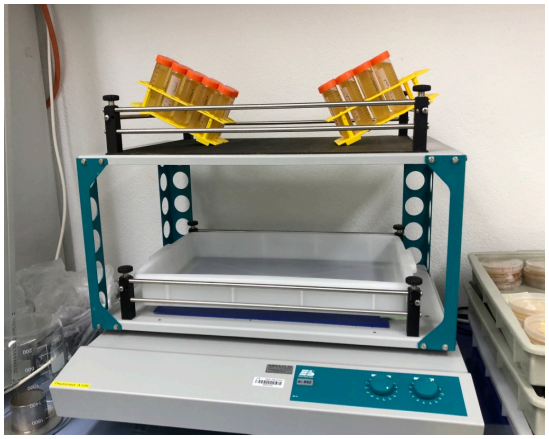


Figure 13: Bacteria incubating in centrifuge tubes on the universal shaker at room temperature of 23°C.

Figure 14: Dissolving bacteria colony with the shaker.

3.5 Experimental design

48h bacterial solutions were applied as treatment. Sterile distilled water and copper solution 0.8% were applied as negative and positive control respectively with 7 replicates each. In total 21 samples for each bacterium tested (Table 2). A total of 24 bacteria strains were tested in the slices assay, which means 504 slice samples analysed. In case of whole tuber assay, 8 bacteria were analysed which correspond to 168 samples. Randomization

Table 2

Experimental design overview

Type of assay	Number of bacteria tested	Treatment group	Code	Repetitions	Total tests per bacteria
Slices	24	Negative control: sterile water	W	7	21
		Positive control: Copper solution 0.8%	Cu		
		Bacterial solution	B		
Whole tuber	6	Negative control: sterile water	W		
		Positive control: Copper solution 0.8%	Cu		
		Bacterial solution	B		

3.6 Slices assay

Slices approximately 0.7cm thick were cut with a sterilized conventional knife from the central part of the tubers (Fig. 15) and placed each of them in 9cm Petri dishes over 3 layers of filter paper, previously moistened with 5ml of sterile distilled water to ensure high relative humidity. Each slice was randomly assigned to the tests.

Inoculation of tuber slices was carried out by cutting agar plugs with a sterile cork borer from a 21-days-old *P. infestans* culture and placed them in the approximate centre of the tuber slice with the help of a inoculation needle. This work was carried out inside the Laminar flow hood to minimize microorganism contamination. The inoculated slices were then incubated for 24h previous to the application of the treatments in a climate chamber either Fitotron or Binder depending on availability, at 18°C and 80% humidity to provide favourable conditions for *Phytophthora* establishment.

After 24h, treatments were administrated on the surface of the slice in 4 points around the agar plug and equidistant to the edge of the tuber slice. 20 microliters of treatment was applied on each point with a micropipette (Fig. 16).

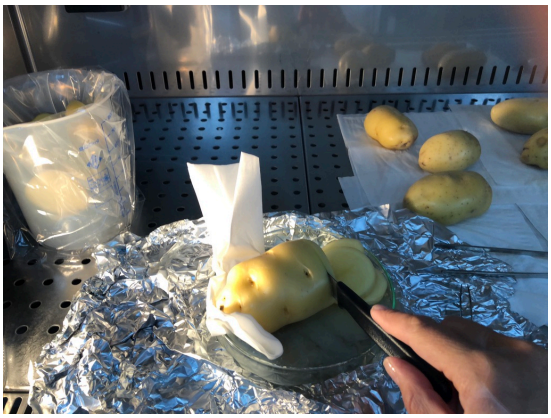


Figure 15. Cutting potato slices on a sterilized glass plate.

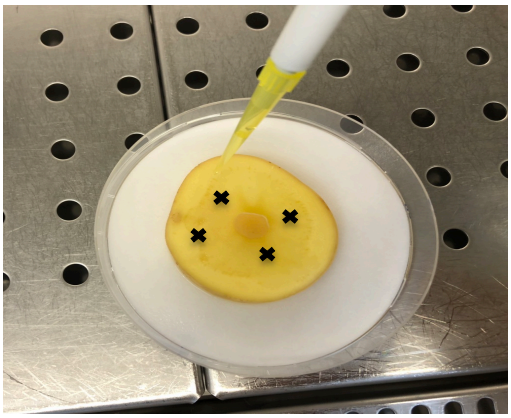


Figure 16. Application of treatment in 4 points of the potato slice surface.

Every plate was labelled with bacteria identification number, treatment identification letter (W=water, Cu=copper, B=bacteria), replicate number (1 to 7) and date of the experimental setting (Fig.). Plates were incubated in a climate chamber at 18°C and 80% Relative Humidity.

Photos from each plate were taken after 6 (±1) days and after 12 (±1) days in order to control evolution of the disease. Measurements were made after 12 days of incubation. The outline of the potato slices, rotten tissue, sporangia and any other visible organism was marked with different colour markers (Fig. 17). Necrotic tissue and sporangia coverage were considered as a measurement of disease development. The different marked areas were measured with a planimeter (Tamaya® Planix 5) and recorded on an Excel document for later statistical analysis. The presence of sprouts was as well recorded and special observations if any were also described. After measurement potato slices were physically inspected to account for toxicity. Surface pressure with fingers in order to account for any decomposition signs and more detailed visual inspections was done.

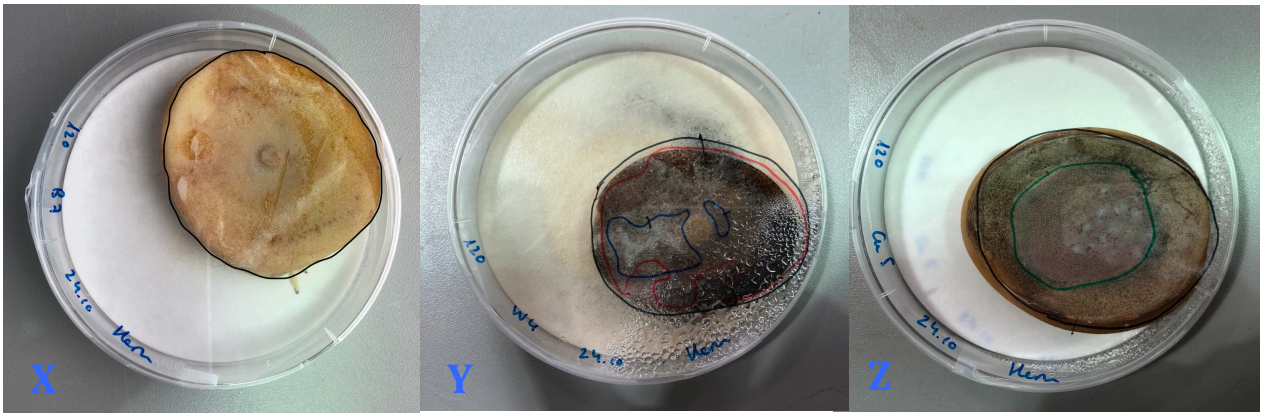


Figure 17. Three randomly selected Petri dishes (X, Y, Z) marked with different colour pens. Black for the outline of the potato slice, blue for delimitation of *Phytophthora* sporangium, red for necrotic tissue and green for other organisms. X. Bacterial treatment (B), Y. Negative control (W) and Z. Positive control (Cu).

3.6 Whole tuber assay

Whole tuber assays were carried out in 2 versions.

- Version 1. Puncture and syringe injection

After the above-described disinfection procedure, each potato tuber was punched with an inoculation needle 0.5 cm deep in one of its eyes and the puncture site was marked (Fig. 18). Tubers were then inoculated with 1ml spore solution by injecting it with a syringe on the wounded eye (Fig. 19).

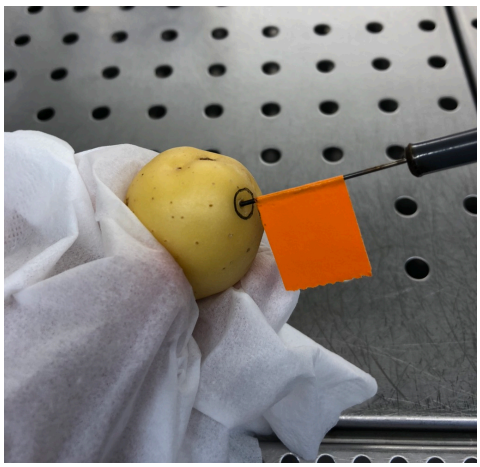


Figure 18: Puncture with sterile needle. The orange marker limits the depth of the puncture.

Figure 19: Injection of treatment in the punctured site.

Tubers were placed on handmade poultry wire mesh supports (Fig. 20) in a plastic container with 30 ml sterile distilled water for favouring saturated conditions, which helped disease establishment (Fig. 21). After closing the containers with the corresponding lid, every sample was labelled with bacteria identification number, treatment identification letter (W=water, Cu=copper, B=bacteria), replicate number (1 to 7) and date of the experimental setting.

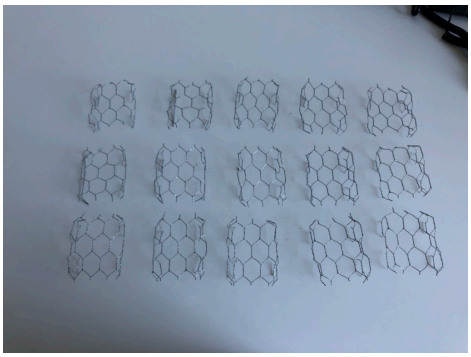


Figure 20: Poultry wire mesh supports.

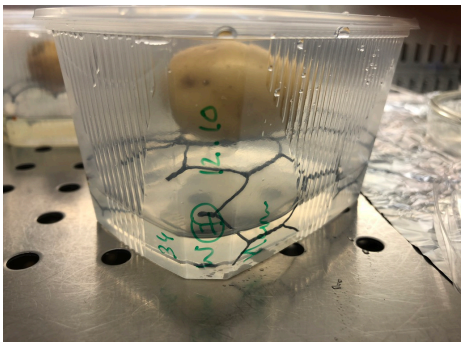


Figure 21: Container with water and tuber.

Containers were then placed in plastic crates in a climate chamber at 18°C and 80% relative humidity in the darkness. After approximately 24 hours, 1ml bacteria treatment was injected as well as 1ml sterile water and 1ml copper 0.8% solution serving as negative and positive control respectively. After this, containers were again placed on the climate chamber at 18°C and 80% relative humidity for 10 days. These conditions were propitious for *P. infestans* development. After ten days, each tuber was visually examined for the presence of late blight lesions on periderm and flesh as well as presence of mycelium. Diameter and depth of lesions was measured with a calliper (Fig. 22). With these two measurements the volume of a cone was calculated as an approximation to the affected tissue. Sporangiophores (Fig. 23), if present, were afterwards analysed under the microscope for confirmation.

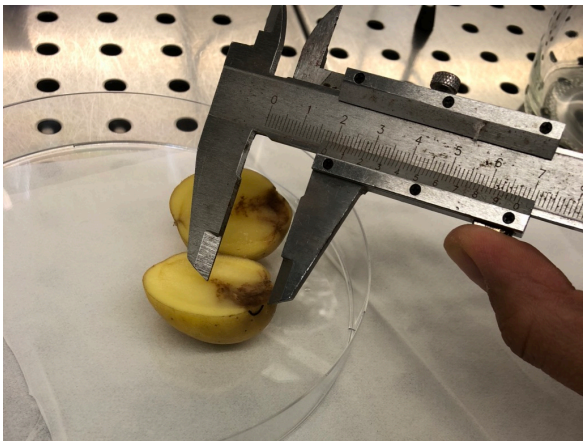


Figure 22: Measurement of the depth of *Phytophthora infestans* symptoms with a calliper.

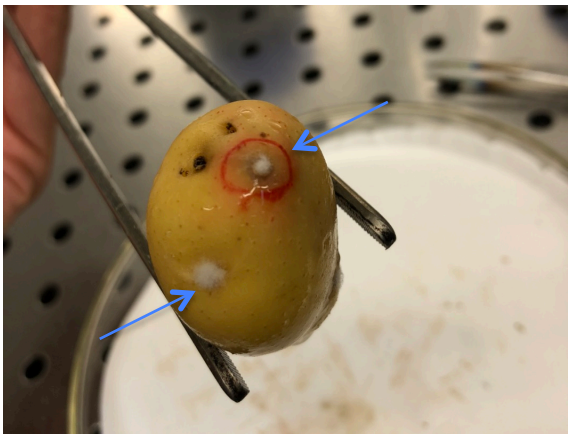


Figure 23: Examination of a tuber 12 days after inoculation. Tuber shows sporangia growth in the injection site and other areas (Blue arrows).

- Version 2. Pipette-tip injection

In this variant of the experiment the inoculum, bacterial treatment and controls were applied through 200µl pipette tips that were previously inserted in the tubers at 1.0 cm depth. Then tubers were placed on a poultry wire mesh support with the pipette tip big orifice facing

straight upward (Fig. 24) and solutions were applied by injecting then with the syringe directly inside the pipette tips (Fig. 25). In this case boxes (container description).

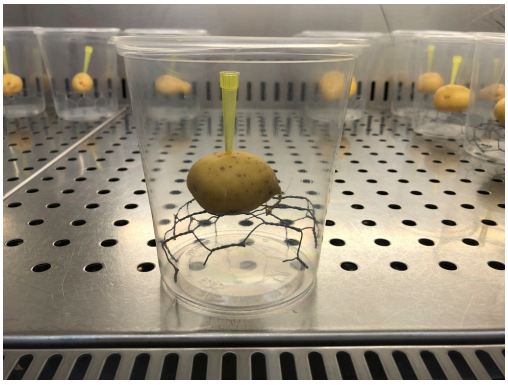


Figure24: Tuber with embedded pipette over wire mesh support.

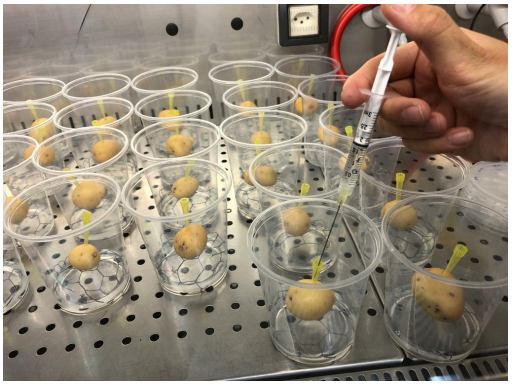


Figure 25: Injection of *Phytophthora infestans* inoculum through the pipette tip.

3.7 Isolation of a possible new strain of *Phytophthora infestans*:

During the growing phase of the potato plants, at the beginning of September, and after a period of rain with a drop of the temperatures, the crop became unexpectedly infected by natural ways with *P. infestans*. Plants showed clear symptoms of late blight in leaves, stems and tubers. The disease spread very rapidly, in 3 to 4 day through the whole area (See figs. in XX Section). This infection only affected at the crop located in free land while the crop located in the green house remained healthy. Given the possibility of being a new strain of *P. infestans*, the pathogen was isolated in the laboratory. 5 potato tubers with evident signs of late blight were incubated in plastic boxes at 18°C and 80% relative humidity. When sporulating sporangia was coming out from the periderm, tubers were transferred to the laminar flow hood. A few tufts were taken with sterile tweezers and gently placed on different PDA and V8 Petri dishes. After closing the Petri dishes and wrapping them with paraffin film, plates were incubated in a climate chamber at 18°C. After approximately 4 days, sporangyophores and conidia from *P. infestans* and other microorganisms was evident. By carefully trying to take pure *P. infestans* material and avoiding secondary microorganisms the oomycete was again inoculated in clean new Petri dishes. This process was repeated until pure culture was obtained. After several tries, a possible new mating type was finally isolated to contribute to the ZHAW collection of microorganisms. Visualizations with the XXX microscope showed the typical lemon shaped sporangia from the *P. infestans* (Figs.). If this new isolate would be confirmed by a genetic analysis it could be very useful for further research on late blight affecting *Solanum tuberosum* because the strains used until now were isolated from *Solanum lycopersicum* (tomato).

3.8 Visualization and confirmation of microorganisms with microscope

With the purpose to confirm the presence of *P. infestans* in the treated samples as well as analysing any other microorganism a Leica DM5500 B microscope was used and pictures were taken in order to leave it documented. Samples were prepared on microscope glass slides with lactophenol cotton blue stain as a dying agent. Mounts were prepare using clear cellophane tape by carefully touching the fungal colony from the culture dish opened in the biological safety cabinet and then placed on the microscope glass slide with a drop of cotton blue.

3.9 Data analysis

The statistic program R (Version 3.2.2) together with RStudio (Version 0.99.485) was used in order to analyse the data. The variables were percentage of necrotic tissue and percentage of sporangia coverage. As data showed neither normality nor homoscedasticity, assumptions for ANOVA test were not fulfil and could not be used as an analysis method. Instead, it made sense to use a non-parametric method like kruskal-Wallis test. P-values were obtained with a significance level $\alpha = 0.05$. Also Pairwise Wilcoxon test was then conducted in order to obtain pairwise comparisons among treatments ($\alpha = 0.05$).

Also, differences between bacterial treatment themselves were compared and ranked with the purpose to see whether one or more bacteria strains had bigger effect than the others. In order to do so, Wilcoxon test was again conducted but this time omitting the method for p adjustment because its use overcorrected p-values and was not possible to get logical values.

Additionally, In order to analyse whether there was a relationship between bacteria treatment and sprout production, chi-squared test was conducted with a significant level $\alpha = 0.05$.

Experimental hypothesis:

- The mean of necrotic area in potato slices under treatment B is lower than the mean of necrotic area in potato slices in the untreated Control group W.
- The mean of the area covered with sporangiophores in potato slices under treatment B (bacteria treatment) is lower than the mean of necrotic area in potato slices in the untreated Control group W.
- The mean of necrotic area in potato slices under treatment B is equal to the mean of necrotic area in potato slices under positive control Cu
- The mean of necrotic area in potato slices under treatment B is equal in all strains.
- Slices treated with B are more likely to produce sprouts

4. RESULTS

4.1 Slice assay

From the total screened bacterial strains, 21 showed clear fungicidal activity against *P. infestans* (Figs. 26 and 27). Potato slices even presented a liquid layer of exudate around the agar plug from the complete lyses of the mycelium (Fig. 28). This fact was also noticed by Ng and Webster (1997) in studies carried out with *X. bovienii* on potato tubers. Bacteria-based treatments supressed the development of late blight lesions with either none or little areas of white sporangia layer as well as low necrosis incidence. Visually, slices treated with bacteria-based solution presented notably healthier appearance compared to positive and negative controls (Fig. 29)

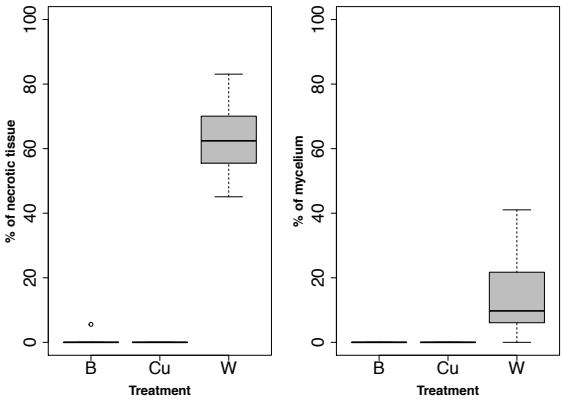


Figure 26: Visualization of the data obtained from strain 34. Percentage of rotting areas for the different treatments, B, Cu and W (Left). Percentage of sporangium coverage (Right). Bigger differences between treatments can be appreciated in the left plot as rotting appears usually before than sporangia.

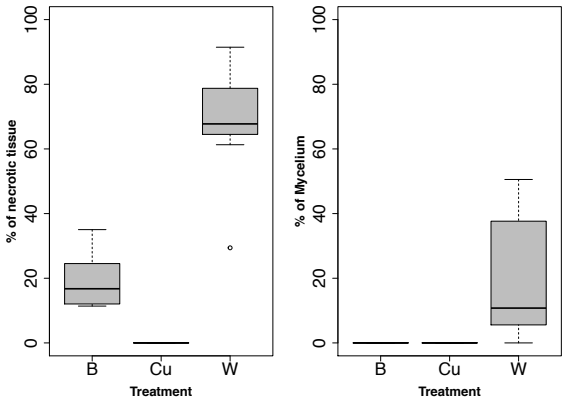


Figure 27: Visualization of the data obtained form strain 102. Left: Percentage of necrotic areas for different treatments (B, Cu and W). Right: Percentage of sporangium coverage. Bigger differences between B-W and Cu-W can be noted for the percentage of necrotic tissue than for sporangia as rotting appears usually before in time.

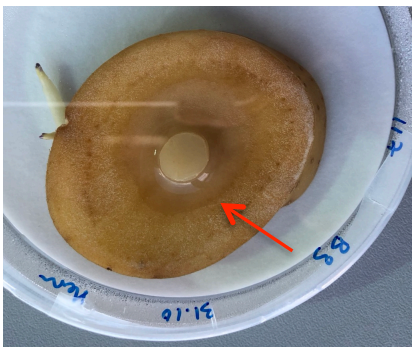


Figure 28: Layer of exudate from the complete lysis of the agar plug mycelia

Figure 29: Seven repetitions of every treatment (W upper line, Cu middle line and B below). It can be visually appreciated the difference in colour and health.

However, none of the treatments did inhibited late blight symptoms completely. Late blight acuteness showed variations between strains with a range of 0.3-67.0% for the means of necrotic areas (Fig. 30 and table 3).

Strains 18, 22, 34, 59, 63, 102, 103, 107, 113, 114, 117,118 and 120 showed significant inhibition effect on necrotic tissue together with mycelium development. Sporangiophores were only found in very small areas and only in a few samples. Moreover, 16 of the total strains showed 100% sporangia inhibition. Additionally, results suggested that strains 18, 22, 31, 34, 45, 49, 83, 114, 117, 118, 120 and 121 even exhibited levels of suppression comparable to those showed by Cu. Strains 34, 114, 117 and 118 seem to yield the best results. Conversely, strains 7, 10, and 16 did not show such as inhibitory effect and results from strain 10 showed that late blight lesions and sporangia coverage were significantly higher that in the negative controls (Table 3).

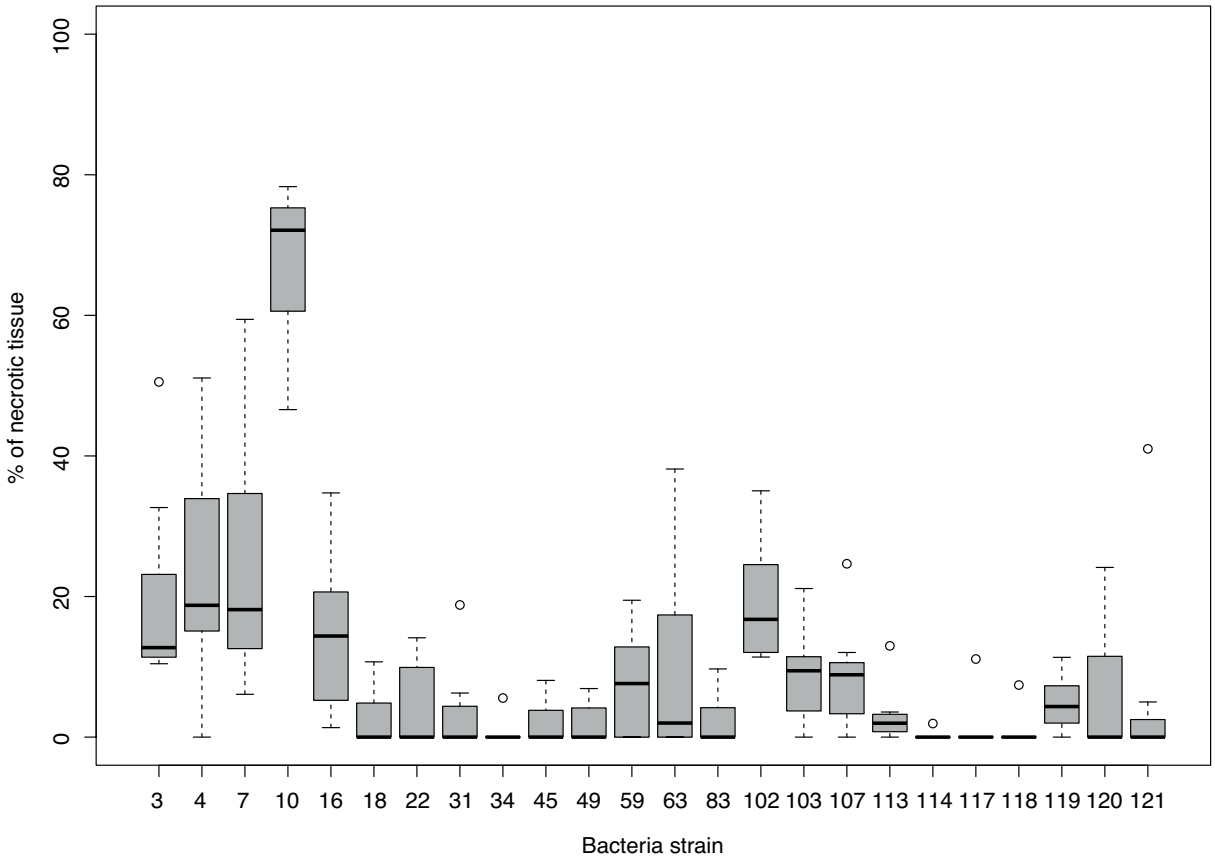


Figure 30: Percentage of necrotic tissue for every bacteria strain. I can be noted that strain 10 gives the worst results. Strains 34,,114, 118 seem to exhibit the best inhibitory effect.

Table 3

Incidence of late blight (percentage of visible rot) and number of samples presenting sprouts in slices treated with bacteria-based solutions, negative controls and positive controls.

	Bact. Treatment			Negative control			Positive control		
	Bight symptoms			Bight symptoms			Bight symptoms		
Str.	Rot	Sporangia coverage	Sprouts	Rot	Sporangia coverage	Sprouts	Rot	Sporangia coverage	Sprouts
3	20.4	0.0	3	51.8	1.0	0	0.0	0.0	0
4	24.0	0.0	4	31.9	12.5	0	0.0	0.3	2
7	25.5	1.2	3	34.8	20.4	1	0.0	8.5	0
10	67.0	18.5	0	52.4	0.0	2	0.0	0.0	2
16	14.6	3.8	3	26.5	21.4	1	0.0	0.0	2
18	2.9	0.0	0	77.1	48.5	0	0.0	0.0	0
22	4.9	0.0	3	67.7	13.3	0	0.0	1.1	0
31	3.9	0.0	3	49.3	2.3	0	0.0	0.0	0
34	0.8	0.0	2	63.1	15.2	0	0.0	0.0	2
45	2.2	3.2	2	15.9	11.2	1	0.0	0.0	1
49	2.2	0.5	2	44.7	4.1	0	0.0	1.4	3
59	7.5	0.0	5	22.0	3.9	2	0.0	8.3	1
63	10.7	4.3	1	40.1	29.5	1	0.0	0.0	3
83	2.6	0.8	3	50.6	14.3	0	0.0	0.0	0
102	19.5	0.0	1	67.9	21.1	0	0.0	0.0	0
103	8.7	0.0	2	57.4	9.6	1	0.0	0.6	0
107	8.8	0.0	1	80.1	20.8	0	0.0	1.5	0
113	3.3	1.5	3	52.9	8.8	1	0.0	0.7	0
114	0.3	0.0	2	63.0	18.7	3	0.0	0.0	0
117	1.6	0.0	2	49.6	13.4	2	0.0	0.0	0
118	1.1	0.0	1	61.4	20.4	0	0.0	0.0	0
119	4.9	0.0	3	33.1	11.5	1	0.0	0.0	0
120	6.7	0.0	5	55.5	14.6	1	0.0	0.0	0
121	6.6	0.0	3	49.1	16.4	1	0.0	0.0	1

Strains 3, 31, 45, 49, 83 and 119 showed significant differences between bacterial treatment and negative controls in the establishment of necrotic areas but not on sporangia growth, as the latter was more delayed. In these trails sporangiophores of the controls were often appearing later in time, after the actual measurements were taken. This was noticed by post observation of the samples at 4 to 8 days after measurements.

After visual and physical inspection of the treated slices at 12±1 days of incubation, phytotoxic symptoms were not apparent as the slices were hard to the touch and not significant changes in the colour of the surface were visible apart from those due to normal

oxidation. Moreover, significant differences between the production of sprouts by slices treated with bacteria-based broth and those treated with negative and positive controls were found (Table 3). Conversely, positive controls, after 2-3 day of application of Cu showed obvious signs of phytotoxicity. 100% of slices treated with Cu presented black burnt areas all over the treated surface and apparent dryness. Surprisingly, these slices showed as well growth of pinkish layer over the black burnt areas (Fig. 31). Microscope visualization showed branching conidiophores with a paintbrush-form-like (or similar to a paintbrush), which together with literature comparison suggested that this organism could belong to the genus *Penicillium* (Fig. 32), however, this has not been yet verified.

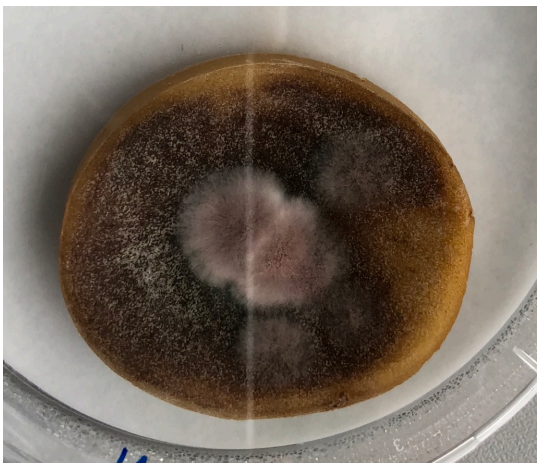


Figure 31: Potato slice treated with Cu. Burnt surface can be appreciated as well as pinkish mycelium.

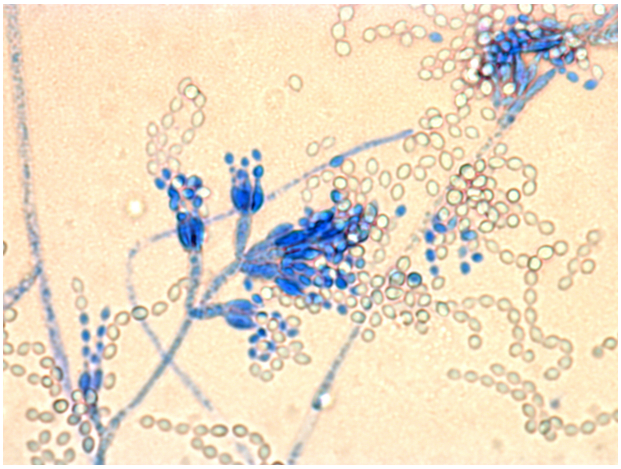


Figure 32: Microscope visualization of pinkish mycelium. Brush-like conidiophores remain to *Penicillium* spp. Kamera= MC170 HD; Mikroskop= DM5500B.

4.2 Whole tuber assay

For the direct-syringe injection version of the tubers assays strains 31, 34, 49, 63, 113 and 118 were tested against *P. infestans*. Strain 31 showed significant fungicidal effect relative to the negative control. Conversely, and against the expected, nor strain 34 neither 49 showed suppression of the disease (Fig. 33 and 34). Trials with strains 63, 113 and 118 did not yield any useful data because the infection protocol was not successful, thus there was not data available. Experiments were then repeated but tubers from both, negative controls and bacteria appeared completely healthy.

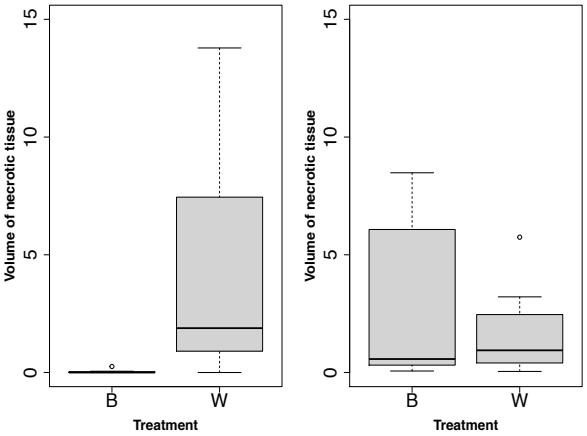


Figure 33: Results of direct-syringe injection assay. Left: Visualization of data from strain 31. There is significant difference between B (bacteria treatment) and W (negative control). Right: Data from strain 34. There is no significant difference between B (bacteria treatment) and W (negative control).

Figure 34: Tuber treated with strain 34. Potato tuber shows large rotting area around the wounded inoculated area. Treatment did not supress the development of *P. infestans*.

Unfortunately, pipette-tip injection did not produce any useful data. Infection of the controls or treatments was neither successful. All potato tubers seemed to be healthy without any signs of late blight in any of the performed tests and repetitions.

4.3 Microscope visualizations

Generally, post-visual analysis of sporangyophores and conidia at microscope confirmed *P. infestans* infection in negative controls showing the typical lemon-shaped sporangia (Fig. 35). However, exceptionally, other species were found. Fig. 36 shows other species distinct from *P. infestans*, however further visualizations and analysis should have been done in order to identify it. Visualizations from strain-10-treated slices also exhibited the characteristic sporangia from *P. infestans*, confirming that this strain did not supress late blight infection development.

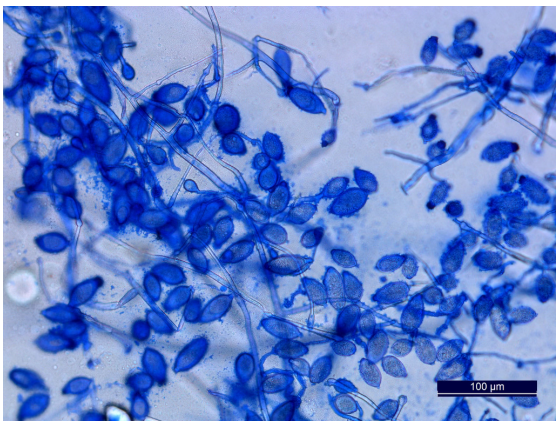


Figure 35: Sporangia from *Phytophthora infestans* from control tests. This confirmed late blight infection. Fruits bodies present the typical lemon-shaped form. Camera MC170 HD; Microscope DM5500B

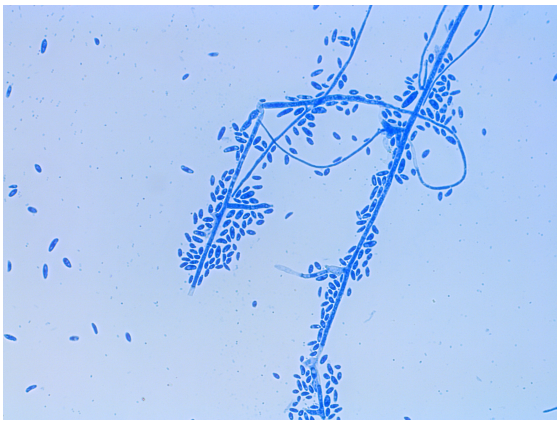


Figure 36: Different fungus specie found on the controls. Hyphae from which phialides extend producing microconidia. Camera MC170 HD; Microscope

Suspicious presence of white layers on the surface of bacteria-treated slices was as well microscopically visualized to confirm the presence of potato starch granules instead of *P. infestans* conidia (as believed in a first look) (Fig. 37a). The characteristic growth rings (Pilling & Smith, 2003) were obvious at a microscope view (Fig. 37b).

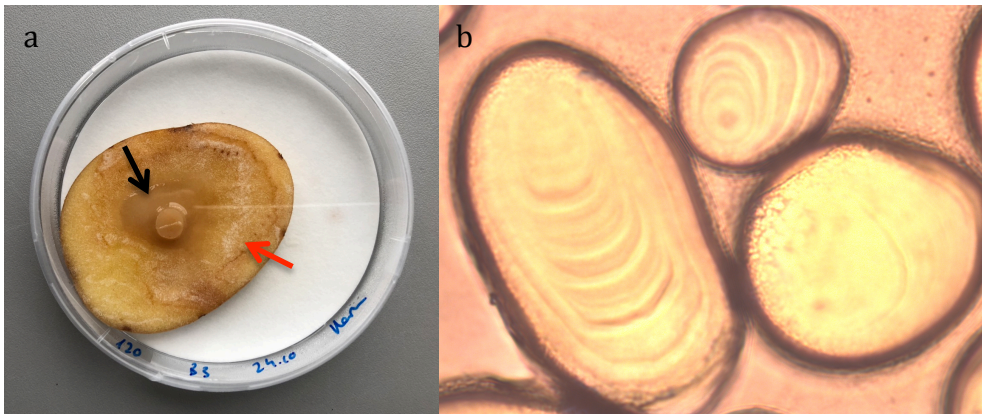


Figure: 37 Potato starch. a. Potato slice inoculated with *P. infestans* agar plug in the centre and treated with bacteria strain 120 showing white layer on the surface (red arrow) and the lysed mycelia (black arrow) after 12 days of inoculation. The white layer corresponds to potato starch and no *P. infestans* sporangia as could be thought on a first visual analysis. b. Potato starch granules showing alternating zones of semicrystalline and amorphous material known as growth rings. Camera MC170 HD; Microscope DM5500B.

6. DISCUSSION

This Master Thesis documented *in vitro* analysis of the antagonistic effect of entomopathogenic bacterial strains against the oomycete *P. infestans* on potato slices and whole tubers. Previous studies have already shown evidence of the capacity of entomopathogenic bacteria and their primary or secondary metabolites to effectively control several plant diseases caused by fungi or fungi-like pathogens (Fang et al., 2011; Jianxiong Li et al., 1995; Ng & Webster, 1997a; Shapiro-Ila et al., 2014; Walch, 2016). However, anti-oomycete effect of the 24 specific entomopathogenic bacterial isolates on potato tubers was not known until this study.

Based on the experimental research carried out in this Thesis, suppression of *P. infestans* by entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* appears to be specially promising. Of the 24 screened strains, 21 showed effective suppression of *P. infestans* with an overall average of 94.1% of the potato areas not presenting any necrotic areas and 99,6% not presenting growing sporangiophores. Subsequently, the first proposed hypothesis was accepted for those 21 strains. Moreover, results suggested that 12 strains even exhibited a level of suppression comparable to those showed for Cu.

Comparatively, results from this Thesis do only partially agree with those studies carried out by Ng & Webster (1997a) with *X. bovienii* strain A2. These authors confirmed 100% inhibition of mycelia outgrowth in *in vitro* and *in vivo* assays with ephemeral phytotoxicity. Experiments carried out by of Ng & Webster (1997a) had anyway a different approach. Bacteria were left to grow for 96h instead of 48 and secondary metabolites were extracted with ethylacetate and subsequently concentrated. Even though these conditions seem to be more favourable (longer growth time, metabolites extraction and concentration) and better results would be expected, comparatively, experimental results obtained with the strains used in this Thesis seem to be very promising. 16 of the 24 tested strains showed 100% sporangia inhibition and 90.2 to 97.7 % rotting inhibition with only half of the incubation time and most likely with less metabolites concentration. Furthermore, these findings claim for a very optimistic future to these specific strain collection. Therefore, analysis, characterization and understanding mechanisms of action of the specific metabolites composition in future studies would be essential .

Separately, experiments carried out by Ng & Webster (1997a) demonstrated that treatments also exhibited fungicidal activity in addiction to fungistatic effect against *P. infestans* (being the first the ability to kill the fungal pathogen whereas the latter only the ability to supress

fungal growth (Graybill et al.,1997)). This fact opened a new question, i. e. whether the mixtures of bacteria and metabolites used in this Thesis have fungicidal or just fungistatic effects towards the pathogen. In order to answer this issue further tests need to be done on this direction. Transferring agar plugs from treated potato slices showing oomycete growth inhibition to PDA or V8-agar Petri dishes in aseptic conditions and subsequent incubation could determinate whether the bacteria strains have fungicide or fungistatic effect (Wenham, et al., 1976). If sporangia were not visible after 15 days of incubation the antifungal metabolites could be considered fungicidal, which would be an important step towards finding commercial compounds. Furthermore, by testing different metabolite concentrations, the minimal fungicidal concentration could as well be determined.

Testing a large amount of different strains allowed to differenciate effects within the same species. In the experimental phase of this Thesis, strain 10 did not show inhibitory effects; moreover, results suggested that this strain even promoted the development of the disease, as symptoms were greater than negative controls. Testing whether primary or secondary form is present in strain 10 could also help to interpret these results. Strain 10 belonging to species *Photorhabdus temperata* has previously demonstrated to have inhibitory effects on *P. infestans*, i.e. *Photorhabdus temperata* strain SN259 has shown to produce stilbene derivates and strong inhibition against different phytopathogenic fungi (Shi et al., 2017), hence, inhibitory effects would be strain specific if primary form of strain 10 would be demonstrated to be present. This led to accept the second proposed hypothesis with caution and subjected to further analysis.

Regarding phytotoxicity, evaluation was made by visual approach. According to optical appreciations bacteria treatments seemed to be innocuous, therefore, the third hypothesis would be accepted, although, chemical and physiological tests should be done in future assays in order to be able to make factual interpretations. This study also assessed the relationship between treatments and sprouts production, with significant results suggesting that slices treated with bacteria solutions are more likely to produce sprouts. This could be interpreted as that bacteria have the capacity of lowering dormancy by an unknown mechanism. On one hand, low dormancy is not beneficial when tubers have to be maintained in chambers for long periods but on the other hand too long dormancy would be an obstacle once they are planted and are expected to produce those sprouts, as Bajji et al. (2007) described. The difference in sprouts production could also be explained as that potato under attack by a pathogen or a toxic compound like a copper-based fungicide, lose or lower their capacity to survive/reproduce in

which case production of sprouts could be interpreted as a sign of health, hence another evidence of low or no toxicity of bacteria treatments. This fact should be more deeply analysed and taken into account for further studies as well.

Microscopically, negative controls occasionally presented secondary infections. This fact was not apparent in bacteria-treated slices suggesting that strains were also able to suppress the development of other microorganisms naturally present in the tubers. This findings support conclusions previously described by Fang, et al., (2014) on studies carried out with *X. nematophila.* Perhaps, this secondary infections could correspond with fungi belonging to the genus *Fusarium* spp. When this occurs, rot development can be enhanced, when *P. infestans* infection is moderate to severe as suggested by Lambert & Currier (1997). Therefore, results on the negative controls and p-values could have been a little affected in some samples; in any case, deviations should not be considerably big. Statistical testing showed that results from strains 31, 83, 103, 107, 113 and 117, where secondary infections were found, did not corresponded with higher necrotic means compared with other negative controls. Possible sources of those microorganisms could be contamination from the laboratory or the soil where potatoes where cultivated. The latter is the most preferred hypothesis, as the experiments were carried out under strict aseptic working conditions. More effective methods of tuber disinfection previous to experimental setting may help to avoid the development of those secondary infections (i. e. use of UVA light), however it has to be taken into account that total disinfection of tuber slices may lead as well in destruction of tissues and thus, *P. infestans* growth might be affected as well.

Regarding whole tubers assays, positive results with strain 31 were obtained, but according to the overall outcome and the lack of consistent data, a more conservative interpretation is preferred concerning the forth hypothesis which was not accepted in this Thesis. One of the main obstacles encountered on whole tuber assays lied on the difficulty to infect tubers with *P. infestans*. Several infection methods were tried but only deep wounding gave acceptable results. Spraying the tubers with spore solution did not give satisfactory infection levels, thus obtaining tuber infection under laboratory conditions became a difficult task. This could be explained by the fact that potato periderm acts as a barrier to *P. infestans* and wounding makes potato more susceptible to infection (Pathak & Clarke, 1987). Conversely, late blight symptoms on tubers from the infected field became very soon apparent. Development stages of the periderm could affect infection under field conditions because maturity of periderm is

related to capacity of penetration of the pathogen. Tuber maturity and soil ecosystem, with its intrinsic interaction between microorganisms, may play as well an important role in pathogen colonization (Nyankanga et al., 2008). Therefore, further research with whole tubers is required in order to establish appropriate infection protocols, which would help to get more reliable results and conclusions. Whole tuber assays without wounding are definitely necessary in order to avoid secondary infections, as pectolytic bacteria or *Fusarium*, which eliminate long-term survival of *P infestans* (D. A. Johnson & Cummings, 2009), thus interfering the results.

7. CONCLUSION

In conclusion, within the framework of increasing demand for environmentally safe products for agriculture plus the progressive development of resistance to the existing ones, there is an imperative need to look for novel and efficacious substitutes. This Thesis has shown evidence that bacterial-based treatments including their organic metabolites exhibit convincing effects against the oomycete *P. infestans* and are able to control the disease in tuber slices with no apparent toxicity. Notwithstanding the above, the indeterminate composition of these metabolites mixture and their unknown effects on humans and ecosystems make these components still not usable in practice and further studies are needed prior to their development by the agrochemical industry (Li et al., 1999).

This Thesis provides support for more future *in vitro* and *in vivo* research of entomopathogenic strains as antifungal agents. Besides this, analysis, extraction and identification of the metabolites involved in these processes are decisive as well as knowing their mechanisms of action. Most promising components seem to be indoles. It is already known for more than two decades that *Xenorhabdus bovienii* synthetize indoles and dithiolopyrrolones, that have demonstrated antagonistic effects on several plant pathogens as *Botrytis cinerea*, *Phytophthora capsici* or *Phytophthora ultimum* (Fang et al., 2014).

Based on the experimental results of this study the tested strains belonging to the genera *Xenorhabdus* and *Photorhabdus* could represent a natural and unique source of novel antibiotics with a broad spectrum of bioactivity and their promising potential for agrochemicals development needs to be fully explored. It is expected that on-going research in this field will help in the battle against disease and pestilence in agriculture.

8. REFERENCES

Abad, Z. G., Abad, J. A., Pathology, P., & Carolina, N. (1997). Special Report Another Look at the Origin of Late Blight of Potatoes , Tomatoes , and Pear Melon in the Andes of South America. *Plant Disease*, *81*(6), 682–688. https://doi.org/10.1094/PDIS.1997.81.6.682

Abu-El Samen, F. M., Secor, G. a., & Gudmestad, N. C. (2003a). Genetic variation among asexual progeny of *Phytophthora infestans* detected with RAPD and AFLP markers. *Plant Pathology*, *52*(3), 314–325. https://doi.org/10.1046/j.1365-3059.2003.00858.x

Abu-El Samen, F. M., Secor, G. A., & Gudmestad, N. C. (2003b). Variability in virulence among asexual progenies of Phytophthora infestans. *Phytopathology*, *93*(3), 293–304. https://doi.org/10.1094/PHYTO.2003.93.3.293

Akhurst, R. J. (1982). Antibiotic Activity of Xenorhabdus spp., Bacteria Symbiotically Associated with Insect Pathogenic Nematodes of the Families Heterorhabditidae and Steinernematidae. *Microbiology*, *128*(12), 3061–3065. https://doi.org/10.1099/00221287-128-12-3061

Akhurst, R. J., & Boemare, N. E. (1988). A numerical taxonomic study of the genus Xenorhabdus (Enterobacteriaceae) and proposed elevation of the subspecies of X. nematophilus to species. *Journal of General Microbiology*, *134*(7), 1835–1845. https://doi.org/10.1099/00221287-134-7-1835

Andrivon, D. (1995). Biology, ecology and epidemiology of the potato late blight pathogen Phytophthora infestans in soil. *Phytopathology*. https://doi.org/10.1094/Phyto-85-1053

Arora, R. K., Sharma, S., & Singh, B. P. (2014). Late blight disease of potato and its management. *Potato Journal*, *41*(1), 16–40.

Bajji, M., M'Hamdi, M., Gastiny, F., Rojas-Beltran, J. A., & du Jardin, P. (2007). Catalase inhibition accelerates dormancy release and sprouting in potato (Solanum tuberosum L.) tubers. *Biotechnololy, Agronomy, Society and Environment*, *11*(2), 121–131.

Birch, P. R. J., & Whisson, S. C. (2001). Pathogen profile Phytophthora infestans enters the genomics era. *Molecular Plant Pathology*, *2*, 257–263.

Bode, H. B. (2009). Entomopathogenic bacteria as a source of secondary metabolites. *Current Opinion in Chemical Biology*, *13*(2), 224–230. https://doi.org/10.1016/j.cbpa.2009.02.037

Brun, L. A., Maillet, J., Richarte, J., Herrmann, P., & Remy, J. C. (1998). Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard

soils. *Environmental Pollution*, *102*(2–3), 151–161. https://doi.org/10.1016/S0269-7491(98)00120-1

Chen, G., Dunphy, G. B., & Webster, J. M. (1994). Antifungal Activity of Two Xenorhabdus Species and Photorhabdus luminescens, Bacteria Associated with the Nematodes Steinernema Species and Heterorhabditis megidis. *Biological Control*, *4*(2), 157–162. https://doi.org/10.1006/bcon.1994.1025

Cohen, Y., Farkash, S., Reshit, Z., & Baider, a. (1997). Oospore Production of Phytophthora infestans in Potato and Tomato Leaves. *Phytopathology*, *87*(2), 191–196. https://doi.org/10.1094/PHYTO.1997.87.2.191

Cooke, L. R., Schepers, H. T. A. M., Hermansen, A., Bain, R. A., Bradshaw, N. J., Ritchie, F., … Nielsen, B. J. (2011). Epidemiology and Integrated Control of Potato Late Blight in Europe. *Potato Research*, *54*(2), 183–222. https://doi.org/10.1007/s11540-011-9187-0

Cunniffe, N. J., Koskella, B., E. Metcalf, C. J., Parnell, S., Gottwald, T. R., & Gilligan, C. A. (2015). Thirteen challenges in modelling plant diseases. *Epidemics*, *10*, 6–10. https://doi.org/10.1016/j.epidem.2014.06.002

Day, J. P., & Shattock, R. C. (1997). Aggressiveness and other factors relating to displacement of populations of Phytophthora infestans in England and Wales. *European Journal of Plant Pathology*, *103*(4), 379–391. https://doi.org/10.1023/A:1008630522139

Drenth, A., Tas, I. C. Q., & Govers, F. (1994). DNA fingerprinting uncovers a new sexually reproducing population of Phytophthora infestans in the Netherlands. *European Journal of Plant Pathology*, *100*(2), 97–107. https://doi.org/10.1007/BF01876244

Engel, M. S., & Grimaldi, D. A. (2004). New light shed on the oldest insect. *Nature*. https://doi.org/10.1038/nature02291

Erwin, T. L. (1996). Biodiversity at its utmost: tropical forest beetles. *Biodiversity II: Understanding and Protecting Our Biological Resources*, 27–40. https://doi.org/10.1016/j.neuroscience.2009.01.029

Fang, X. L., Li, Z. Z., Wang, Y. H., & Zhang, X. (2011). In vitro and in vivo antimicrobial activity of Xenorhabdus bovienii YL002 against Phytophthora capsici and Botrytis cinerea. *Journal of Applied Microbiology*, *111*(1), 145–154. https://doi.org/10.1111/j.1365-2672.2011.05033.x

Fang, X., Zhang, M., Tang, Q., Wang, Y., & Zhang, X. (2014). Inhibitory effect of Xenorhabdus nematophila TB on plant pathogens Phytophthora capsici and Botrytis cinerea in vitro and in planta. *Scientific Reports*, *4*, 1–7. https://doi.org/10.1038/srep04300

Filippov, A., Rogozhin, A., Kuznetsova, M., Statsyuk, N., Ronis, A., & Platt, H. W. (Bud). (2015). Efficiency of a computerised decision support system as a tool to reduce fungicide input for the control of potato late blight. *Zemdirbyste-Agriculture*, *102*(4), 449–456. https://doi.org/10.13080/z-a.2015.102.058

Finckh, M. R., Hayer, F., Schulte-Geldermann, E., & Bruns, C. (2008). Diversität, Pflanzenernährung und Prognose: Ein integriertes Konzept zum Management der Kraut- und Knollenfäule in der Ökologischen Landwirtschaft. *Gesunde Pflanzen*, *60*(4), 159–170. https://doi.org/10.1007/s10343-008-0192-4

Fischer-Le Saux, M., Viallard, V., Brunel, B., Normand, P., & Boemare, N. E. (1999). Polyphasic classification of the genus Photorhabdus and proposal of new taxa: P. luminescens subsp. luminescens subsp. nov., P. luminescens subsp. akhurstii subsp. nov., P. luminescens subsp. laumondii subsp. nov., P. temperata sp. nov., P. temperata subs. *International Journal of Systematic Bacteriology*, *49*(4), 1645–1656. https://doi.org/10.1099/00207713-49-4-1645

Flemming, C. A., & Trevors, J. T. (1989). Copper toxicity and chemistry in the environment: a review. *Water, Air, and Soil Pollution*. https://doi.org/10.1007/BF00228784

Flier, W. G., Turkensteen, L. J., Van Den Bosch, G. B. M., Vereijken, P. F. G., & Mulder, A. (2001). Differential interaction of Phytophthora infestans on tubers of potato cultivars with different levels of blight resistance. *Plant Pathology*, *50*(3), 292–301. https://doi.org/10.1046/j.1365-3059.2001.00574.x

Forst, S., Dowds, B., Boemare, N., & Stackebrandt, E. (1997). Xenorhabdus and Photorhabdus spp.: bugs that kill bugs. *Annual Review of Microbiology*, *51*, 47–72. https://doi.org/10.1146/annurev.micro.51.1.47

Forst, S., & Nealson, K. (1996). Molecular biology of the symbiotic-pathogenic bacteria Xenorhabdus spp. and Photorhabdus spp. *Microbiological Reviews*, *60*(1), 21–43.

Fry, W. E., Birch, P. R. J., Judelson, H. S., Grünwald, N. J., Danies, G., Everts, K. L., … Smart, C. D. (2015). Five Reasons to Consider *Phytophthora infestans* a Reemerging Pathogen. *Phytopathology*, *105*(7), 966–981. https://doi.org/10.1094/PHYTO-01-15-0005-FI

Gent, D. H., Mahaffee, W. F., McRoberts, N., & Pfender, W. F. (2013). The Use and Role of Predictive Systems in Disease Management. *Annual Review of Phytopathology*, *51*(1), 267–289. https://doi.org/10.1146/annurev-phyto-082712-102356

Ghosh, S., Molla, K. A., Karmakar, S., Datta, S. K., & Datta, K. (2016). Enhanced resistance to late blight pathogen conferred by expression of rice oxalate oxidase 4 gene in

transgenic potato. *Plant Cell, Tissue and Organ Culture*, *126*(3), 429–437. https://doi.org/10.1007/s11240-016-1011-8

Gómez-Alpizar, L., Carbone, I., & Ristaino, J. B. (2007). An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene genealogies. *Proceedings of the National Academy of Sciences*, *104*(9), 3306–3311. https://doi.org/10.1073/pnas.0611479104

Goodrich-Blair, H., & Clarke, D. J. (2007). Mutualism and pathogenesis in Xenorhabdus and Photorhabdus: Two roads to the same destination. *Molecular Microbiology*, *64*(2), 260–268. https://doi.org/10.1111/j.1365-2958.2007.05671.x

Goodwin, S. B., Sujkowski, L. S., & Fry, W. E. (1996). Widespread Distribution and Probably Origin of Resistance to Metalaxyl in Clonal Genotypes of Phytopthora infestans in the United States and Western Canada. *Ecology and Epidemiolgy*.

Goss, E. M., Tabima, J. F., Cooke, D. E. L., Restrepo, S., Fry, W. E., Forbes, G. A., … Grunwald, N. J. (2014). The Irish potato famine pathogen Phytophthora infestans originated in central Mexico rather than the Andes. *Proceedings of the National Academy of Sciences*, *111*(24), 8791–8796. https://doi.org/10.1073/pnas.1401884111

Gouge, D. H., & Snyder, J. L. (2006). Temporal association of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) and bacteria. *Journal of Invertebrate Pathology*, *91*(3), 147–157. https://doi.org/10.1016/j.jip.2005.12.003

Graybill, J. R., Burgess, D. S., & Hardin, T. C. (1997). Key issues concerning fungistatic versus fungicidal drugs. In *European Journal of Clinical Microbiology and Infectious Diseases*. https://doi.org/10.1007/BF01575120

Gu, Y. H., Yoo, S. J., Park, C. J., Kim, Y. H., Park, S. K., Kim, J. S., & Lim, J. H. (2016). BLITE-SVR: New forecasting model for late blight on potato using support-vector regression. *Computers and Electronics in Agriculture*, *130*, 169–176. https://doi.org/10.1016/j.compag.2016.10.005

Gualtieri, M., Aumelas, A., & Thaler, J. O. (2009). Identification of a new antimicrobial lysine-rich cyclolipopeptide family from xenorhabdus nematophila. *Journal of Antibiotics*, *62*(6), 295–302. https://doi.org/10.1038/ja.2009.31

Haas, B. J., Kamoun, S., Zody, M. C., Jiang, R. H. Y., Handsaker, R. E., Cano, L. M., … Nusbaum, C. (2009). Genome sequence and analysis of the Irish potato famine pathogen Phytophthora infestans. *Nature*, *461*(7262), 393–398. https://doi.org/10.1038/nature08358

HARRISON, J. G. (1992). Effects of the aerial environment on late blight of potato foliage–a

review. *Plant Pathology*. https://doi.org/10.1111/j.1365-3059.1992.tb02435.x

Hartill, W. F. T., Young, K., Allan, D. J., & Henshall, W. R. (1990). Effects of temperature and leaf wetness on the potato late blight. *New Zealand Journal of Crop and Horticultural Science*, *18*(4), 181–184. https://doi.org/10.1080/01140671.1990.10428093

Haverkort, A. J., Boonekamp, P. M., Hutten, R., Jacobsen, E., Lotz, L. A. P., Kessel, G. J. T., … Van Der Vossen, E. A. G. (2008). Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. *Potato Research*, *51*(1), 47–57. https://doi.org/10.1007/s11540-008-9089-y

Haverkort, A. J., Boonekamp, P. M., Hutten, R., Jacobsen, E., Lotz, L. A. P., Kessel, G. J. T., … Visser, R. G. F. (2016). Durable Late Blight Resistance in Potato Through Dynamic Varieties Obtained by Cisgenesis: Scientific and Societal Advances in the DuRPh Project. *Potato Research*, *59*(1), 35–66. https://doi.org/10.1007/s11540-015-9312-6

Haverkort, A. J., Struik, P. C., Visser, R. G. F., & Jacobsen, E. (2009). Applied biotechnology to combat late blight in potato caused by phytophthora infestans. *Potato Research*, *52*(3), 249–264. https://doi.org/10.1007/s11540-009-9136-3

Hohl, H. R., & Iselin, K. (1984). Strains of *Phytophthora infestans* from Switzerland with A2 mating type behaviour. *Transactions of the British Mycological Society*, *83*(3), 529–531. https://doi.org/10.1016/S0007-1536(84)80057-1

Hu, K., Li, J., Wang, W., Wu, H., Lin, H., & Webster, J. M. (1998). Comparison of metabolites produced in vitro and in vivo by *Photorhabdus luminescens*, a bacterial symbiont of the entomopathogenic nematode *Heterorhabditis megidis*. *Canadian Journal of Microbiology*, *44*(11), 1072–1077. https://doi.org/10.1139/cjm-44-11-1072

Jang, E. K., Ullah, I., Lim, J. H., Lee, I. J., Kim, J. G., & Shin, J. H. (2012). Physiological and molecular characterization of a newly identified entomopathogenic bacteria, Photorhabdus temperata M1021. *Journal of Microbiology and Biotechnology*. https://doi.org/10.4014/jmb.1203.03068

Johnson, D. A., & Cummings, T. F. (2009). Latent Infection of Potato Seed Tubers by *Phytophthora infestans* During Long-Term Cold Storage. *Plant Disease*, *93*(9), 940–946. https://doi.org/10.1094/PDIS-93-9-0940

Johnson, D. a., Cummings, T. F., & Geary, B. (2000). Postinfection Activity of Selected Late Blight Fungicides. *Plant Disease*, *84*(10), 1116–1120. https://doi.org/10.1094/PDIS.2000.84.10.1116

Judelson, H. S. (1997). The genetics and biology of Phytophthora infestans: Modern approaches to a historical challenge. *Fungal Genetics and Biology*.

https://doi.org/10.1006/fgbi.1997.1006

Judelson, H. S., & Blanco, F. A. (2005). The spores of Phytophthora: Weapons of the plant destroyer. *Nature Reviews Microbiology*, *3*(1), 47–58. https://doi.org/10.1038/nrmicro1064

Keller, A. A., Adeleye, A. S., Conway, J. R., Garner, K. L., Zhao, L., Cherr, G. N., … Zuverza-Mena, N. (2017). Comparative environmental fate and toxicity of copper nanomaterials. *NanoImpact*, *7*, 28–40. https://doi.org/10.1016/j.impact.2017.05.003

Krasomil-Osterfeld, K. C. (1995). Influence of osmolarity on phase shift in Photorhabdus luminescens. *Applied and Environmental Microbiology*, *61*(10), 3748–3749.

Lal, M., Arora, R. K., Maheshwari, U., Rawal, S., & Yadav, S. (2016). Impact of late blight occurrence on potato productivity during 2013-14. *International Journal of Agricultural and Statistical Sciences*, *12*(1), 187–192.

Lambert, D. H., & Currier, A. I. (1997). Differences in tuber rot development for North American clones of Phytophthora infestans. *American Potato Journal*. https://doi.org/10.1007/BF02849171

Lengyel, K., Lang, E., Fodor, A., Szállás, E., Schumann, P., & Stackebrandt, E. (2005). Description of four novel species of Xenorhabdus, family Enterobacteriaceae: Xenorhabdus budapestensis sp. nov., Xenorhabdus ehlersii sp. nov., Xenorhabdus innexi sp. nov., and Xenorhabdus szentirmaii sp. nov. *Systematic and Applied Microbiology*, *28*(2), 115–122. https://doi.org/10.1016/j.syapm.2004.10.004

Li, J., Chen, G., & Webster, J. M. (1997). Nematophin, a novel antimicrobial substance produced by Xenorhabdus nematophilus (Enterobactereaceae). *Can J Microbiol*, *43*(8), 770–773. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9304787

Li, J., Chen, G., Webster, J. M., & Czyzewska, E. (1995). Antimicrobial metabolites from a bacterial symbiont. *Journal of Natural Products*, *58*(7), 1081–1086. https://doi.org/10.1021/np50121a016

Li, J., Hu, K., & Webster, J. M. (1999). (REVIEW), *34*(1), 1331–1339.

Maridueña-Zavala, M. G., Freire-Peñaherrera, A., Cevallos-Cevallos, J. M., & Peralta, E. L. (2017). GC-MS metabolite profiling of Phytophthora infestans resistant to metalaxyl. *European Journal of Plant Pathology*, *149*(3), 563–574. https://doi.org/10.1007/s10658-017-1204-y

Martin, M. D., Vieira, F. G., Ho, S. Y. W., Wales, N., Schubert, M., Seguin-Orlando, A., … Gilbert, M. T. P. (2016). Genomic characterization of a south American phytophthora hybrid mandates reassessment of the geographic origins of phytophthora infestans.

Molecular Biology and Evolution, *33*(2), 478–491. https://doi.org/10.1093/molbev/msv241

Maziero, J. M. N., Maffia, L. a., & Mizubuti, E. S. G. (2009). Effects of Temperature on Events in the Infection Cycle of Two Clonal Lineages of Phytophthora infestans Causing Late Blight on Tomato and Potato in Brazil. *Plant Disease*, *93*(5), 459–466. https://doi.org/10.1094/PDIS-93-5-0459

Mcinerney, B. V., Gregson, R. P., Lacey, M. J., Akhurst, R. J., Lyons, G. R., Rhodes, S. H., … White, A. H. (1991). Biologically active metabolites from xenorhabdus spp., part 1. dithiolopyrrolone derivatives with antibiotic activity. *Journal of Natural Products*. https://doi.org/10.1021/np50075a005

Montes, M. S., Nielsen, B. J., Schmidt, S. G., Bødker, L., Kjøller, R., & Rosendahl, S. (2016). Population genetics of Phytophthora infestans in Denmark reveals dominantly clonal populations and specific alleles linked to metalaxyl-M resistance. *Plant Pathology*, *65*(5), 744–753. https://doi.org/10.1111/ppa.12462

Nechwatal, J., & Zellner, M. (2015). Potential suitability of various leaf treatment products as copper substitutes for the control of late blight (Phytophthora infestans) in organic potato farming. *Potato Research*, *58*(3), 261–276. https://doi.org/10.1007/s11540-015-9302-8

Ng, K. K., & Webster, J. M. (1997a). Antimycotic activity of Xenorhabdus bovienii (Enterobacteriaceae) metabolites against Phytophthora infestans on potato plants. *Canadian Journal of Plant Pathology*. https://doi.org/10.1080/07060669709500540

Ng, K. K., & Webster, J. M. (1997b). Antimycotic activity of Xenorhabdus bovienii (Enterobacteriaceae) metabolites against Phytophthora infestans on potato plants. *Canadian Journal of Plant Pathology*, *19*(2), 125–132. https://doi.org/10.1080/07060669709500540

Nowicki, M., Foolad, M. R., Nowakowska, M., & Kozik, E. U. (2012). Potato and Tomato Late Blight Caused by *Phytophthora infestans* : An Overview of Pathology and Resistance Breeding. *Plant Disease*, *96*(1), 4–17. https://doi.org/10.1094/PDIS-05-11-0458

Nyankanga, R. O., Olanya, O. M., Wien, H. C., El-Bedewy, R., Karinga, J., & Ojiambo, P. S. (2008). Development of tuber blight (Phytophthora infestans) on potato cultivars based on in vitro assays and field evaluations. *HortScience*, *43*(5), 1501–1508.

Olanya, O. M., Starr, G. C., Honeycutt, C. W., Griffin, T. S., & Lambert, D. H. (2007). Microclimate and potential for late blight development in irrigated potato. *Crop Protection*, *26*(9), 1412–1421. https://doi.org/10.1016/j.cropro.2006.12.002

Orbegozo, J., Roman, M. L., Rivera, C., Gamboa, S., Tovar, J. C., Forbes, G. A., … Ghislain, M. (2016). Rpi-blb2 gene from Solanum bulbocastanum confers extreme resistance to late blight disease in potato. *Plant Cell, Tissue and Organ Culture*, *125*(2), 269–281. https://doi.org/10.1007/s11240-016-0947-z

Pathak, N., & Clarke, D. D. (1987). Studies on the resistance of the outer cortical tissues of the tubers of some potato cultivars to Phytophthora infestans. *Physiological and Molecular Plant Pathology*. https://doi.org/10.1016/0885-5765(87)90011-7

Pilling, E., & Smith, A. M. (2003). Growth Ring Formation in the Starch Granules of Potato Tubers 1, *132*(May), 365–371. https://doi.org/10.1104/pp.102.018044.plants

Pirozzi, A. V. A., Stellavato, A., La Gatta, A., Lamberti, M., & Schiraldi, C. (2016). Mancozeb, a fungicide routinely used in agriculture, worsens nonalcoholic fatty liver disease in the human HepG2 cell model. *Toxicology Letters*. https://doi.org/10.1016/j.toxlet.2016.03.004

Poinar, G. O., & Grewal, P. S. (2012). History of entomopathogenic nematology. *Journal of Nematology*, *44*(2), 153–161.

Rossman, A. Y. (2009). The impact of invasive fungi on agricultural ecosystems in the United States. *Ecological Impacts of Non-Native Invertebrates and Fungi on Terrestrial Ecosystems*, 97–107. https://doi.org/10.1007/978-1-4020-9680-8_7

Savary, S., Bregaglio, S., Willocquet, L., Gustafson, D., Mason D'Croz, D., Sparks, A., … Garrett, K. (2017). Crop health and its global impacts on the components of food security. *Food Security*, *9*(2), 311–327. https://doi.org/10.1007/s12571-017-0659-1

Shapiro-Ilan, D. I., Bock, C. H., & Hotchkiss, M. W. (2014). Suppression of pecan and peach pathogens on different substrates using Xenorhabdus bovienii and Photorhabdus luminescens. *Biological Control*, *77*, 1–6. https://doi.org/10.1016/j.biocontrol.2014.05.010

Shi, D., An, R., Zhang, W., Zhang, G., & Yu, Z. (2017). Stilbene derivatives from Photorhabdus temperata SN259 & their antifungal activities against phytopathogenic fungi. *Journal of Agricultural and Food Chemistry*, *65*(1), 60–65. https://doi.org/10.1021/acs.jafc.6b04303

Sicard, M., Brugirard-Ricaud, K., Pagès, S., Lanois, A., Boemare, N. E., Brehélin, M., & Givaudan, A. (2004). Stages of infection during the tripartite interaction between Xenorhabdus nematophila, its nematode vector, and insect hosts. *Applied and Environmental Microbiology*, *70*(11), 6473–6480. https://doi.org/10.1128/AEM.70.11.6473-6480.2004

Skelsey, P., Kessel, G. J. T., Holtslag, A. A. M., Moene, A. F., & van der Werf, W. (2009). Regional spore dispersal as a factor in disease risk warnings for potato late blight: A proof of concept. *Agricultural and Forest Meteorology*, *149*(3–4), 419–430. https://doi.org/10.1016/j.agrformet.2008.09.005

Small, I. M., Joseph, L., & Fry, W. E. (2015). Development and implementation of the BlightPro decision support system for potato and tomato late blight management. *Computers and Electronics in Agriculture*, *115*, 57–65. https://doi.org/10.1016/j.compag.2015.05.010

Tailliez, P., Pagès, S., Ginibre, N., & Boemare, N. (2006). New insight into diversity in the genus Xenorhabdus, including the description of ten novel species. *International Journal of Systematic and Evolutionary Microbiology*, *56*(12), 2805–2818. https://doi.org/10.1099/ijs.0.64287-0

Taylor, M. C., Hardwick, N. V., Bradshaw, N. J., & Hall, A. M. (2003). Relative performance of five forecasting schemes for potato late blight (Phytophthora infestans) I. Accuracy of infection warnings and reduction of unnecessary, theoretical, fungicide applications. *Crop Protection*, *22*(2), 275–283. https://doi.org/10.1016/S0261-2194(02)00148-5

THOMAS, G. M., & POINAR, G. O. (1979). Xenorhabdus gen. nov., a Genus of Entomopathogenic, Nematophilic Bacteria of the Family Enterobacteriaceae. *International Journal of Systematic Bacteriology*. https://doi.org/10.1099/00207713-29-4-352

Tosi, S., Costa, C., Vesco, U., Quaglia, G., & Guido, G. (2018). Supplementary material of "A 3 - year survey of Italian honey bee collected pollen reveals widespread contamination by agricultural pesticides." *Science of The Total Environment*, *615*, 208–218.

Walch, B. (2016). Screening for Antagonistic Effects against the Late Blight Disease of Tomato in Bacteria , Mutualistically Associated with Entomopathogenic Nematodes Author, (March).

Wang, Y., Fang, X., An, F., Wang, G., & Zhang, X. (2011). Improvement of antibiotic activity of Xenorhabdus bovienii by medium optimization using response surface methodology. *Microbial Cell Factories*, *10*, 1–15. https://doi.org/10.1186/1475-2859-10-98

Wenham, H. T., MacKintosh, B. L., & Bolkan, H. A. (1976). Evaluation of fungicides for control of potato black scurf disease. *New Zealand Journal of Experimental Agriculture*, *4*(1), 97–101. https://doi.org/10.1080/03015521.1976.10425852

Yang, X., Qiu, D., Yang, H., Liu, Z., Zeng, H., & Yuan, J. (2011). Antifungal activity of xenocoumacin 1 from Xenorhabdus nematophilus var. pekingensis against Phytophthora infestans. *World Journal of Microbiology and Biotechnology*, *27*(3), 523–528. https://doi.org/10.1007/s11274-010-0485-5

Judelson, H. S., & Blanco, F. A. (2005) Course of infection by *Phytophthora infestans* (Format) Retrieved from The spores of Phytophthora: Weapons of the plant destroyer. *Nature Reviews Microbiology*, *3*(1), 47–58. https://doi.org/10.1038/nrmicro1064

Judelson, H. S., & Blanco, F. A. (2005) Stages of the spore cycles of *Phytophthora infestans* (Format) Retrieved from The spores of Phytophthora: Weapons of the plant destroyer. *Nature Reviews Microbiology*, *3*(1), 47–58. https://doi.org/10.1038/nrmicro1064

North Dakota State University. Late-blight infected tuber [Digital image] Retrieved March 12, 2018 from URL (https://www.ag.ndsu.edu/extplantpath/plant-pest-alerts/potato-tomato-late-blight-start-monitoring-early)

Robinson, A. Late blight lesions may be present on petioles and stems of the plant, especially in new growth where moisture persists [Digital image] Retrieved March 12, 2018 from URL (https://www.ag.ndsu.edu/publications/crops/late-blight-in-potato)

Schumann, G.L. and C. J. D'Arcy. 2000. Simplified disease cycle for late blight of potato [Digital image] Retrieved April 6, 2018 from URL (https://www.apsnet.org/edcenter/intropp/lessons/fungi/Oomycetes/Pages/LateBlight.aspx)

APPENDIX I

POSTER

Fungicidal activity of entomopathogenic bacteria against *Phytophthora infestans* in potato tubers

Marta Hernández-Pinzón Maldonado
Zürich University of Applied Sciences / School of life sciences and facility management / Institute of environment and natural resources
Grüental 8820 Wädenswil, Switzerland.

INTRODUCTION

• Late blight caused by the fungus-like microorganism (oomycete) named *Phytophthora infestans* (Figure 1) is an aggressive, destructive and dreaded plant pathogen considered the one that has most greatly impacted humanity to date (Goss et al., 2014). It has the capacity to attack potato aerial plant and tubers and can devastate crops within 5 to 7 days (Bode, 2009) (Figures 2–5). Nowadays still endangers the global potato cultivation (Judelson & Blanco, 2005).

• Chemically based fungicides are extensively applied to combat the disease, however, they are known to produce negative effects on human health, environment and ecosystems. *Phytophthora infestans* is also rapidly able to develop resistance to those chemically based products. Therefore, looking for new environmentally safe products is an imperative need in agriculture.

• Entomopathogenic bacteria from the two genera *Xenorhabdus* and *Photorhabdus* spp. belonging to the family Enterobacteriaceae, mutualistically associated with insect-pathogenic nematode species (Thomas & Poinar, 1979) (Figure 6), are able to produce several secondary metabolites, which are known to act against a variety of fungal diseases, *Phytophthora infestans* among them (Akhurst, 1982; Chen et al., 1994; J Li et al., 1997; Mcinerney et al., 1991; Ng & Webster, 1997b). These bacterial groups can be isolated from their hosts and artificially cultivated as independent organisms in a controlled environment. Possibilities of secondary metabolite mass production can be more deeply explored as an alternative in disease control for agriculture (Jang et al., 2012).

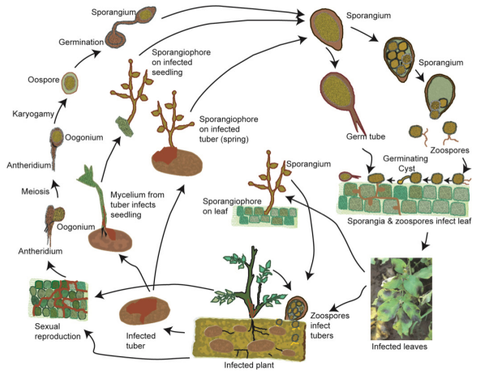


Figure 1: Phytophthora infestans life's cycle on potato plant. (Source: 2012, Vetukuri R.)

AIMS OF THE STUDY

• Evaluate and analyse the inhibitory effect of 24 selected entomopathogenic bacteria strains from the genera *Xenorhabdus* and *Photorhabdus* against *Phytophthora infestans* on potato tubers under *in vitro* conditions.

• Setting up a base for further in vivo and field research intended to give response to the demands of the agroindustry within a contest where the development of safe commercial formulations and treatments is absolutely necessary for the protection of environmental resources and human health.

Figure 2: Potato field totally devastated by *Phytophthora infestans* in the facilities of ZHAW, Campus Grüental during cultivation phase of the Master's Thesis.

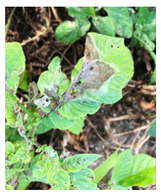


Figure 3: Late blight lesions on potato leaves. Detail of figure 2.

Figure 4: Late blight lesions on potato tubers. These potatoes were harvested from the field in figure 2.

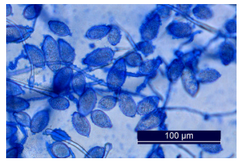


Figure 5: *Phytophthora infestans* fruits bodies.

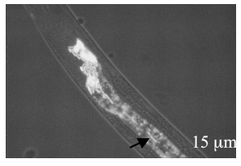


Figure 6: *Xenorhabdus nematophila* cells in the intestines of nematode *Steinernema carpocapsae* (Source: 2004, Sicard M.).

Formulated hypothesis:

1 The 24 selected strains from the genera *Xenorhabdus* and *Photorhabdus* suppress the development of *P. infestans* in inoculated potato tuber slices.
2 Antimycotic effect is strain specific (different strains of the same species have different antimycotic effects).
3 Strains suppressing the pathogen do not produce phytotoxicity on the potato slices.
4 Bacteria strains have as well antimycotic effect in a whole tuber system.

METHODS

• *Solanum tuberculosum* L. tubers cv. Bintje were use to carry out the experiments.

• Preparation of inoculum solution was done by scraping and filtering *Phytophthora infestans* cultures from V8-agar Petri dishes in aseptic conditions.

• Bacterial treatments were prepared by transferring single colonies from agar Petri dishes to centrifuge tubes with LB broth medium in aseptic conditions and subsequent incubation on a Universal Shaker for 48h at 23 °C.

• Concentration of cells (Optical density) was measured by spectrophotometry.

• Potato slices were inoculated with the *Phytophthora* inoculum solution, then treated with the bacteria-based solution with a pipette (figure 7) and finally incubated in chambers inside sealed Petri dishes for 12 days at 18°C and relative humidity = 80.

• Whole tubers were inoculated with the *Phytophthora* inoculum solution, then treated with the bacteria-based solution by injecting it with a syringe (figure 8) and finally incubated in chambers inside plastic boxes with a wire mesh support for 12 days at 18°C and relative humidity = 80.

• Sterile distiled water and copper-based solution were used as negative and positive control respectevely.

• A planimeter was used to measure areas presenting late blight symptoms in slices.

• A calliper was used to measure depth and diameter of rotten tissue in whole tubers.

• Statistic program "R" with support of "Rstudio" was used to analyse data.

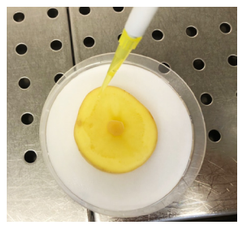


Figure 7: Application of the bacteria-based treatment over a potato slice.

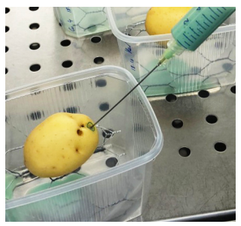


Figure 8: Application of treatment by direct injection.

Figure 9: Set of potato tubers inside boxes.

RESULTS

This study has provided with quantification and evaluation of the inhibitory effect of 24 selected entomopathogenic bacteria strains against *P. infestans.*

• Significant differences were found between bacteria treatments and negative controls in 21 of the 24 bacteria. In figure 10 results from strain 34 are displayed.

• Strains of the same species show different effects. This fact suggest that the fungicidal activity is strain specific (figure 11).

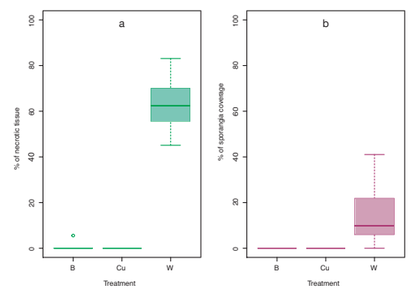


Figure 10: Visualisation of the results from strain 34 shows significant fungicidal effect compared to negative controls. a: percentage of necrotic tissue. b: percentage of sporangia coverage. B = bacteria treatment, Cu = copper based fungicide (positive control), W = sterile distilled water (negative control)

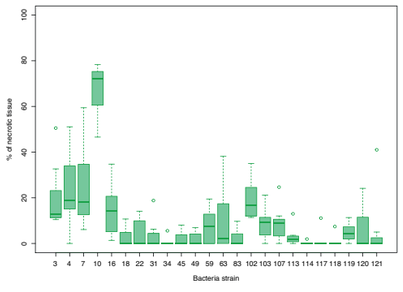


Figure 11: Percentage of necrotic tissue for every bacteria strain. While strain 10 does not inhibit the disease, strains 34, 114, 117, 118 seem to visually provide the best inhibition rates.

• Phytotoxicity on the bacteria treated slices was not apparent while it was very evident in copper-treated slices (figures 12 and 13).

• No significant differences between bacteira-based treatments and positive control was found in 12 of the 24 strains (100% of sporangia inhibition).

• Bacteria-treated slices seem to be more likely to produce sprouts.

• Whole tuber assays did not provide consistent results as tubers presented difficulties when tryed to be infected. More studies need to be done in this direction to establish good infection protocols.

Figure 12: General view of experiments carried out with strain 34 after 12 days of incubation. (A) potato slice treated with negative control (water). Necrotic tissue is evident owing to *Phytophthora infestans* growth between potato cells. (B) slice treated with positive control (Cu) Signs of phytotoxicity are visible. (C) slice treated with strain 31 showing healthy surface.

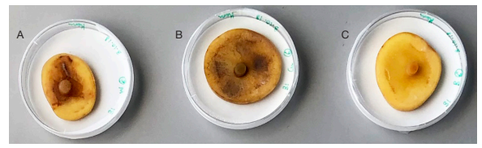


Figure 13: Detail of potato slices after 12 days of incubation (strain 31). (A) potato slice treated with negative control (water). Necrotic tissue is evident owing to *Phytophthora infestans* growth between potato cells. (B) slice treated with positive control (Cu) Signs of phytotoxicity are visible. (C) slice treated with strain 31 showing healthy surface.

CONCLUSIONS

• The genera *Xenorhabdus* and *Photorhabdus* represent a natural and unique source of novel antimycotic compounds with a broad spectrum of bioactivity and they have a promising future in the battle against late blight disease.

• Further research on the identification and carachterization, as well as mechanism of action, of those compounds is required.

• Additionally, future research on whole potato and fection protocols is also needed, as well as *in vivo* tests.

• It is expected that future findings in this field will help mankind in the battle against crop disease and colaborate with the improvement of food safety and agriculture.

References

Akhurst, R. J. (1982). Antibiotic Activity of *Xenorhabdus* spp., Bacteria Symbiotically Associated with Insect Pathogenic Nematodes of the Families Heterorhabditidae and Steinernematidae. *Microbiology*, *128*(12), 3061–3065.

Bode, H. B. (2009). Entomopathogenic bacteria as a source of secondary metabolites. *Current Opinion in Chemical Biology*, *13*(2), 224–230.

Chen, G., Dunphy, G. B., & Webster, J. M. (1994). Antifungal Activity of Two *Xenorhabdus* Species and *Photorhabdus luminescens*, Bacteria Associated with the Nematodes Steinernema Species and Heterorhabditis megidis. *Biological Control*, *4*(2), 157–162.

Goss, E. M., Tabima, J. F., Cooke, D. E. L., Restrepo, S., Fry, W. E., Forbes, G. A., … Grunwald, N. J. (2014). The Irish potato famine pathogen *Phytophthora infestans* originated in central Mexico rather than the Andes. *Proceedings of the National Academy of Sciences*, *111*(24), 8791–8796.

Jang, E. K., Ullah, I., Lim, J. H., Lee, I. J., Kim, J. G., & Shin, J. H. (2012). Physiological and molecular characterization of a newly identified entomopathogenic bacteria, *Photorhabdus temperata* M1021. *Journal of Microbiology and Biotechnology*.

Judelson, H. S., & Blanco, F. A. (2005). The spores of *Phytophthora infestans* Weapons of the plant destroyer. *Nature Reviews Microbiology*, *3*(1), 47–58.

Li, J., Chen, G., & Webster, J. M. (1997). Nematophin, a novel antimicrobial substance produced by *Xenorhabdus* nematophilus (Enterobactereaceae). *Can J Microbiol*, *43*(8), 770–773.

Mcinerney, B. V., Gregson, R. P., Lacey, M. J., Akhurst, R. J., Lyons, G. R., Rhodes, S. H., … White, A. H. (1991). Biologically active metabolites from *Xenorhabdus* spp., part 1. dithiolopyrrolone derivatives with antibiotic activity. *Journal of Natural Products*.

Ng, K. K., & Webster, J. M. (1997). Antimycotic activity of *Xenorhabdus bovienii* (Enterobacteriaceae) metabolites against *Phytophthora infestans* on potato plants. *Canadian Journal of Plant Pathology*.

Thomas, G. M., & Poinar, G. O. (1979). *Xenorhabdus* gen. nov., a Genus of Entomopathogenic, Nematophilic Bacteria of the Family Enterobacteriaceae. *International Journal of Systematic Bacteriology*.

APPENDIX II

Raw data

Slices assays

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
3	B	15.4	2.1	0	0	13.64	0.00	1
3	B	18.8	9.5	0	0	50.53	0.00	1
3	B	18.2	1.9	0	0	10.44	0.00	1
3	B	17.8	2.1	0	0	11.80	0.00	0
3	B	16.5	2.1	0	0	12.73	0.00	0
3	B	19.9	6.5	0	0	32.66	0.00	0
3	B	19.2	2.1	0	0	10.94	0.00	0
3	Cu	19.1	0	0	0.4	0.00	0.00	0
3	Cu	19.2	0	0	1.1	0.00	0.00	0
3	Cu	19.1	0	0	0.3	0.00	0.00	0
3	Cu	15.5	0	0	0.3	0.00	0.00	0
3	Cu	15.1	0	0	0	0.00	0.00	0
3	Cu	17.8	0	0	0	0.00	0.00	0
3	Cu	17.9	0	0	0.6	0.00	0.00	0
3	W	17.1	13.2	0	0	77.19	0.00	0
3	W	17.9	13	0	0	72.63	0.00	0
3	W	19.9	9.5	0	0	47.74	0.00	0
3	W	19	10.9	0	0	57.37	0.00	0
3	W	15.6	3.8	0	0	24.36	0.00	0
3	W	18	8.8	1.2	0	48.89	6.67	0
3	W	15.2	5.2	0	0	34.21	0.00	0
4	B	18.4	9.4	0	0	51.09	0.00	1
4	B	18.6	8.1	0	0	43.55	0.00	1
4	B	21.8	5.3	0	0	24.31	0.00	1
4	B	22.9	0	0	0	0.00	0.00	1
4	B	24.4	4.3	0	0	17.62	0.00	0
4	B	21.5	2.7	0	0	12.56	0.00	0
4	B	20.8	3.9	0	0	18.75	0.00	0
4	Cu	25.4	0	0	0.6	0.00	0.00	0
4	Cu	24.3	0	0	0.6	0.00	0.00	0
4	Cu	24.2	0	0	0.9	0.00	0.00	1
4	Cu	23.3	0	0	1.1	0.00	0.00	1
4	Cu	20.9	0	0	1.2	0.00	0.00	0
4	Cu	21.8	0	0	0	0.00	0.00	0
4	Cu	21.8	0	0.4	0.4	0.00	1.83	0
4	W	19.4	8.8	3.8	0	45.36	19.59	0
4	W	23.8	3.8	3.2	0	15.97	13.45	0
4	W	25.3	6.1	1.5	0	24.11	5.93	0
4	W	25.3	5.9	4	0	23.32	15.81	0
4	W	26.5	12.6	4.5	0	47.55	16.98	0
4	W	34.1	10.7	4.2		31.38	12.32	0
4	W	24	8.5	0.8	0	35.42	3.33	0
7	B	19.7	1.2	0	0	6.09	0.00	0
7	B	20.4	3.7	0	0	18.14	0.00	1
7	B	23.7	2.5	0	0	10.55	0.00	0
7	B	24.6	3.6	0	0	14.63	0.00	0
7	B	25.8	10.9	2.2	0	42.25	8.53	1

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
7	B	24.4	14.5	0	0	59.43	0.00	0
7	B	17	4.6	0	0	27.06	0.00	1
7	Cu	21.5	0	0	0.2	0.00	0.00	0
7	Cu	17.5	0	0	1	0.00	0.00	0
7	Cu	17.3	0	0.8	0.8	0.00	4.62	0
7	Cu	19.2	0	4.6	0	0.00	23.96	0
7	Cu	21.9	0	0	0.2	0.00	0.00	0
7	Cu	22.5	0	7	0.3	0.00	31.11	0
7	Cu	22.1	0	0	1.2	0.00	0.00	0
7	W	23.9	3.6	0	0	15.06	0.00	0
7	W	23.1	4.9	0	0	21.21	0.00	0
7	W	22.7	15	14.1	0	66.08	62.11	0
7	W	18.6	14.9	15	0	80.11	80.65	0
7	W	16.3	2.7	0		16.56	0.00	0
7	W	19.1	3.6	0	0	18.85	0.00	1
7	W	18.3	4.7	0	0	25.68	0.00	0
10	B	19	13.7	10.2	0	72.11	53.68	0
10	B	14.7	9.6	1.5	0	65.31	10.20	0
10	B	17.6	8.2	0	0	46.59	0.00	0
10	B	18.2	13.4	2.5	0	73.63	13.74	0
10	B	20.4	11.4	2.3	0	55.88	11.27	0
10	B	20.4	15.7	2.3	0	76.96	11.27	0
10	B	18.9	14.8	5.5	0	78.31	29.10	0
10	Cu	17.8	0	0	0.4	0.00	0.00	0
10	Cu	16.7	0	0	1	0.00	0.00	0
10	Cu	23.9	0	0	0	0.00	0.00	1
10	Cu	21.9	0	0	1	0.00	0.00	0
10	Cu	20.8	0	0	0.6	0.00	0.00	0
10	Cu	19.8	0	0	1.6	0.00	0.00	0
10	Cu	16.4	0	0	0.2	0.00	0.00	1
10	W	24.4	14.1	0	0	57.79	0.00	0
10	W	24.2	12.7	0	0	52.48	0.00	0
10	W	24.3	8.8	0	0	36.21	0.00	1
10	W	24	14.4	0	0	60.00	0.00	0
10	W	23.2	13	0	0	56.03	0.00	0
10	W	17.7	14.1	0	0	79.66	0.00	0
10	W	18.2	4.5	0	0	24.73	0.00	1
16	B	26.8	6.4	6.7	0	23.88	25.00	0
16	B	29.6	0.4	0	0	1.35	0.00	0
16	B	17.8	3.1	0	0	17.42	0.00	0
16	B	21.8	0.5	0	0	2.29	0.00	1
16	B	15.9	1.3	0	0	8.18	0.00	1
16	B	16	2.3	0	0	14.38	0.00	1
16	B	21.3	7.4	0.4	0	34.74	1.88	0
16	Cu	22.4	0	0	3.8	0.00	0.00	1
16	Cu	22.2	0	0	2.2	0.00	0.00	0

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
16	Cu	21.9	0	0	0.8	0.00	0.00	0
16	Cu	21.3	0	0	1.9	0.00	0.00	0
16	Cu	20	0	0	1.9	0.00	0.00	0
16	Cu	22.9	0	0	2.8	0.00	0.00	0
16	Cu	27.7	0	0	0.7	0.00	0.00	1
16	W	28.3	6.4	1	0	22.61	3.53	1
16	W	26.1	9.2	12.5	0	35.25	47.89	0
16	W	35.3	2.9	0	0	8.22	0.00	0
16	W	29.2	3.7	18.3	0	12.67	62.67	0
16	W	29.1	4.4	1.8	0	15.12	6.19	0
16	W	35.3	25.7	10.5	0	72.80	29.75	0
16	W	30.9	5.9	0	0	19.09	0.00	0
18	B	17.9	0.9	0	0	5.03	0.00	0
18	B	19.3	0.9	0	0	4.66	0.00	0
18	B	19	0	0	0	0.00	0.00	0
18	B	14	0	0	0	0.00	0.00	0
18	B	13	0	0	0	0.00	0.00	0
18	B	16.8	1.8	0	0	10.71	0.00	0
18	B	16.5	0	0	0	0.00	0.00	0
18	Cu	22.4	0	0	0.6	0.00	0.00	0
18	Cu	21.5	0	0	4.9	0.00	0.00	0
18	Cu	19.4	0	0	9.6	0.00	0.00	0
18	Cu	16.3	0	0	1.9	0.00	0.00	0
18	Cu	19.7	0	0	4.3	0.00	0.00	0
18	Cu	19	0	0	2.3	0.00	0.00	0
18	Cu	19.3	0	0	3.6	0.00	0.00	0
18	W	26	20	6.1	0	76.92	23.46	0
18	W	25.7	12.2	2.6	0	47.47	10.12	0
18	W	16.3	16	2	0	98.16	12.27	0
18	W	16.7	14	12.6	0	83.83	75.45	0
18	W	21.6	19	19	0	87.96	87.96	0
18	W	19.4	18.1	18.1	0	93.30	93.30	0
18	W	16.8	8.8	6.2	0	52.38	36.90	0
22	B	22.9	2.5	0	0	10.92	0.00	1
22	B	19	0	0	0	0.00	0.00	0
22	B	20.2	1.8	0	0	8.91	0.00	0
22	B	21.7	0	0	0	0.00	0.00	1
22	B	17.7	0	0	0	0.00	0.00	1
22	B	16	0	0	0	0.00	0.00	0
22	B	18.4	2.6	0	0	14.13	0.00	0
22	Cu	20.9	0	0	2.8	0.00	0.00	0
22	Cu	24.1	0	1.2	4	0.00	4.98	0
22	Cu	22.1	0	0	3	0.00	0.00	0
22	Cu	21.4	0	0	1.3	0.00	0.00	0
22	Cu	22.1	0	0.6	3	0.00	2.71	0
22	Cu	19.3	0	0	3.2	0.00	0.00	0
22	Cu	22.5	0	0	2.9	0.00	0.00	0

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
22	W	18	15.6	2	0	86.67	11.11	0
22	W	17.7	15	2.2	0	84.75	12.43	0
22	W	16.1	6.7	0	0	41.61	0.00	0
22	W	14.9	13.3	2.8	0	89.26	18.79	0
22	W	15.7	12	4	0	76.43	25.48	0
22	W	14.2	7.2	1.5	0	50.70	10.56	0
22	W	22.1	9.8	3.3	0	44.34	14.93	0
31	B	25.6	0	0	0	0.00	0.00	0
31	B	25.5	1.6	0	0	6.27	0.00	0
31	B	25.1	0	0	0	0.00	0.00	1
31	B	19.9	0.5	0	0	2.51	0.00	1
31	B	23.4	4.4	0	0	18.80	0.00	0
31	B	22.7	0	0	0	0.00	0.00	1
31	B	18.8	0	0	0	0.00	0.00	0
31	Cu	22.4	0	0	0	0.00	0.00	0
31	Cu	16.5	0	0	1.3	0.00	0.00	0
31	Cu	20.1	0	0	0.8	0.00	0.00	0
31	Cu	25.8	0	0	0.8	0.00	0.00	0
31	Cu	26.1	0	0	0.7	0.00	0.00	0
31	Cu	27.4	0	0	0.7	0.00	0.00	0
31	Cu	28.3	0	0	0.9	0.00	0.00	0
31	W	27.3	3.7	0	0	13.55	0.00	0
31	W	26.3	12.3	0.9	0	46.77	3.42	0
31	W	25.1	14.4	0	0	57.37	0.00	0
31	W	23.6	13.8	0.7		58.47	2.97	0
31	W	20.7	8.3	0	0	40.10	0.00	0
31	W	18.3	14	1.8	0	76.50	9.84	0
31	W	18.9	9.9	0	0	52.38	0.00	0
34	B	16.2	0	0	0	0.00	0.00	0
34	B	16.3	0	0	0	0.00	0.00	0
34	B	17.6	0	0	0	0.00	0.00	1
34	B	19.6	0	0	0	0.00	0.00	0
34	B	19.2	0	0	0	0.00	0.00	0
34	B	18.8	0	0	0	0.00	0.00	1
34	B	16.2	0.9	0	0	5.56	0.00	0
34	Cu	12.9	0	0	1.2	0.00	0.00	0
34	Cu	11.9	0	0	0.6	0.00	0.00	0
34	Cu	17.3	0	0	0	0.00	0.00	0
34	Cu	14.9	0	0	0.7	0.00	0.00	1
34	Cu	16	0	0	1.5	0.00	0.00	1
34	Cu	15.9	0	0	1.4	0.00	0.00	0
34	Cu	17.2	0	0	0.7	0.00	0.00	0
34	W	19.5	16.2	1.9	0	83.08	9.74	0
34	W	17.3	7.8	7.1	0	45.09	41.04	0
34	W	19.1	14.1	5.5	0	73.82	28.80	0
34	W	19.8	11.1	2.9	0	56.06	14.65	0
34	W	18.7	12.4	0	0	66.31	0.00	0

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
34	W	18.2	10	1	0	54.95	5.49	0
34	W	16.5	10.3	1.1	0	62.42	6.67	0
45	B	25.2	0	0	0	0.00	0.00	0
45	B	26.7	0	0	0	0.00	0.00	1
45	B	24.9	1.9	2.4	0	7.63	9.64	0
45	B	25.8	0	0	0	0.00	0.00	0
45	B	24.8	0	0	0	0.00	0.00	0
45	B	22.3	1.8	2.9	0	8.07	13.00	0
45	B	17.8	0	0	0	0.00	0.00	1
45	Cu	22.8	0	0	1.6	0.00	0.00	0
45	Cu	20.3	0	0	1.6	0.00	0.00	0
45	Cu	20.5	0	0	1.3	0.00	0.00	0
45	Cu	30.2	0	0	2.1	0.00	0.00	1
45	Cu	24.4	0	0	4.4	0.00	0.00	0
45	Cu	28.2	0	0	2.8	0.00	0.00	0
45	Cu	29.4	0	0	4.5	0.00	0.00	0
45	W	29	7.2	10.4	0	24.83	35.86	na
45	W	30.8	4.6	0.7	0	14.94	2.27	0
45	W	31.2	3.7	2.7	0	11.86	8.65	1
45	W	29.8	2.5	0	0	8.39	0.00	0
45	W	29.1	9.8	9.2	0	33.68	31.62	0
45	W	28.4	2.6	0	0	9.15	0.00	0
45	W	26.8	2.2	0	0	8.21	0.00	0
49	B	18.8	1.3	0	0	6.91	0.00	0
49	B	17.8	0	0	0	0.00	0.00	0
49	B	22.4	0	0	0	0.00	0.00	1
49	B	23.9	0	0.8	0	0.00	3.35	0
49	B	18.8	0	0	0	0.00	0.00	1
49	B	24.3	0.9	0	0	3.70	0.00	0
49	B	23.9	1.1	0	0	4.60	0.00	0
49	Cu	19.4	0	0	0	0.00	0.00	0
49	Cu	19.1	0	0	1	0.00	0.00	1
49	Cu	21	0	0.4	0.3	0.00	1.90	0
49	Cu	19.9	0	0	0	0.00	0.00	1
49	Cu	23.4	0	0	0	0.00	0.00	1
49	Cu	22.5	0	1.7	0	0.00	7.56	0
49	Cu	20.5	0	0	0	0.00	0.00	0
49	W	25.3	2	0	0	7.91	0.00	0
49	W	21.4	14.9	0	0	69.63	0.00	0
49	W	20.9	18.1	1.8	0	86.60	8.61	0
49	W	25.6	3.2	0	0	12.50	0.00	0
49	W	24.8	7.2	1.4	0	29.03	5.65	0
49	W	20.7	14.2	1.9	0	68.60	9.18	0
49	W	20.7	8	1.1	0	38.65	5.31	0
59	B	15.9	2.6	0	0	16.35	0.00	2
59	B	22.2	0	0	0	0.00	0.00	1
59	B	22.6	4.4	0	0	19.47	0.00	0

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
59	B	18.6	0	0	0	0.00	0.00	1
59	B	17.7	0	0	0	0.00	0.00	0
59	B	21	1.6	0	0	7.62	0.00	1
59	B	20.4	1.9	0	0	9.31	0.00	1
59	Cu	23.4	0	1	0.3	0.00	4.27	0
59	Cu	21.4	0	0	1	0.00	0.00	1
59	Cu	22.8	0	1.2	2.7	0.00	5.26	0
59	Cu	22.7	0	9	0.9	0.00	39.65	0
59	Cu	24	0	1.3	0	0.00	5.42	0
59	Cu	22	0	0.8	1.3	0.00	3.64	0
59	Cu	23.1	0	0	1.3	0.00	0.00	0
59	W	19.7	3	0	0	15.23	0.00	0
59	W	21.2	3.2	0.6	0	15.09	2.83	1
59	W	20.3	4.4	1.3	0	21.67	6.40	1
59	W	22.1	7.4	1.8	0	33.48	8.14	0
59	W	21.8	3.6	0	0	16.51	0.00	0
59	W	18	5.2	0	0	28.89	0.00	0
59	W	18.5	4.3	1.8	0	23.24	9.73	0
63	B	21.5	8.2	6.5	0	38.14	30.23	0
63	B	13.6	0	0	0	0.00	0.00	0
63	B	20	0.4	0	0	2.00	0.00	0
63	B	22.6	5.4	0	0	23.89	0.00	0
63	B	23	2.5	0	0	10.87	0.00	0
63	B	22.5	0	0	0	0.00	0.00	0
63	B	23	0	0	0	0.00	0.00	1
63	Cu	23.5	0	0	2.6	0.00	0.00	0
63	Cu	21.4	0	0	3.5	0.00	0.00	0
63	Cu	20	0	0	2.1	0.00	0.00	1
63	Cu	20.7	0	0	0.8	0.00	0.00	1
63	Cu	26.5	0	0	1.7	0.00	0.00	1
63	Cu	25	0	0	6.2	0.00	0.00	0
63	Cu	22.2	0	0	2	0.00	0.00	0
63	W	30.6	2.2	0	0	7.19	0.00	1
63	W	32.6	19.8	17.7	0	60.74	54.29	0
63	W	30.8	17.6	10.2	0	57.14	33.12	0
63	W	29.5	12.3	5.3	0	41.69	17.97	0
63	W	17.1	5.9	3.1	0	34.50	18.13	0
63	W	17.5	11.1	14.5	0	63.43	82.86	0
63	W	20.8	3.3	0	0	15.87	0.00	0
83	B	16	0.5	0	0	3.13	0.00	1
83	B	15.2	0	0	0	0.00	0.00	0
83	B	20.3	0	0	0	0.00	0.00	1
83	B	20.9	0	0	0	0.00	0.00	0
83	B	15.4	0	0.9	0	0.00	5.84	0
83	B	21	1.1	0	0	5.24	0.00	0
83	B	16.5	1.6	0	0	9.70	0.00	1
83	Cu	17.2	0	0	0	0.00	0.00	0

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
83	Cu	17.2	0	0	0.4	0.00	0.00	0
83	Cu	16.9	0	0	0.6	0.00	0.00	0
83	Cu	18.6	0	0	0	0.00	0.00	0
83	Cu	15.6	0	0	0.3	0.00	0.00	0
83	Cu	17.3	0	0	0	0.00	0.00	0
83	Cu	17.3	0	0	0.7	0.00	0.00	0
83	W	18.7	3.5	0	0	18.72	0.00	0
83	W	16.9	11.9	0	0	70.41	0.00	0
83	W	18.1	11.2	5.6	0	61.88	30.94	0
83	W	17.9	13.5	5.3	0	75.42	29.61	0
83	W	18.9	10.6	3.1	0	56.08	16.40	0
83	W	17.4	8.4	4	0	48.28	22.99	0
83	W	19	4.4	0	0	23.16	0.00	0
102	B	26.1	6.6	0	0	25.29	0.00	0
102	B	25.2	3.1	0	0	12.30	0.00	1
102	B	19.1	3.2	0	0	16.75	0.00	0
102	B	22.7	5.4	0	0	23.79	0.00	0
102	B	15.8	1.8	0	0	11.39	0.00	0
102	B	22.9	2.7	0	0	11.79	0.00	0
102	B	25.4	8.9	0	0	35.04	0.00	0
102	Cu	21.7	0	0	2.2	0.00	0.00	0
102	Cu	21.9	0	0	1.7	0.00	0.00	0
102	Cu	16.4	0	0	1.9	0.00	0.00	0
102	Cu	17.5	0	0	2.9	0.00	0.00	0
102	Cu	24	0	0	3	0.00	0.00	0
102	Cu	18.7	0	0	4.6	0.00	0.00	0
102	Cu	24.1	0	0	3.2	0.00	0.00	0
102	W	18.2	14.9	9.2	0	81.87	50.55	0
102	W	16.4	15	6.2	0	91.46	37.80	0
102	W	16	12.1	6	0	75.63	37.50	0
102	W	15.5	10.5	1.3	0	67.74	8.39	0
102	W	13	8.8	1.4	0	67.69	10.77	0
102	W	26.1	16	0	0	61.30	0.00	0
102	W	25.5	7.5	0.7	1	29.41	2.75	0
103	B	13.1	0.6	0	0	4.58	0.00	0
103	B	16.4	2	0	0	12.20	0.00	1
103	B	17.5	3.7	0	0	21.14	0.00	0
103	B	17.8	1.9	0	0	10.67	0.00	0
103	B	17.5	0.5	0	0	2.86	0.00	1
103	B	18	1.7	0	0	9.44	0.00	0
103	B	19.4	0	0	0	0.00	0.00	0
103	Cu	12.2	0	0	3.1	0.00	0.00	0
103	Cu	14.3	0	0	3.8	0.00	0.00	0
103	Cu	15.5	0	0	5.7	0.00	0.00	0
103	Cu	17	0	0	1.3	0.00	0.00	0
103	Cu	18.1	0	0.7	2.9	0.00	3.87	0
103	Cu	16.9	0	0	4.1	0.00	0.00	0

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
103	Cu	17.8	0	0	1.4	0.00	0.00	0
103	W	15.1	3.8	1.1	0	25.17	7.28	0
103	W	16.9	4.4	1.4	0	26.04	8.28	0
103	W	12.8	9.2	1.4	0	71.88	10.94	0
103	W	14.8	11.6	4.1	0	78.38	27.70	0
103	W	17.2	10.1	0	0	58.72	0.00	1
103	W	18.2	12.9	1.4	0	70.88	7.69	0
103	W	19.7	13.9	1	0	70.56	5.08	0
107	B	17.8	0	0	0	0.00	0.00	0
107	B	21.1	0	0	0	0.00	0.00	0
107	B	21.1	1.4	0	0	6.64	0.00	1
107	B	22.3	5.5	0	0	24.66	0.00	0
107	B	21.6	2.6	0	0	12.04	0.00	0
107	B	20.3	1.8	0	0	8.87	0.00	0
107	B	19.7	1.8	0	0	9.14	0.00	0
107	Cu	18.4	0	0	1.4	0.00	0.00	0
107	Cu	17.7	0	0	2.5	0.00	0.00	0
107	Cu	18.1	0	1.5	4.2	0.00	8.29	0
107	Cu	13.5	0	0.3	4.1	0.00	2.22	0
107	Cu	19.2	0	0	1.8	0.00	0.00	0
107	Cu	14	0	0	1.3	0.00	0.00	0
107	Cu	19.1	0	0	1.2	0.00	0.00	0
107	W	17.3	13.6	0	2.6	78.61	0.00	0
107	W	15.1	11.9	4.1	0	78.81	27.15	0
107	W	18.8	15	6.7	0	79.79	35.64	0
107	W	18.5	15.6	5	0	84.32	27.03	0
107	W	17.5	14.7	3.6	0	84.00	20.57	0
107	W	18.3	14.9	4.3	0	81.42	23.50	0
107	W	15.3	11.3	1.8	0	73.86	11.76	0
113	B	24	0.7	0	0	2.92	0.00	0
113	B	25.2	0.5	0	0	1.98	0.00	0
113	B	26	0.4	0	0	1.54	0.00	1
113	B	25.2	0.9	0	0	3.57	0.00	0
113	B	26	0	0.4	0	0.00	1.54	1
113	B	23.3	0	0	0	0.00	0.00	1
113	B	20.8	2.7	1.8	0	12.98	8.65	0
113	Cu	17.9	0	0	1.8	0.00	0.00	0
113	Cu	18.2	0	0.5	0.7	0.00	2.75	0
113	Cu	17.2	0	0	1.2	0.00	0.00	0
113	Cu	16.8	0	0	1.9	0.00	0.00	0
113	Cu	15.8	0	0.3	3.7	0.00	1.90	0
113	Cu	18.3	0	0	2.4	0.00	0.00	0
113	Cu	16.6	0	0	2.8	0.00	0.00	0
113	W	20.5	10.9	3	0	53.17	14.63	0
113	W	18.9	8.5	0.8	0	44.97	4.23	0
113	W	17.2	6.4	0.3	0	37.21	1.74	0
113	W	19.2	15.4	4.8	0	80.21	25.00	0

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
113	W	17.8	8.5	1.3	0	47.75	7.30	0
113	W	14.5	12.1	0.5	0	83.45	3.45	0
113	W	19.7	4.6	1	0	23.35	5.08	1
114	B	19.1	0	0	0	0.00	0.00	0
114	B	20.7	0.4	0	0	1.93	0.00	0
114	B	19.5	0	0	0	0.00	0.00	0
114	B	12.5	0	0	0	0.00	0.00	0
114	B	14.2	0	0	0	0.00	0.00	0
114	B	16.9	0	0	0	0.00	0.00	1
114	B	17.8	0	0	0	0.00	0.00	1
114	Cu	17.3	0	0	3.5	0.00	0.00	0
114	Cu	17.6	0	0	1.9	0.00	0.00	0
114	Cu	14.4	0	0	6.1	0.00	0.00	0
114	Cu	12.6	0	0	5.3	0.00	0.00	0
114	Cu	18.1	0	0	1.8	0.00	0.00	0
114	Cu	19.7	0	0	0.7	0.00	0.00	0
114	Cu	18.8	0	0	3.5	0.00	0.00	0
114	W	18.5	13.2	4.8	0	71.35	25.95	0
114	W	11	6.9	0	0	62.73	0.00	0
114	W	16.4	10.3	2.5	0	62.80	15.24	0
114	W	15.9	10.6	3.1	0	66.67	19.50	1
114	W	16.8	11.7	5.2	0	69.64	30.95	1
114	W	17.8	12.2	4.9	0	68.54	27.53	0
114	W	19.1	7.5	2.3	0	39.27	12.04	1
117	B	19.4	0	0	0	0.00	0.00	0
117	B	17.6	0	0	0	0.00	0.00	0
117	B	17.6	0	0	0	0.00	0.00	1
117	B	16.2	1.8	0	0	11.11	0.00	1
117	B	18.6	0	0	0	0.00	0.00	0
117	B	16.2	0	0	0	0.00	0.00	0
117	B	18.5	0	0	0	0.00	0.00	0
117	Cu	17.3	0	0	2.5	0.00	0.00	0
117	Cu	19.1	0	0	0	0.00	0.00	0
117	Cu	13.9	0	0	0.7	0.00	0.00	0
117	Cu	15	0	0	0	0.00	0.00	0
117	Cu	18	0	0	2	0.00	0.00	0
117	Cu	17.2	0	0	0	0.00	0.00	0
117	Cu	17.8	0	0	1.5	0.00	0.00	0
117	W	19.8	10.2	4.5	0	51.52	22.73	0
117	W	19.1	15.6	7	0	81.68	36.65	0
117	W	17.1	8.5	2.7	0	49.71	15.79	0
117	W	19.2	12.7	2	0	66.15	10.42	0
117	W	16.9	9.2	0.3	0	54.44	1.78	0
117	W	16.4	3.1	1.1	0	18.90	6.71	1
117	W	16	4	0	0	25.00	0.00	1
118	B	21.6	1.6	0	0	7.41	0.00	0
118	B	21	0	0	0	0.00	0.00	0

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
118	B	21.7	0	0	0	0.00	0.00	0
118	B	22.3	0	0	0	0.00	0.00	0
118	B	22.6	0	0	0	0.00	0.00	0
118	B	22	0	0	0	0.00	0.00	1
118	B	21	0	0	0	0.00	0.00	0
118	Cu	21.9	0	0	3.8	0.00	0.00	0
118	Cu	19.6	0	0	4.3	0.00	0.00	0
118	Cu	20.7	0	0	2	0.00	0.00	0
118	Cu	22.6	0	0	1.8	0.00	0.00	0
118	Cu	22.4	0	0	1	0.00	0.00	0
118	Cu	21.9	0	0	2.3	0.00	0.00	0
118	Cu	17.9	0	0	2.5	0.00	0.00	0
118	W	14.9	9.1	4	0	61.07	26.85	0
118	W	15.2	9.2	2.3	0	60.53	15.13	0
118	W	13.3	8.6	4.1	0	64.66	30.83	0
118	W	16.6	10.8	4.2	0	65.06	25.30	0
118	W	16.2	8.6	3.8	0	53.09	23.46	0
118	W	13.7	8.4	1.7	0	61.31	12.41	0
118	W	15.8	10.1	1.4	0	63.92	8.86	0
119	B	17.8	0	0	0	0.00	0.00	0
119	B	20	0	0	0	0.00	0.00	0
119	B	18.5	2.1	0	0	11.35	0.00	1
119	B	17.6	0.7	0	0	3.98	0.00	1
119	B	18.4	0.8	0	0	4.35	0.00	0
119	B	16.8	0.8	0	0	4.76	0.00	2
119	B	18.3	1.8	0	0	9.84	0.00	0
119	Cu	16.4	0	0	0	0.00	0.00	0
119	Cu	15.7	0	0	7.6	0.00	0.00	0
119	Cu	17	0	0	1.9	0.00	0.00	0
119	Cu	14	0	0	3.3	0.00	0.00	0
119	Cu	15.2	0	0	3.1	0.00	0.00	0
119	Cu	15.2	0	0	1	0.00	0.00	0
119	Cu	16.8	0	0	1.4	0.00	0.00	0
119	W	15.2	6.5	2.2	0	42.76	14.47	0
119	W	23.6	2	0	0	8.47	0.00	0
119	W	23.3	2.2	0	0	9.44	0.00	0
119	W	21.1	5.3	0	0	25.12	0.00	1
119	W	20.8	10.8	3	0	51.92	14.42	0
119	W	25.1	3.6	0	0	14.34	0.00	0
119	W	18.3	14.6	9.4	0	79.78	51.37	0
120	B	14.5	3.5	0	0	24.14	0.00	0
120	B	23.5	0	0	0	0.00	0.00	1
120	B	24.3	2.1	0	0	8.64	0.00	0
120	B	21.6	3.1	0	0	14.35	0.00	1
120	B	19.3	0	0	0	0.00	0.00	1
120	B	24	0	0	0	0.00	0.00	1
120	B	23.2	0	0	0	0.00	0.00	1

Bacteria strain	Treatment	Slice surface (cm2)	Necrotic area (cm2)	Sporangia coverage (cm2)	Other mycelium area (cm2)	% of Necrotic area	% of sporangia coverage	Presence of Sprouts
120	Cu	25.7	0	0	3	0.00	0.00	0
120	Cu	26.3	0	0	1.7	0.00	0.00	0
120	Cu	23.7	0	0	0.7	0.00	0.00	0
120	Cu	17.5	0	0	4.1	0.00	0.00	0
120	Cu	22.1	0	0	0.9	0.00	0.00	0
120	Cu	21.9	0	0	1.1	0.00	0.00	0
120	Cu	19.8	0	0	1.2	0.00	0.00	0
120	W	21.4	8.2	1.8	0	38.32	8.41	1
120	W	17.8	10.5	4.1	0	58.99	23.03	0
120	W	18.6	9	1.6	0	48.39	8.60	0
120	W	18.8	14.5	2.7	0	77.13	14.36	0
120	W	16.7	7.9	1.2	0	47.31	7.19	0
120	W	18.1	9.3	3.5	0	51.38	19.34	0
120	W	17.1	11.4	3.6	0	66.67	21.05	0
121	B	16	0.8	0	0	5.00	0.00	1
121	B	20.1	0	0	0	0.00	0.00	0
121	B	17.1	0	0	0	0.00	0.00	1
121	B	17.8	7.3	0	0	41.01	0.00	0
121	B	19.6	0	0	0	0.00	0.00	1
121	B	17.6	0	0	0	0.00	0.00	0
121	B	17.9	0	0	0	0.00	0.00	0
121	Cu	18	0	0	1	0.00	0.00	0
121	Cu	18.7	0	0	4.7	0.00	0.00	0
121	Cu	14.9	0	0	0.9	0.00	0.00	0
121	Cu	15	0	0	1.1	0.00	0.00	0
121	Cu	16.9	0	0	0.8	0.00	0.00	0
121	Cu	14	0	0	3.5	0.00	0.00	1
121	Cu	18.9	0	0	0	0.00	0.00	0
121	W	19.9	10.8	1.9	0	54.27	9.55	0
121	W	15.2	13.1	5.9	0	86.18	38.82	0
121	W	20.8	6.3	1.2	0	30.29	5.77	0
121	W	18.9	2.8	0	0	14.81	0.00	1
121	W	15	11.1	0.7	0	74.00	4.67	0
121	W	15	9.3	8.4	0	62.00	56.00	0
121	W	15.6	3.4	0	0	21.79	0.00	0

APPENDIX III

Raw data

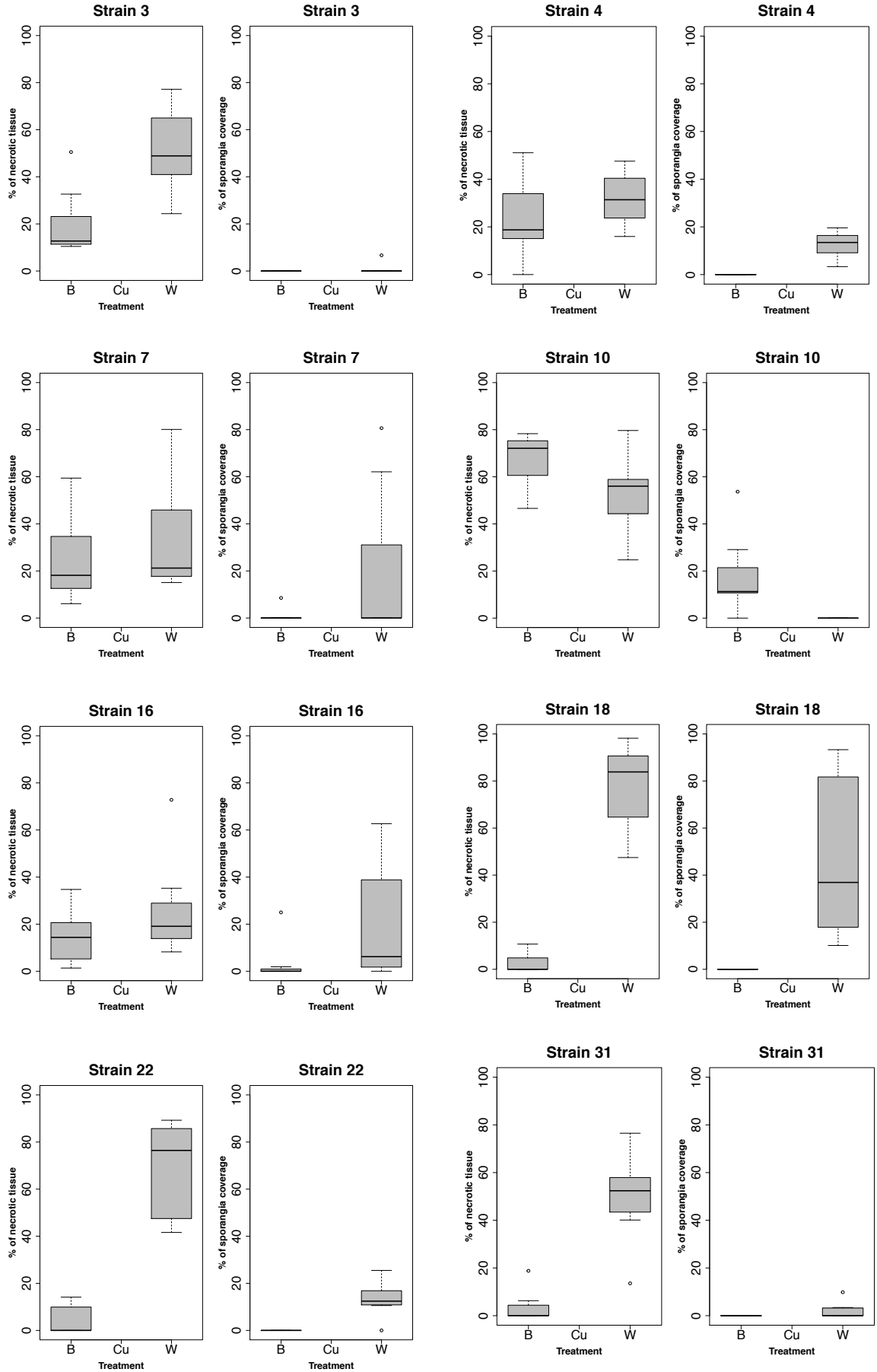
Tuber assays

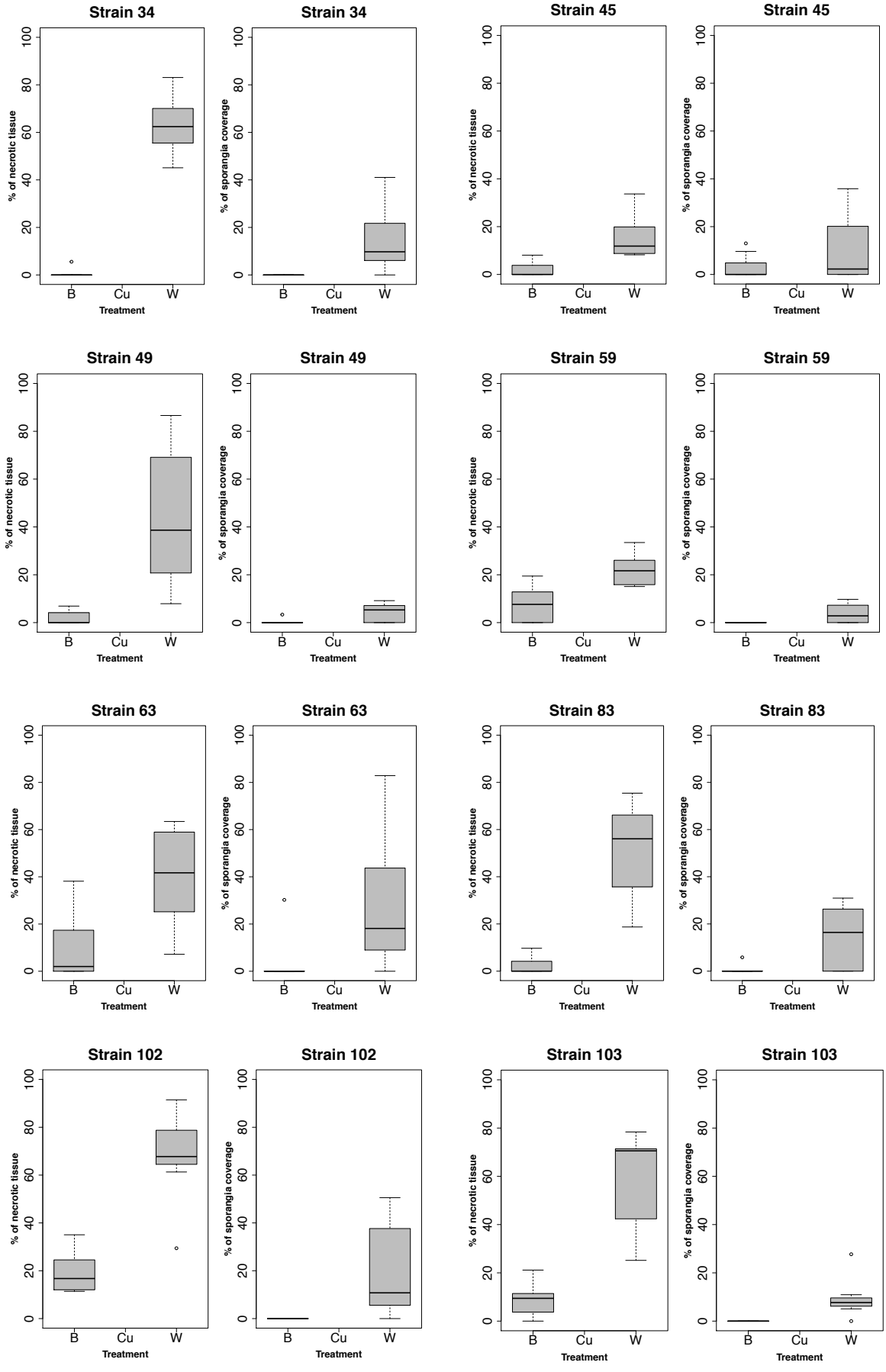
Strain	Treatment	Surface lesion diameter	Necrosis depth	radio	Cone volume
31	B	0.70	2.00	0.35	0.26
31	B	0.00	0.00	0.00	0.00
31	B	0.00	0.00	0.00	0.00
31	B	0.20	2.00	0.10	0.02
31	B	0.30	0.00	0.15	0.00
31	B	0.30	2.30	0.15	0.05
31	B	0.30	0.00	0.15	0.00
31	W	4.50	2.60	2.25	13.78
31	W	1.70	1.30	0.85	0.98
31	W	3.20	2.90	1.60	7.77
31	W	2.00	1.80	1.00	1.88
31	W	4.00	1.70	2.00	7.12
31	W	0.00	0.00	0.00	0.00
31	W	1.20	2.20	0.60	0.83
34	B	3.20	2.50	1.60	6.70
34	B	3.40	1.80	1.70	5.45
34	B	0.70	0.80	0.35	0.10
34	B	2.70	0.30	1.35	0.57
34	B	1.00	2.00	0.50	0.52
34	B	3.60	2.50	1.80	8.48
34	B	0.30	2.70	0.15	0.06
34	W	0.50	0.70	0.25	0.05
34	W	1.20	2.00	0.60	0.75
34	W	1.20	2.50	0.60	0.94
34	W	1.90	3.40	0.95	3.21
34	W	1.50	2.90	0.75	1.71
34	W	2.80	2.80	1.40	5.75
34	W	0.60	0.60	0.30	0.06
49	B	1.80	2.30	0.90	1.95
49	B	2.50	1.50	1.25	2.45
49	B	2.10	2.40	1.05	2.77
49	B	1.50	2.00	0.75	1.18
49	B	1.70	1.50	0.85	1.13
49	B	1.50	2.00	0.75	1.18
49	B	1.00	1.50	0.50	0.39
49	W	2.00	1.00	1.00	1.05
49	W	3.70	2.40	1.85	8.60
49	W	3.00	2.30	1.50	5.42
49	W	1.50	2.50	0.75	1.47
49	W	3.10	1.50	1.55	3.77
49	W	1.20	1.50	0.60	0.57
49	W	3.20	2.50	1.60	6.70

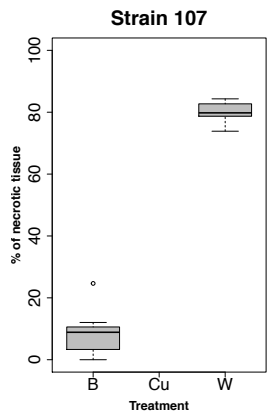
APPENDIX IV

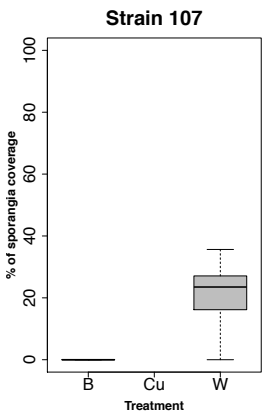
Boxplots

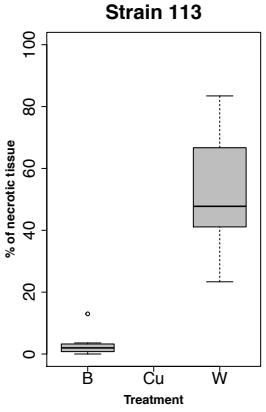
Slice assays

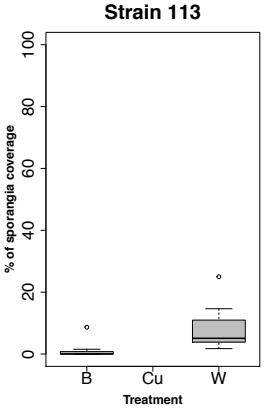

Strain 3
% of necrotic tissue
Treatment
B
Cu
W
Strain 3
% of sporangia coverage
Strain 4
Strain 4
Strain 7
Strain 7
Strain 10
Strain 10
Strain 16
Strain 16
Strain 18
Strain 18
Strain 22
Strain 22
Strain 31
Strain 31

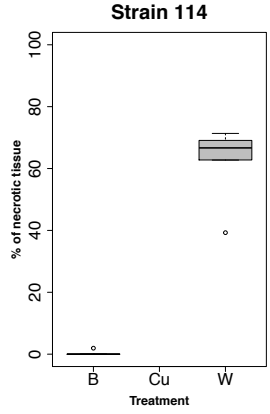

Strain 34
% of necrotic tissue
Treatment
B
Cu
W
Strain 34
% of sporangia coverage
Strain 45
Strain 45
Strain 49
Strain 49
Strain 59
Strain 59
Strain 63
Strain 63
Strain 83
Strain 83
Strain 102
Strain 102
Strain 103
Strain 103

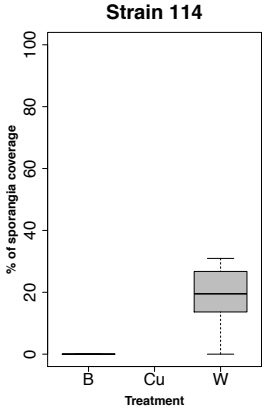

Strain 107
% of necrotic tissue
0
20
40
60
80
100
B
Cu
W
Treatment

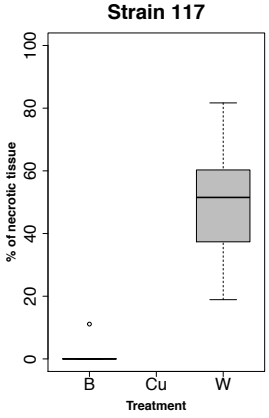

Strain 107
% of sporangia coverage
0
20
40
60
80
100
B
Cu
W
Treatment

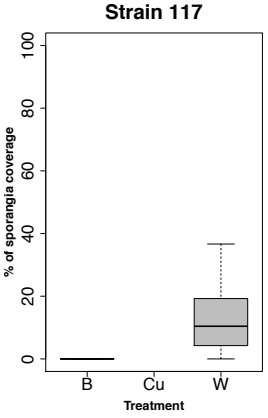

Strain 113
% of necrotic tissue
0
20
40
60
80
100
B
Cu
W
Treatment

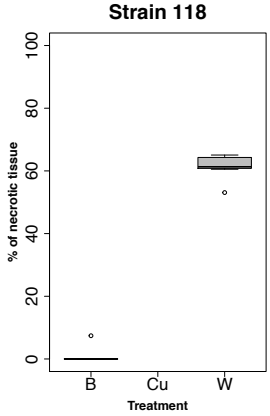

Strain 113
% of sporangia coverage
0
20
40
60
80
100
B
Cu
W
Treatment

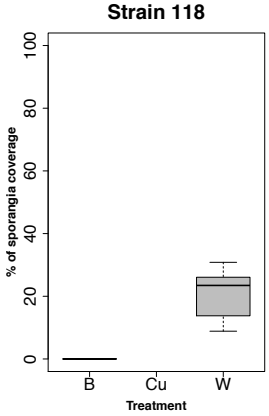

Strain 114
% of necrotic tissue
0
20
40
60
80
100
B
Cu
W
Treatment

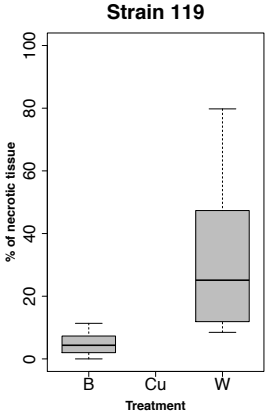

Strain 114
% of sporangia coverage
0
20
40
60
80
100
B
Cu
W
Treatment

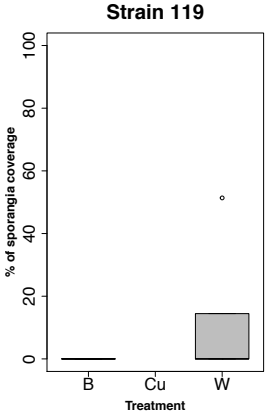

Strain 117
% of necrotic tissue
0
20
40
60
80
100
B
Cu
W
Treatment

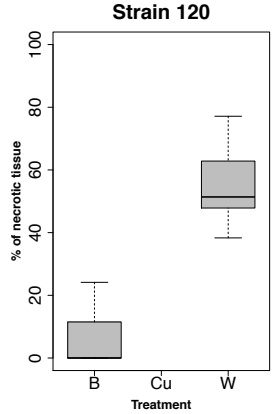

Strain 117
% of sporangia coverage
0
20
40
60
80
100
B
Cu
W
Treatment

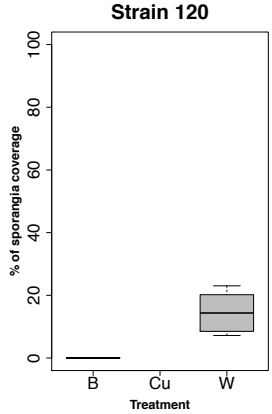

Strain 118
% of necrotic tissue
0
20
40
60
80
100
B
Cu
W
Treatment

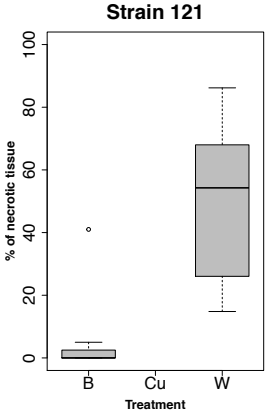

Strain 118
% of sporangia coverage
0
20
40
60
80
100
B
Cu
W
Treatment

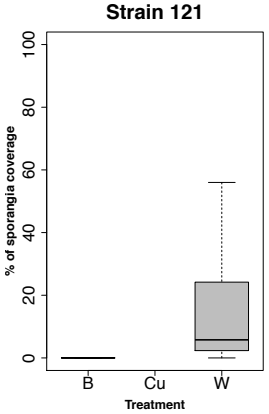

Strain 119
% of necrotic tissue
0
20
40
60
80
100
B
Cu
W
Treatment


Strain 119
% of sporangia coverage
0
20
40
60
80
100
B
Cu
W
Treatment


Strain 120
% of necrotic tissue
0
20
40
60
80
100
B
Cu
W
Treatment


Strain 120
% of sporangia coverage
0
20
40
60
80
100
B
Cu
W
Treatment

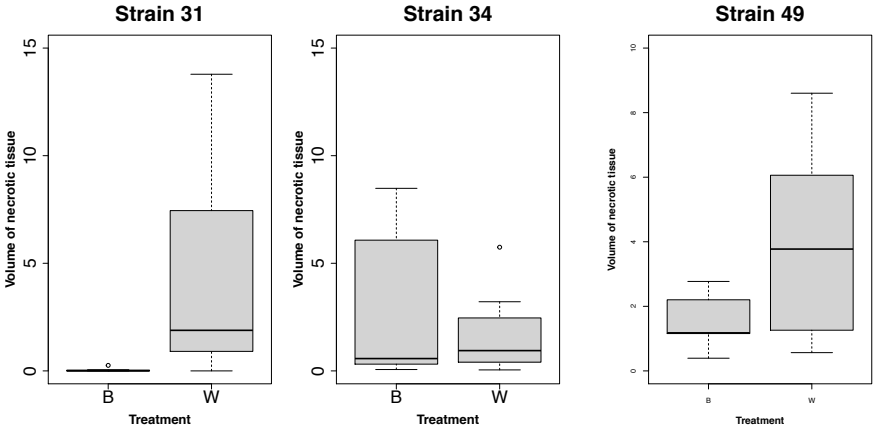

Strain 121
% of necrotic tissue
0
20
40
60
80
100
B
Cu
W
Treatment


Strain 121
% of sporangia coverage
0
20
40
60
80
100
B
Cu
W
Treatment

APPENDIX V

Boxplots

Tuber assays


Strain 31
Volume of necrotic tissue
0
5
10
15
B
W
Treatment
Strain 34
Volume of necrotic tissue
0
5
10
15
B
W
Treatment
Strain 49
Volume of necrotic tissue
0
2
4
6
8
10
B
W
Treatment

APPENDIX VI

Declaration of originality

Zurcher Hochschule
für Angewandte Wissenschaften

zh
aw

Life Sciences und
Facility Management

DECLARATION OF ORIGINALITY

Master's Thesis for the School of Life Sciences and Facility Management

By submitting this Master's thesis, the student attests of the fact that all the work included in the assignment is their own and was written without the help of a third party.

The student declares that all sources in the text (including Internet pages) and appendices have been correctly disclosed. This means that there has been no plagiarism, i.e. no sections have been partially or wholly taken from other texts and represented as the student's own work or included without being correctly referenced.

Any misconduct will be dealt with according to paragraphs 39 and 40 of the General Academic Regulations for Bachelor's and Master's Degree Courses at the Zurich University of Applied Sciences (dated 29 Januar 2008) and subject to the provisions for disciplinary action stipulated in the University regulations (Rahmenprüfungsordnung ZHAW (RPO)).

Town/City, Date: Signature:

ZÜRICH

06.01.2019 ...

The original signed and dated document (no copies) must be included in the appendix of the ZHAW version of all Master's theses submitted.