



Natural resources and bioeconomy studies 13/2024

Blackleg and Soft Rot of potatoes

Two decades of research and developments in Finland

Yeshitila Degefu

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Referencing instructions:

Degefu, Y. 2024. Blackleg and Soft Rot of potatoes : Two decades of research and developments in Finland. Natural Resources and Bioeconomy Studies 13/2024. Natural Resources Institute Finland. Helsinki. 29 p.

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ISBN 978-952-380-879-9 (Online)

ISSN 2342-7639 (Online)

URN urn.fi/URN:ISBN:978-952-380-879-9

Copyright: Natural Resources Institute Finland (Luke)

Authors: Yeshitila Degefu

Publisher: Natural Resources Institute Finland (Luke), Helsinki 2024

Year of publication: 2024

Cover picture: Yeshitila Degefu

Abstract

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Potato is the third food crop after rice and wheat in the world, recommended by the United Nations (FAO) as the global food security crop. Despite such high promises, potato suffers from production constraints which result in reduced yield and quality of harvest. Diseases are among the most crucial factors in the potato production. Potato is attacked by about 160 diseases and disorders which are fungal, bacterial, and viral origin and many other unknown causes. Late Blight caused by the Oomycete *Phytophthora infestans*, the most devastating disease that led to the infamous Irish Famine in the mid 19th century (1845-1850), is remembered as one of the tragic events in human history and the unsolved problem of Plant Pathology in the 21st century.

Blackleg and soft rot of potatoes are diseases caused by the bacteria in the genera *Dickeya* and *Pectobacterium* are very destructive diseases which are consistent threats to potato production in the world. The bacteria are among the top ten bacteria in economic importance. This report is a brief highlight of a two-decade research advances about these pathogens in the Natural Resources Institute Finland (Luke) and the former Agrifood Research Finland (MTT). It covers major outcomes and achievements in molecular diagnostics (method developments, standardization for research and end user applications), monitoring and surveillance of *Dickeya* and *Pectobacterium* aimed at safeguarding the EU granted High-Grade status of Finland for seed potato production and some remarks and recommendations for the management of emerging and re-emerging species to support policy formulation and effective blackleg disease management strategies in Finland. Information and knowledge generated during the last twenty years have been published in international scientific journals and professional Newsletters and disseminated to stakeholders in the potato value chain for awareness and active engagement in disease control. The report does not include descriptions of methodological protocols due to space limitations but interested readers could refer to our relevant publications listed in the reference section of this report where the experimental procedures are described in detail. The report is a useful reference document for young researchers who would like to know about blackleg and blackleg research in Finland-current status and future prospects and build projects to tackle the many potential challenges posed by blackleg to Finnish and global food security. Furthermore, the report is a brief synthesis of selected topics presented in a form simple to understand not only to experts in Plant Pathology but also to those who are involved in formulation of Phytosanitary policy guidelines or decision making.

Keywords: Potato, Blackleg, Soft rot, Molecular diagnostics, High-Grade status, *Dickeya* spp, *Pectobacterium* spp.

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1. Introduction

1.1. Background

Potato (*Solanum tuberosum*) is the third most important food crop in the world after rice and wheat in terms of human consumption. According to the recent FAO data published in 2023 (<https://www.potatonewstoday.com/2023/01/21/global-potato-statistics-latest-fao-data-published/>) a total of 376 million tonnes of potatoes were produced worldwide. China (94 million tonnes) and India (54 million tonnes) were the largest potato producing countries. FAO figures indicate that the total area harvested globally in 2021 was 18,132,694 hectares. Potato is recommended by the FAO as a global future food and food security crop as the world faces a growing population and shortage of food supply (Devaux et al. 2014)

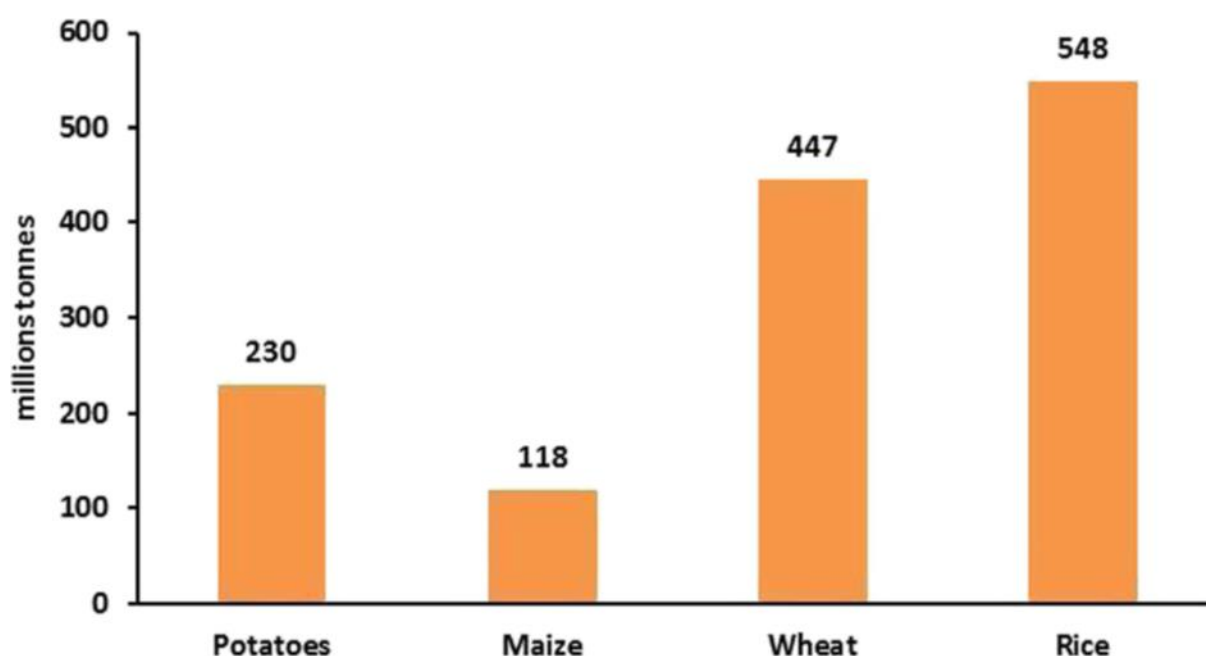


Figure 1. Potato production shift. Source: FAO 2014.

Despite such high potential of being the future food of the world, there are several production constraints which affect the yield and quality of potato. One of the major constraints are potato diseases. Potato is affected by about 160 diseases and disorders of which fifty are fungal, ten bacterial, forty viral origins. There are also tens of disorders of unknown causes.

An estimated 22% of potatoes are lost per year to viral, bacterial, and fungal diseases and pests, which is equivalent to an annual loss of over 65 million tonnes (Ross 1986), (International Potato Centre, Lima, Peru <http://www.cipotato.org/>, Food and Agriculture Organization of the United Nations <http://www.fao.org/>).

Among the topmost diseases of potato, the potato late blight caused by the Oomycete fungus like plant pathogen *Phytophthora infestans*, is a devastating disease worldwide and led to the ill-reputed Irish Potato Famine in 1845–1852 (Bourke 1964, Savary et al. 2017) that resulted in the death and displacement of millions of people. Late blight is still the unsolved

problem of Plant Pathology even in the 21st century causing losses of billions of Dollars and high risk of environmental hazards from excessive use chemicals.

Blackleg and bacterial soft rot of potatoes have long been recognized as important bacterial diseases causing heavy economic losses on potatoes. Bacteria in the family Pectobacteriaceae and the genera *Dickeya* and *Pectobacterium* are identified as the causal agents of blackleg and soft rot on potatoes. The bacteria are listed among the ten top economically important bacterial plant pathogens (Mansfield et al. 2002). To date five species, namely, *D. solani*, *P. atrosepticum*, *P. brasiliense*, *P. carotovorum* and *P. parmentieri* (formerly *P. wasabiae*) are commonly occurring on potatoes. Over the last two decades economic losses and general threat from these pathogens have been increasing in Finland and other north European countries because of the emergence of new species and the north ward extension of the causal agents exacerbated by climate change and expanding international trade. (Hickling et al. 2006, Bebber et al. 2013).

This report is built on core advances and findings of research and developments on the survival, pathogenesis, diagnostics, monitoring and surveillance, emergence and re-emergence and the main route of introduction and spread of species of *Dickeya* and *Pectobacterium* in Finland (Degefu et al. 2009, Degefu et al. 2013, Degefu 2016a, Degefu 2016b, Degefu & Virtanen 2015, Degefu 2015, Degefu et al. 2016, Degefu 2021, Degefu 2024, Manuscript in press) are discussed. The body of knowledge recorded, and the lessons learnt during the long period of research in Finland, strategies for sustainable management of blackleg and soft rot are highlighted. A model system for sustainable management of the disease and formulations of policy framework are proposed. In general pathogens emerge without warning and often discovered after they have been introduced and started causing losses as was the case with *D. solani* in Finland (Degefu et al. 2013) and other countries of Europe (Toth et al. 2010). This is due to the globalization of trade and free movement of planting materials across geographical boundaries (Andersson et al. 2004). The emergence and spread of pathogens are very complex that advances in biological research and Plant Pathology alone, though play crucial role, are not enough to bring about sustainable solutions (Almeida 2018, Jeger et al. 2021). Thus, multi actor (multi-actor projects: scientists and farmers creating solutions together, (<https://ec.europa.eu/eip/agriculture/en/about/multi-actor-projects-scientists-and-farmers>) and applying well executed multifaceted stakeholder engagement strategies are good alternatives to combat the current persistent threat of emerging and re-emerging disease risks to food security (Murray-Watson et al. 2022, Sherman et al. 2019, Ristainoa, et al. 2021)

Therefore, alternative options and prospects in sustainable management of emerging *Dickeya* and *Pectobacterium* and the need for integration of scientific research and stakeholder engagement as well as the roles of Natural and Social Sciences such as Citizen Science (Ryan et al. 2018, Meentemeyer, et al. 2015) in sustainable management of emerging species of *Dickeya* and *Pectobacterium* are emphasized.

Topics included in this report are subject areas investigated during the two decades of research and development work where significant advances have been achieved.

1.1.1. Blackleg a disease complex (syndrome)

Blackleg is a disease of potato caused by pectolytic (ability to hydrolyse pectin) Gram-negative bacteria characterized by wilting, stunting, chlorosis, necrosis and death of the potato plant (Fig. 2). The term "blackleg" originates from the typical blackening and decay of the lower stem portion, or "leg", of the plant.



Figure 2. Typical symptoms of blackleg and soft rot on potatoes caused by *Dickeya* and *Pectobacterium* species. Poor drainage and the waterlogged condition create an anaerobic condition that favour disease outbreak and spread (Photos and composition by Yeshitila Degefu, Luke)

The bacteria belong to the family Pectobacteriaceae and the genera *Dickeya* and *Pectobacterium*. Currently five species namely, *Dickeya solani*, *Pectobacterium atrosepticum*, *Pectobacterium brasiliense*, *Pectobacterium carotovorum* and *Pectobacterium parmentieri* (formerly *P. wasabiae*) are detected on potatoes posing a threat to potato production in Finland (Degefu, 2021, Degefu 2015, Degefu & Virtanen 2015) causing typical blackleg symptoms indistinguishable from each other. Hence, blackleg is a disease complex. In a disease complex, different pathogen species cause similar symptoms on a common host plant species (Le May et al. 2009). Co-occurring pathogens may affect each other through antagonism and/or synergism thereby limiting or favouring plant damages and pathogens reproduction. Thus, co-occurrence is believed as one of the main forces that shape pathogen community structures and therefore the dynamics of diseases in field. Complex diseases are often more difficult to manage than one pathogen one disease pathosystem especially if the co-occurring species require different management measures. The blackleg Pectobacteriaceae infect potato simultaneously (infection by one or species) on the field. The frequency of co-occurrence depends on the species and weather condition. Co-occurrence among *Pectobacterium* species is more

frequent while there is rare co-occurrence between *D. solani* and *Pectobacterium* species, *P. atrosepticum* in particular (Degefu 2021). The phenomenon is very interesting but the mechanism behind it is not clear. However, it has been understood that *D. solani* is highly competitive with unique ability of overtaking the *Pectobacterium* species (Toth et al. 2010) including under field infection conditions. In addition, some preliminary studies (Motyka-Pomagruk et al. 2021) revealed some peculiar gene pools, specific to *D. solani*, which might contribute to the occupation of an isolated ecological niche. A study by Garland and co-workers, 2013 highlighted the existence of toxic secondary metabolites in *Dickeya* species that may confer high adaptation to unique environment and ability to spread fast in the potato ecosystem which may suggest its competitive nature in the natural ecosystem. This subject is valuable to deepen understanding of the ecology and epidemiology of the blackleg and soft rot diseases and cast light on the possible role of this selection mechanism on the cycle of emergence and re-emergence of this group of pathogens..

1.1.2. Bacteria, taxonomy and selective isolation

The taxonomy of blackleg and soft rot Pectobacteriaceae has been under continuous transformation in recent decades (Toth et al. 2021). Following the advances in genomics and sequencing technologies, new species have been identified (Pasanen et al. 2021, Dees et al. 2017, Sarfras et al. 2018) and old taxonomic classifications have been re-evaluated (Samson et al. 2005, Zhang et al. 2016) resulting in increased understanding of the group (Nabhan et al. 2012).

The bacteria causing blackleg and soft rot on potatoes belong to the genera *Pectobacterium* and *Dickeya*. They are pectolytic bacteria forming characteristics deep sunken cavities on a pectin amended Crystal Violet Poly pectate (CVP) agar plates, a semi selective medium commonly used for routine isolation and characterization (Fig. 3).

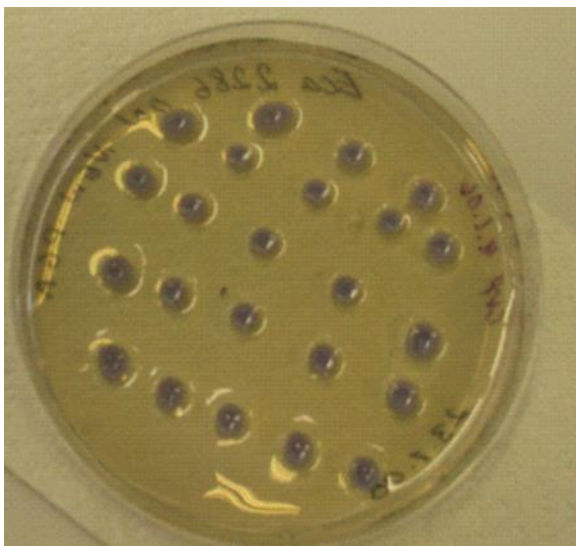


Figure 3. Cavity formation by *Pectobacterium* and *Dickeya* species on a pectin amended Crystal Violet Poly pectate (CVP) agar plates. (Photo: Yeshitila Degefu, Luke).

In addition to cavity formation on CVP, *Dickeya* spp is characterized by the production of dark blue pigmentation (Fig. 4) on a differential medium consisting of 23 g of nutrient agar, 10 ml glycerol (1% v/v) and 0.4 g $MnCl_2 \cdot 4H_2O$ (2 mM) commonly known as NGM (Lee & Yu 2006). The glycerol induces pigment production and the 2 mM $MnCl_2 \cdot 4H_2O$, that further

enhances colour development. The pigment production on the NGM medium is a very stable property specific only to *Dickeya* species. The *Pectobacterium* spp do not possess the ability. Therefore, the pigment production on NGM serves as a phenotypic property to differentiate *Dickeya* spp from the other Pectolytic *Pectobacterium* spp. Pigment production results from the expression of indigoidine biosynthesis gene.

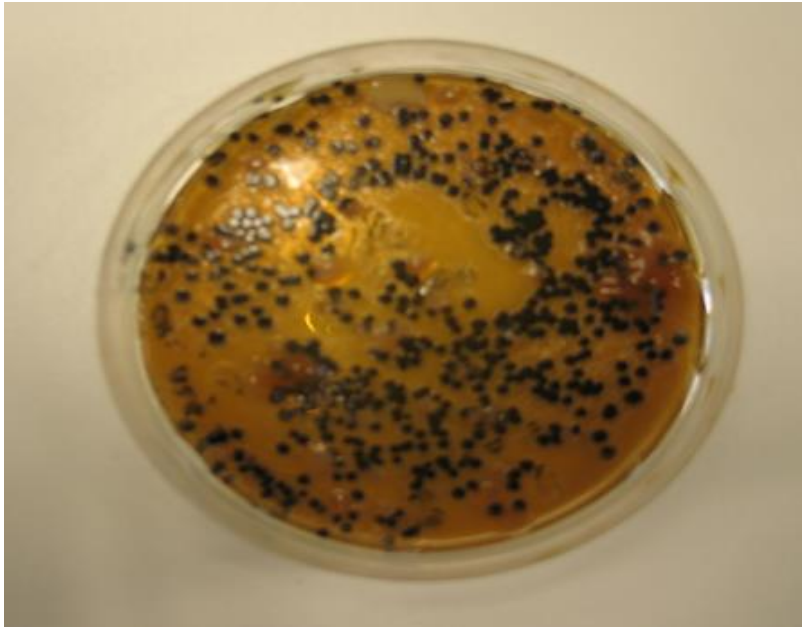


Figure 4. Blue colonies of *Dickeya* species on the NGM differential medium. The ability to produce blue pigmentation on the medium is typical to *Dickeya* species not that of *Pectobacterium* species. (Photo: Yeshitila Degefu, Luke).

1.1.3. Sources of inoculum and overwintering

Since over fifty years, it has been widely recognized that the main source of inoculum for infection of potatoes by blackleg and soft rot Pectobacteriaceae is latently contaminated seed (mother) tuber (Pérombelon 1974). The survival of the bacteria in soil is very short, often ranging from few days to few weeks after planting which then disappear very quickly. (Toth et al. 2021, Pérombelon & Hyman 1988, Czajkowski et al. 2011 and references therein). Bacteria in tubers left over from harvest and exposed to cold Finnish winters (Fig. 5A) are dead and non-viable (Degefu, unpublished), so is the bacteria residing in such tubers while tubers deeply buried in soil overwinter and grow into normal volunteer potato plants and, if infected, could serve as a source of infection (Fig. 5B)



Figure 5. Tubers left over during harvest exposed to cold winter (A), The tubers are dead and no viable *Dickeya* or *Pectobacterium* spp were detected. Volunteer potato (B) originating from tubers deeply buried in the ground and developing into normal potato plant surviving the Finnish winter.(Photo: Yeshitila Degefu, Luke).

1.1.4. Pathogenesis and mechanisms of disease transmission

Pathogenesis is defined as infection, colonization, pathogen reproduction and spread within the host plant. When infected mother tuber is planted, it often rots in the soil and bacteria are liberated and infect the potato plant. The bacteria from the mother plant move upwards the aerial part of the plant and infect the stem. Once in the stems, the bacteria may cause stem rot or typical blackleg visible at the base of the stem or above ground (Fig. 6) and in the meantime moves downwards via infected stolon and infect the daughter tubes which, depending on the weather conditions, may start rotting in field or later in poorly ventilated storage. On the other hand, if conditions favourable for disease development are not met, the bacteria may remain latent in the contaminated tuber for longer period than one season (Pérombelon 1974, Pérombelon & Kelman 1980, Pérombelon 1992).

The bacterial population created at the rhizosphere due to the disintegrated mother tuber is often transmitted by soil water to contaminate neighbouring plants leading to disease progression within the potato field. It has been demonstrated that crop contamination may also occur from air borne sources such as aerosols produced by rain splash from infected plants and haulm pulverization prior to harvest (Pérombelon 1992, Harrison et al. 1987, Pérombelon et al. 1979, Graham 1976). Furthermore, findings from a recent study indicated that soft rot *Pectobacteriaceae* are carried by several species of insects on the potato field (Rossmann et al. 2018) implicating insects as potential agents of transmission of *Dickeya* and *Pectobacterium* species from infected to healthy potato crop.

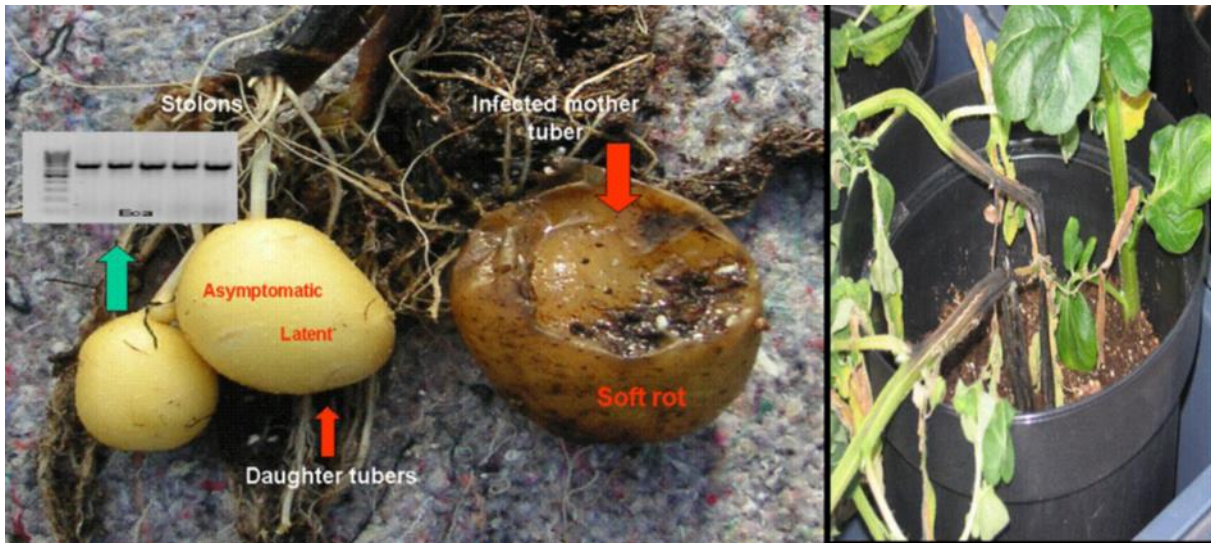


Figure 6. Infection and pathogenesis (infection, colonization, and pathogen reproduction) of blackleg on potatoes (Yeshitila Degefu. Luke).

Species of *Dickeya* have been isolated from water sources in Finland and Poland (Laurila et al. 2008, Potrykus et al. 2016) implicating river and surface water used for irrigation purposes is likely one way of transmission. However, knowledge about inoculum sources other than the seed tuber and their role in the blackleg disease on potatoes is very limited. Investigation in the subject will be very useful to answer some the longstanding questions such as "how do potatoes developing from pathogen free high class and first-generation mini tuber seed potato get infected resulting in diseased potato crop on field?" For up-to-date information readers are advised to refer to the review by Toth et al. 2021, https://doi.org/10.1007/978-3-030-61459-1_3.

1.1.5. Seed system and blackleg management

Latently contaminated seed tubers have been recognized as the main source of inoculum for infection of potato by blackleg and soft rot Pectobacteriaceae as discussed above. Therefore, avoidance of seed contamination through inspection and use of disease-free mini tubers schemes have been central in blackleg and soft rot management/control strategy and they have helped to minimize the problem over the years. Field inspection by visual observation has one major drawback, that it fails to detect the bacteria latent in the tuber. The advent of molecular biology has provided more advantages over older methods such as serology and biochemical tests. During the last two decades, the use of PCR detection has become widespread and the rapid and specific detection of the blackleg and soft rot Pectobacteriaceae has been readily achieved and the technology is disseminated to end user laboratories for routine seed testing and certification replacing the old and less reliable serological and visual seed inspection systems. The Molecular Biology Laboratory at Luke, Oulu has been providing PCR based seed tuber analysis using conventional or end point and Quantitative Real Time PCR (QPCR) for about twenty years supporting seed potato companies and farmers to know the health status of their seed lots and avoid financial losses resulting from rejection and downgrading of their seeds. The blackleg on potatoes is a syndrome very difficult to control since chemotherapy and thermotherapy are practically difficult because the bacteria are deep seated in the tuber and not easily accessible. Thus, starting healthy, in other words planting pathogen free seed tubers and staying healthy during the growing season by consistent

monitoring and surveillance are key practices in the management of the disease and minimizing losses. It has been evident that there is no single solution to the problem of blackleg and soft rot in potatoes. Therefore, besides using healthy seeds, cultural and sanitary methods such as avoiding seed contamination during grading and planting, avoiding poorly drained and waterlogged fields and poorly ventilated storage facilities are integral practices in the integrated system of management of the potato blackleg complex. For more insight, please see Czajkowski et al. 2011, <https://doi.org/10.1111/j.1365-3059.2011.02470.x>.

2. Blackleg research and development in Finland

The research and development work over the past two decades at then Agrifood Research Finland (MTT) and currently Natural Resources Institute Finland (LUKE) has focused on four major areas. First, the development of validated and optimized molecular detection and diagnostic methods for the blackleg and soft rot *Pectobacteriaceae* commonly found on potatoes in Finland suited for research and routine seed testing and certifications. Second, carry out constant surveillance and monitor the introduction, establishment and spread of emerging and re-emerging species of *Dickeya* and *Pectobacterium* in the High-Grade and other potato growing areas of Finland. Third, establish international and national network for collaborative research and exchange of expertise. A case in point is the Euphresco *Dickeya* and *Pectobacterium* network that was very successful and productive research collaborative network of scientists from seventeen European countries. Four, work on a strategy of stakeholders' engagement in combating the threat of especially emerging and re-emerging *Dickeya* and *Pectobacterium* species Finland and especially in the High-Grade seed potato production zone (the municipality of Tyrnävä and Liminka in North Finland (Fig. 7).

2.1. Safeguarding the High-Grade (HG) zone

2.1.1. The rationale of the HG status

Finland is one of the five countries in Europe (Germany, England, Ireland, and the Azores Archipelago in Portugal) (Fig. 7) which are granted the High-Grade status for seed potato production. The rationale of the High-Grade status is the privilege and obligations of applying stringent measures to keep the designated area clean from invasion by dangerous pests and pathogens of potatoes (Pohto 2002). Some of these measures include the controlled importation of seed potatoes from infected countries to these areas, the use of high-class certified seeds for potato production in the zone and reducing the number of food potato fields (area) in the designated High-Grade localities.



Figure 7. Countries in Europe with High-Grade status for seed potato production (Marked with black circle). Map: mapswire.com (CC-BY 4.0.)

2.1.2. Challenges of maintaining the HG status

While the High-Grade status offers a potential economic benefits to the Finnish seed potato sector and future food security, the safety of the region and the status has been threatened because of the trends of the northward extension of plant pathogens in general (Bebber et al. 2013) and the blackleg soft rot *Dickeya* and *Pectobacterium* species in particular. The drivers for this phenomenon are believed to be the combined effect of climate change, globalization and increasing free international trade and fast transportation (Anderson et al. 2004). In just less than two decades, Finland has experienced the emergence of two new species, namely *D. solani* and *P. brasiliense* and the re-emergence of *P. parmentieri* (formerly *P. wasabiae*). While *D. solani* (Toth et al. 2010) and *P. brasiliense* (Duarte et al. 2004, van der Merwe et.al. 2010) were previously confined to warmer climate, *P. parmentieri* is reported to have been present in Finland since 1980's (Nykyri et al. 2012) but increased in incidence and virulence due to the observable climate change taking place in Finland.

Finland is warming. According to the report by the Finnish meteorological Institute (Helsingin Sanomat International Edition), climate change is already affecting Finland. Atmosphere is more humid than before, sea levels are rising, and extreme weather phenomena increase. Summers have been exceptionally warm. The average temperature has risen at a rate of about one degree in one hundred years. Snow is melting earlier than before. The number of "hot days", according to Finnish weather classification, temperature ≥ 25 °C is increasing significantly giving more opportunities for warm weather species to establish in Finland. Therefore, coupled with globalization, increasing free international trade and fast transportation, it is not surprising that new species of the blackleg and soft rot Pectobacteriaceae continue to emerge and invade adding to the etiological diversity and complexity of the blackleg disease syndrome challenging food security. These circumstances call for tighter regulatory measures at the port of entry and obligatory testing of planting materials before distribution to farmers. Unfortunately, such guidelines do not exist for *Dickeya* and *Pectobacterium* species in all EU member states including Finland. Thus, testing seeds for *Dickeya* and *Pectobacterium* species in Finland until this day is not mandatory but voluntary that depends on the good will of the individual farmers. National level discussions strongly recommend the need for tighter phytosanitary guidelines including mandatory seed testing since long time, but the formulation of policy guidelines often take long time since decisions on policy matters are dependent on social, economic, and political factors. Thus, until such policies are in place, in addition to scientific research and knowledge production, raising the awareness of stakeholders and creating shared understanding of blackleg disease management solutions are necessary for collective actions and plausible integrated management of emerging and re-emerging species of *Dickeya* and *Pectobacterium* and the emerging potato disease risks.

2.2. Molecular detection and diagnosis of the blackleg and soft rot Pectobacteriaceae.

Healthy and physiological sound seed tuber is fundamental for sustainable potato production. Latent bacteria present in the tuber are the main source of infection by the blackleg and soft rot bacteria. Therefore, accurate and rapid detection is key to managing the spread of these pathogens. The main selling point in the effort has been the standardization and validation of the PCR technology for end user laboratory applications. As well stated by Hoorfar et al. 2004, in diagnostic PCR validation and sample preparation are two sides of the same

coin. Most importantly the sample must be representative of the seed lot. In addition, the standardized and robust PCR seed testing technology must be specific and predictive. In other words, lab test results should correlate positively with field infections. Therefore, we gave due consideration to the various pre-PCR sample preparation processes in our scheme of diagnostic PCR as presented in Figure 8. Both conventional (end point) and Real time detection systems were developed for detection of the blackleg and soft rot bacteria present on potatoes in Finland and have been used successfully in seed certification and monitoring of *Pectobacterium* and *Dickeya* species. Moreover, a high throughput system, theoretically unlimited multiplexing ability, commonly known as detection microarray was evaluated in detail for the detection of the major bacterial pathogens (Degefu et al. 2016). Unfortunately, the technology proved to be less sensitive and inconsistent for routine detection purposes. The details are described below.

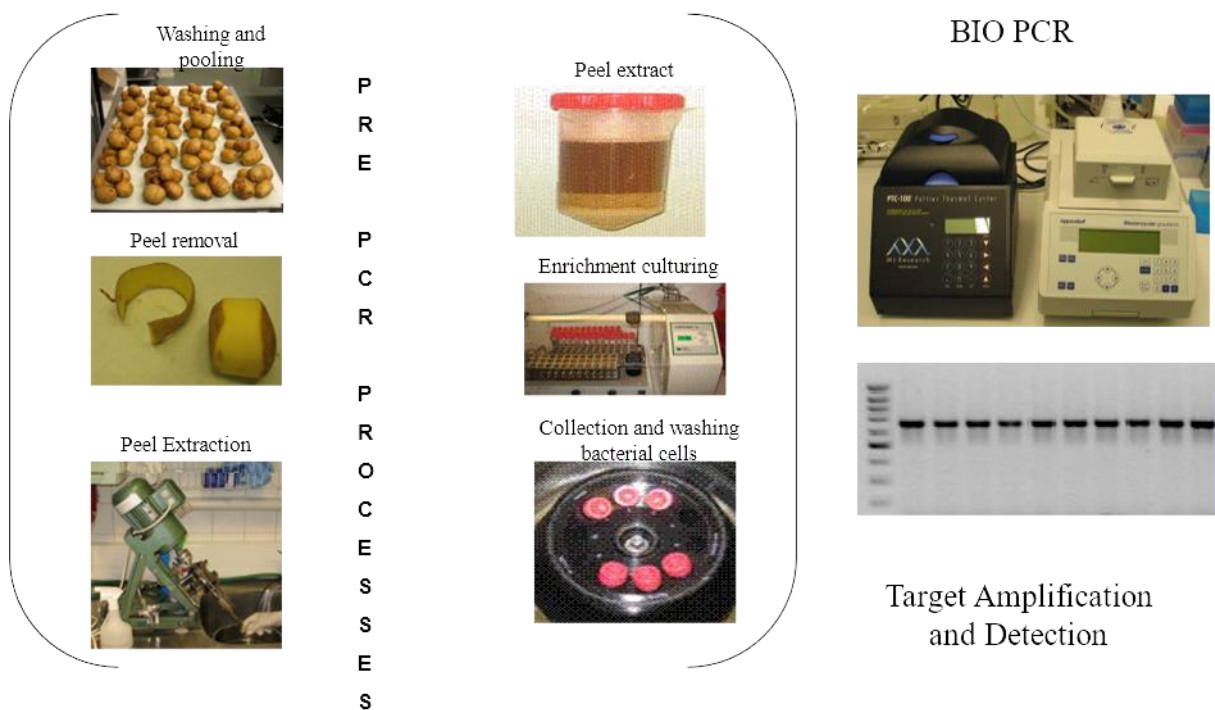


Figure 8. Schematic presentation of pre-PCR and target amplification and detection of the blackleg and soft rot bacterial species in potatoes (adapted from Degefu et al. 2009).

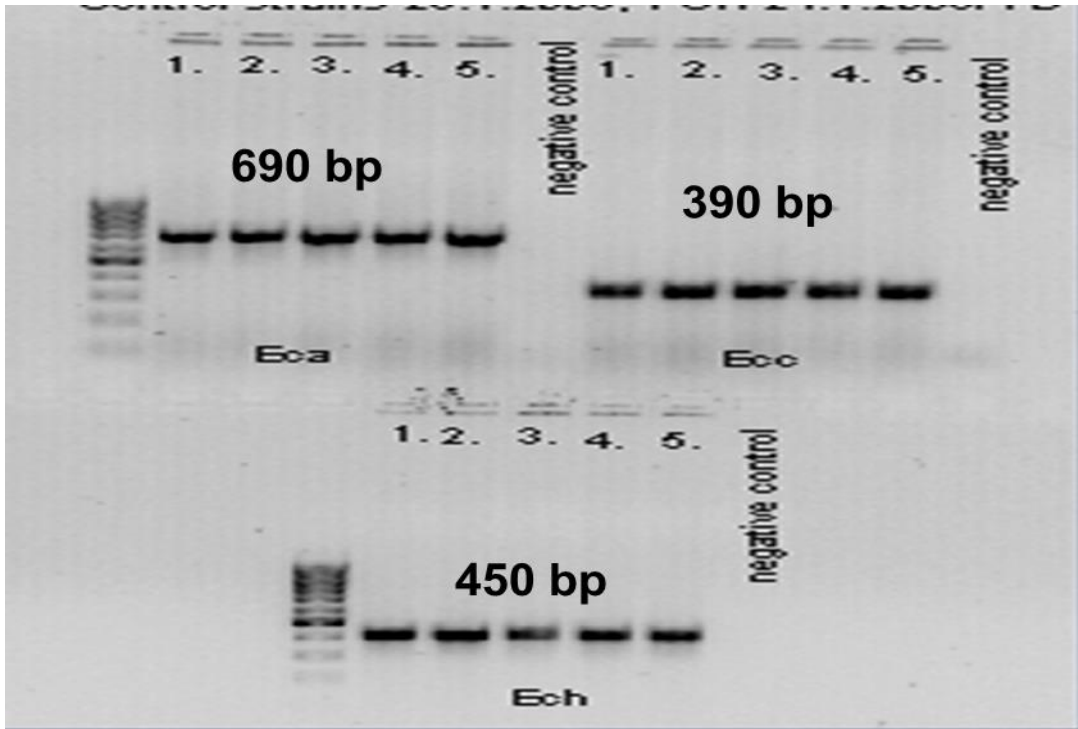
2.2.1. An overview of conventional (end point) and real time PCR detection of the blackleg and soft rot bacteria in potatoes

Currently the use of PCR in pathogen detection has become widespread. Although wide varieties of PCR variants are available for use, the conventional or end point PCR and the real time PCR which are well suited for routine detection have been used in our laboratory. It is not my intention to describe the basic mechanics and the different chemistries of PCR in this report, but it is important to highlight what are the shared elements of all PCR variants. All PCR techniques use primers or short synthetic single stranded Oligonucleotide fragments of complementary DNA that bind to templates to amplify defined sequences million/billion-fold during successive rounds of denaturation, annealing and polymerization. Each step is initiated by the cyclical changes of temperature in an instrument called the thermal cycler. Diagnostic PCR at Luke Oulu has been upgraded from conventional to a real time detection

chemistry for specificity, sensitivity, and cost effectiveness. Examples of the specific detection of the blackleg and soft rot *Dickeya* and *Pectobacterium* by conventional or end point PCR (Fig. 9) and Real Time PCR (Fig.10), respectively are presented.

SYBR Green and TaqMan are two methods employed to detect or monitor the amplification process in Real Time PCR. SYBR Green is a method based on intercalating nucleic acid staining dye while TaqMan is a method based on hydrolysis probe. Both technologies are designed to generate fluorescence during the PCR, which allows the real-time PCR machine to monitor the reaction in "real time". SYBR Green method is carried out using a fluorescent dye called SYBR green and detects the amplification by binding the dye to the amplified double stranded DNA.

The most important difference between the TaqMan and SYBR Green dye chemistries is that the SYBR Green I dye chemistry will detect all double-stranded DNA, including non-specific reaction products. A well-optimized reaction is therefore essential for accurate results. The specificity of the SYBR green Real Time PCR chemistry is often confirmed by a process known as melting curve analysis and sometimes by gel electrophoresis (Fig. 11). Melting curve analysis takes place after a qPCR run and serves to verify that the fluorescence detected during the run come from a single amplicon. The nonspecific nature of SYBR Green as a fluorophore makes melting curve analysis one of the very few ways to confirm that target-specific amplification occurred. Melt curve analysis is performed in a closed vessel at the end of the PCR protocol, the temperature of the vessel is slowly raised. Fluorescence measurements are taken at 2 degrees Celsius increments. A melting curve analysis can be thought of as a simpler version of an agarose gel. While both techniques easily demonstrate the number and size of PCR products, agarose gels require extra reagents, extra time, and an increased risk of contamination of the workspace.



PCR detection of the three strains of soft rot and blackleg *Erwinia* from potato

Figure 9. Conventional or end point PCR detection of *Dickeya* spp (Ech), *Pectobacterium atrosepticum* (Eca) and *Pectobacterium carotovorum* (Ecc) using primers specific to the species. Amplified genomic DNA fragments separated by Gel Electrophoresis, 1.2% agarose and visualized by ethidium bromide staining.

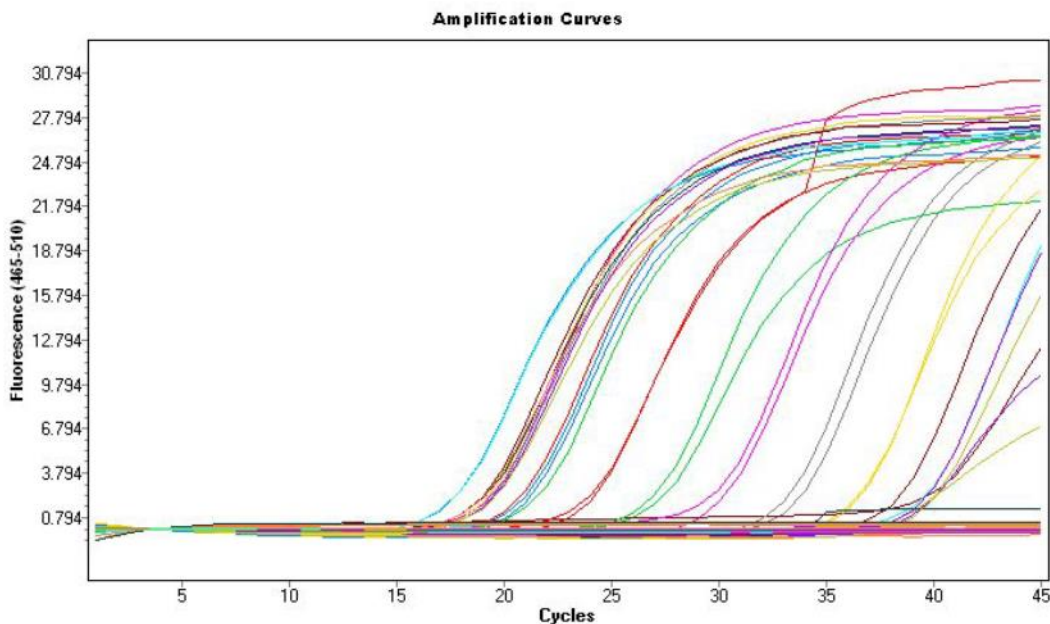


Figure 10. Amplification curves of real time PCR detection of *Pectobacterium atrosepticum*.

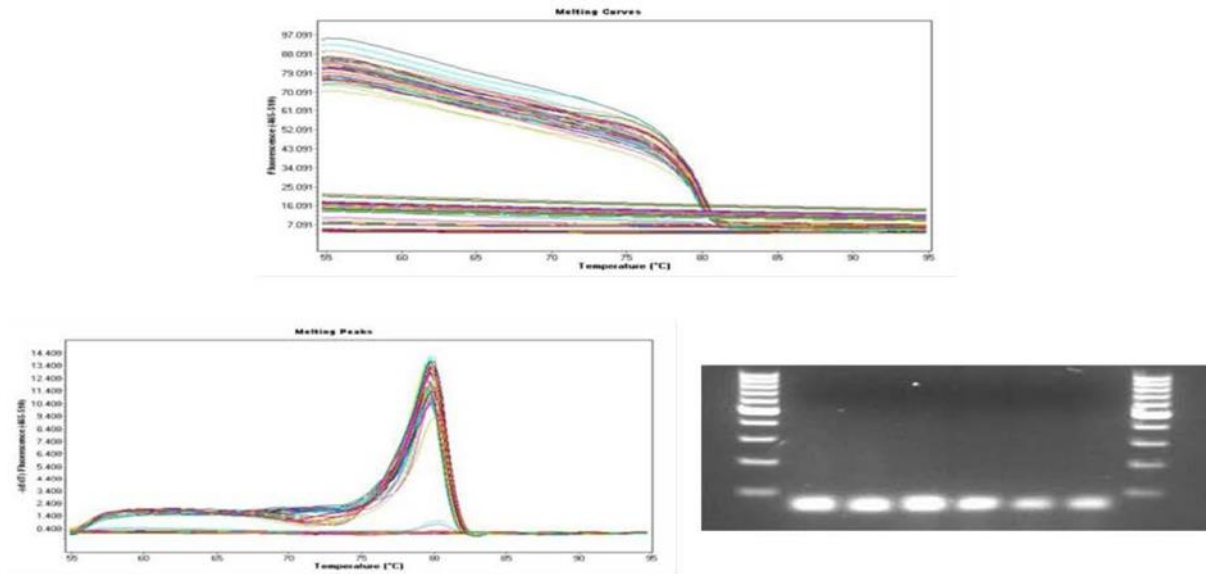


Figure 11. Validation of SYBR Green based Real Time PCR detection of the blackleg and soft rot bacteria, *Pectobacterium atrosepticum* in potatoes. The melting curve analysis confirms the specificity of the reaction by the observed result of a single target amplicon of the right size and only one peak without primer dimer formation.

2.2.2. Detection Microarrays: A hype than opportunity

Microarrays offer a platform for unlimited multiplexing and potentially enable the parallel detection of diverse organisms (Wang, et al. 2007, Kostić et al. 2017). However, our study on the evaluation and validation of the technology for routine detection of major bacterial pathogens of potatoes including the blackleg and soft rot *Pectobacteriaceae* (Degefu et al. 2016) directly from tubers with enrichment proved that the level of analytical sensitivity is not sufficient for routine detection of bacteria and results were very inconsistent. The system works well when abundant DNA, about 500 ng, from pure culture of the bacteria was used for labeling and hybridisation (Aittemaa et al. 2008) or when the procedure is coupled with pre-PCR amplification of the target pathogen DNA which is not economically feasible in seed certification. Advances in genomics and sequencing has allowed the designing of specific probes possible. We have achieved this and designed and used 15 208 probes which is the maximum number of user-defined probes in an Agilent $8 \times 15K$ custom array. The probes were specific for detection of the bacterial species (Fig.12). However, in addition to the cost and other technical issues of data analyses, the low sensitivity and inconsistencies detected especially in detecting latent bacteria from potato tubers are constraints for establishing the platform for routine use in end user seed certification laboratories. Therefore, despite the high expectations as high throughput detection platform, microarray turned out to be a hype than opportunity (Degefu 2016). As a result of these drawbacks, the technology is rapidly replaced by the fast-growing sequencing technologies (Bumgarner 2013) and almost no advances are observed in detection microarrays currently.

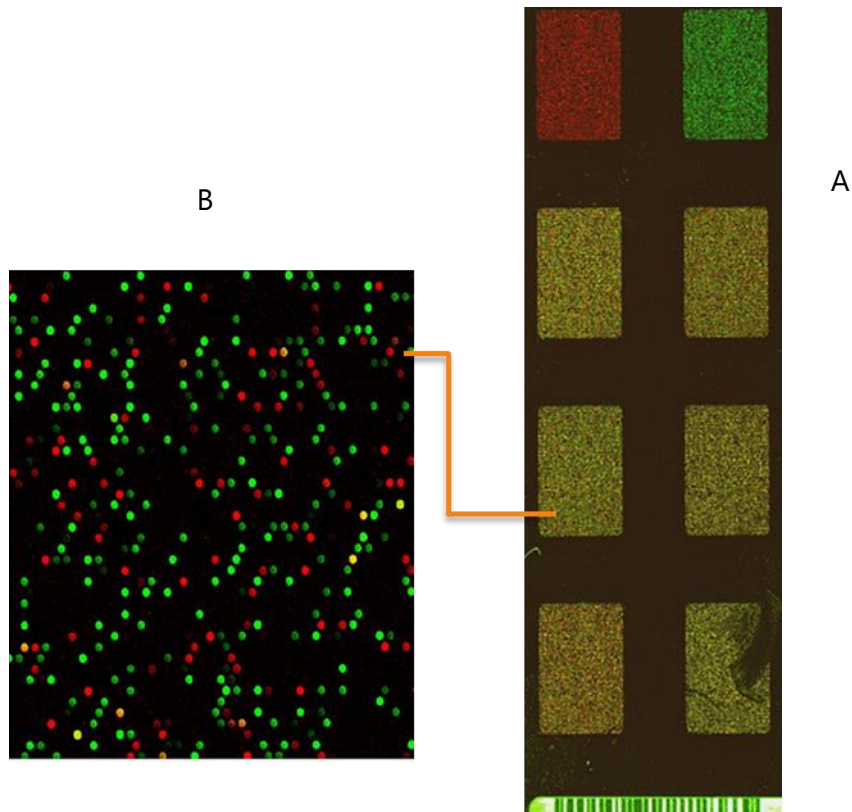


Figure 12. Scanned images of the signals detected on the microarray. A view of the whole microarray with eight subarrays (A) and areas covered by 1 528 probes of one subarray is shown at higher magnification (B). Two samples were hybridized on each of the eight subarrays. The sample labelled with Cy5 (red) in all eight subarrays and the other samples labelled with Cy3 (green)

3. Past lessons and future prospects

As described earlier, the past two decades have been an era of extensive investigation in the history research and development on blackleg and soft rot of potatoes in Finland. The avenues of introduction and spread of the causal bacteria are well known, rapid and sensitive methods of detection and diagnosis are standardized and validated and made available for routine analysis in research and seed certification replacing the old system of inspection based on visual observation of symptoms. The later does not give any information about latent bacteria present in the tuber which is the main source of infection and dissemination. Methodological advances have led to increased understanding of the blackleg complex and the pathogens involved. Methodological advancements in molecular biology and genomics have greatly facilitated our depth of knowledge and understanding of the genetics and diversity (Toth et al. 2021) and pathogenesis (Van Gijsegem et al. 2021) of *Dickeya* and *Pectobacterium* useful to draw valid conclusions and recommendations. The Euphresco *Dickeya* and *Pectobacterium* network where experts from seventeen European countries had focused on joint effort of generating knowledge and understanding about blackleg disease complex is one example how networking and cooperation are effective options in current situation where global funding in agriculture and expert capacity building is very limited. One of the most important achievements of the network has been the publication of the only book "Plant Diseases Caused by *Dickeya* and *Pectobacterium* Species" (van Gisejem et al. 2021, https://doi.org/10.1007/978-3-030-61459-1_3), where the author of this report is one of the contributors and active member of the research team representing Finland through Luke.

3.1. Emerging and re-emerging *Dickeya* and *Pectobacterium* species: Persistent threat

A current and growing challenge for the discipline of plant pathology is the introduction and spread of pathogens to new locations, and emergence or re-emergence of new pathogens in a changing climate. One common feature is pathogens emerge without warning, and they are often noticed after they have established and started causing damages. Furthermore, newly emerged pathogens are often very aggressive and spread rapidly since plants are new and may not have the least resistance to these new encounters. The lesson learnt from the emergence and spread of *D. solani* in Finland and the rest of Europe in early 2000s and *P. brasiliense* just recently are consistent to these hypotheses. Records showed that *D. solani* was very aggressive, causing infection from small amount of inoculum and spreading rapidly overtaking the other blackleg Pectobacteriaceae (Toth et al. 2010). The same trend is unfolding with *P. brasiliense*.

As stated earlier, Finland has experienced two newly emerging and one re-emerging blackleg bacterial species during the last two decades. The phenomena have been triggered by climate change, increased international trade and the lack of policy for mandatory seed testing for *Dickeya* and *Pectobacterium* species for imported or locally produced seed potato lots. Despite expert discussions on national and international level which strongly justified the need for implementation of mandatory testing policy is still not in place. It is still voluntary. In the absence of such stringent policy guidelines, it is very important to work on raising the awareness of stakeholders and adopt a concerted engagement strategy through shared understanding of management solutions especially for emerging pathogens since the problem

is more complex. The lesson learnt from the emergence, spread, and decline of *D. solani* in Finland (Degefu, 2024 Manuscript in press) showed that the concerted engagement and adoption of a stringent practice or “zero tolerance” to *D. solani* by growers and seed potato companies led to the successful step wise elimination of the pathogen from the seed system. As a result, the pathogen that, once overwhelmed farmers, and seed potato companies in Finland (Paassilta 2016, Degefu 2016a), is no more a threat at the moment. The strategy adopted is recommended as a model system for the fight against especially emerging and re-emerging *Dickeya* and *Pectobacterium* species including *P. brasiliense* and *P. parmentieri* (formerly *P. wasabiae*) whose incidence is increasing in Finland in recent years. Networking, and cooperation on national and European level are key for joint action against these pathogens which threaten the production of potato, a crop believed to be the future food of the world. Moreover, in an increasingly connected world this effectively brings the need for a global surveillance system, highlighting the need to access skills and technologies to increase baseline knowledge of the pathogen across the globe.

3.2. Thinking and acting outside the box

The discipline of plant pathology has an expanding sphere of activity requiring a multi-faceted, interdisciplinary approach to address the complexity of interactions for any given disease, disease complex or syndrome. Current research in Plant Pathology recognizes some key elements that are believed to be faced to meet the food security and environmental challenges that may emerge over the coming decades. Under such circumstances the further challenge is for the Plant Pathology community is to respond to a broader forum for multi-disciplinary research, recognising that impact will depend not just on advances in the Plant Pathology discipline and biological research alone, but on interactions more broadly with other agricultural sciences and issues of social dimensions (Jeger et al. 2021, Almeida 2018, Mills et al. 2011, Murray-Watson et al. 2022, Meentemeyer 2015) supported by national and global policies and regulations. The potential of this broader concept in the management of especially emerging and re-emerging plant pathogens is recognized and is receiving full attention. Several excellent reviews have been published recently (Brown et al. 2020, Jeger et al. 2021, Mills et al. 2021, Murray-Watson et al. 2022) providing useful insights.

The growing number of multi-actor EU funded projects: under the main theme “scientists and farmers creating solutions together” (<https://ec.europa.eu/eip/agriculture/en/about/multi-actor-projects-scientists-and-farmers>) and the proposal for integrating natural and social science perspectives (Mills et al. 2011) are among some examples of steps towards a paradigm shift in plant disease management approaches.

Furthermore, the requirements for inclusion of Citizen Science as part of the Horizon 2022 emerging PestRisk project call is a recognition that Citizen Science and public participation give better prediction of emerging pathogens (Meentemeyer 2015). In the global challenges facing Plant Pathology, such multidisciplinary approaches are believed to offer better option to address the food security and environmental challenges (Jeger et al. 2021).

Our success story with management of the newly emerged virulent blackleg bacteria, *D. solani* in Finland (Degefu 2024, article in press) is a useful model system of multifaceted stakeholder engagement strategy based on shared understanding of management solutions and coordinated cooperation for effectively preventing the spread of blackleg disease outbreak in Finland. In addition to scientific advances in diagnostics and detection technologies

for knowledge production which are very crucial, the creation of effective network with seed potato companies and potato farmers and establishing effective communication for dissemination of knowledge and raising awareness at grass root level were the key to success. The strategy is an important input for formulation of an integrated management policy guidelines to combat the threat of emerging and re-emerging blackleg and soft rot Pectobacteriaceae and a good example of "farmers and scientists solving agricultural problems together".

4. Acknowledgements

The Finnish Ministry of Agriculture and Forestry is acknowledged for financially supporting the projects which led to this success.

I thank Senior Scientist and Head of the Plant Health Group (KAST) Marja Jalli for encouraging me to write this synthesis report and her thoughtful support and guidance throughout the writing process. I also thank my colleagues, Senior Scientist Lea Hiltunen, Research Assistant Tiina Väyrynen, and Research Assistant Anne-Maria Möttönen for their kind cooperation and support in this long-term research work.

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