Euphresco

Final Report

For more information and guidance on completion and submission of the report contact the Euphresco Call Secretariat (bgiovani@euphresco.net).

Project title (Acronym)
Assessment of *Dickeya* sp. and *Pectobacterium* spp. on potatoes and ornamentals (Dickeya)

Project duration:

Start date:	1 March 2013
End date:	1 March 2015

1. Research consortium partners

Coordinator – Partner 1			
Organisation	Wageningen UR		
Name of Contact (incl. Title)	Dr Jean Martin van der Wolf	**Gender**:	Male
Job Title	Bacteriologist		
Postal Address	P.O. Box 16, 6700 AA Wageningen, The Netherlands		
E-mail	Jan.vanderWolf@wur.nl		
Phone	+31.317.480598		

Partner 2			
Organisation	NVWA		
Name of Contact (incl. Title)	Dr. Maria Bergsma-Vlami	**Gender**:	Female
Job Title	Bacteriologist		
Postal Address	Geertjesweg 15, Wageningen, The Netherlands		
E-mail	m.vlami@nvwa.nl		
Phone	+31.6 15891907		

Partner 3			
Organisation	Natural Resources Institute Finland		
Name of Contact (incl. Title)	Dr. Yeshitila Degefu	**Gender**:	Male
Job Title	Docent, Principal Research Scientist		
Postal Address	Paavo Haavaksen tie 3		
E-mail	yeshitila.degefu@luke.fi		
Phone	+358295326068		

Partner 4			
Organisation	Julius Kühn-Institute		
Name of Contact (incl. Title)	Dr. Petra Müller	**Gender**:	Female
Job Title	scientist		
Postal Address	Stahnsdorfer Damm 81, 14532 Kleinmachnow, Germany		
E-mail	Petra.mueller@julius-kuehn.de		
Phone	+49(0)33203 48 377		

Partner 5			
Organisation	FN3PT-RD3PT		
Name of Contact (incl. Title)	Dr V. Hélias	**Gender**:	Female
Job Title	Research Engineer		
Postal Address	UMR 1349 IGEPP INRA - Agrocampus Ouest Rennes - Université Rennes 1 BP 35327 F-35653 LE RHEU FRANCE		
E-mail	Valerie.helias@fnpppt.fr		
Phone	+33 (0)2 23 48 51 86		

Partner 6			
Organisation	Science and Advice for Scottish Agriculture		
Name of Contact (incl. Title)	Prof Gerry Saddler	**Gender**:	Male
Job Title	Deputy Head of SASA. Head of Potato and Plant Health		
Postal Address	Roddinglaw Road, Edinburgh, Scotland, UK EH12 9FJ		
E-mail	Gerry.saddler@sasa.gsi.gov.uk		
Phone	+44 (0) 131 244 8925		

Partner 7			
Organisation	Bern University of Applied Sciences, School of Agricultural, Forest and Food Sciences HAFL		
Name of Contact (incl. Title)	Dr. Patrice de Werra and Dr. Andreas Keiser	**Gender**:	Male
Job Title	Scientist and Professor		
Postal Address	Länggasse 85, 3052 Zollikofen, Switzerland		
E-mail	patrice.dewerra@bfh.ch and andreas.keiser@bfh.ch		
Phone	+41 (0)31 910 21 11		

Partner 8			
Organisation	Agroscope		
Name of Contact (incl. Title)	Brice Dupuis and Dr. Santiago Schaerer	**Gender**:	Male
Job Title	Scientist and Group leader		
Postal Address	Route de Duillier 50, 1260 Nyon1, Switzerland		
E-mail	brice.dupuis@agroscope.admin.ch and santiago.schaerer@agroscope.admin.ch		
Phone	+41 (0)58 363 44 44		

Partner 9			
Organisation	University of Helsinki		
Name of Contact (incl. Title)	Dr. Minna Pirhonen	**Gender**:	Female
Job Title	University lecturer, Associate professor		
Postal Address	Department of Agricultural Sciences, PO Box 27, 00014 University of Helsinki, Finland		
E-mail	minna.pirhonen@helsinki.fi		
Phone	+358 504922077		

Partner 10			
Organisation	Department of Plant Pathology and Weed Research, Agricultural Research Organization, Gilat Research Center, Israel		
Name of Contact (incl. Title)	Dr. Leah Tsror	**Gender**:	Female
Job Title	Researcher		
Postal Address			
E-mail	tsror@volcani.agri.gov.il		
Phone			

Partner 11			
Organisation	Instituut voor Landbouw en Visserij Onderzoek (ILVO) Onderzoekseenheid Plant-Gewasbescherming		
Name of Contact (incl. Title)	Dr. Johan van Vaerenbergh /Dr. Martine Maes	**Gender**:	Male/Female
Job Title	Researcher/Scientific Director		
Postal Address	Burgemeester Van Gansberghelaan 96, Merelbeke, Flanders		
E-mail	johan.vanvaerenbergh@ilvo.vlaanderen.be		
Phone	+32 9 2722476		

Partner 12			
Organisation	Biotechnology University of Gdansk		
Name of Contact (incl. Title)	Prof Ewa Lojkowska	**Gender**:	Female
Job Title	Professor		
Postal Address	Abrahama 58, 80-307 Gdansk,Poland		
E-mail	ewa.lojkowska@biotech.ug.edu.pl		
Phone	+48 58 5236345		

Partner 13			
Organisation	Department of Plant Science, University of Pretoria, South Africa		
Name of Contact (incl. Title)	Jacquie van der Waals	**Gender**:	Female
Job Title	Professor		
Postal Address			
E-mail	Jacquie.vanderWaals@up.ac.za		
Phone			

Partner 14			
Organisation	Institute of Ecology and Environmental Sciences, INRA-UPMC Université Paris, France		
Name of Contact (incl. Title)	Dr. Frédérique van Gijseghem	**Gender**:	Female
Job Title	Researcher		
Postal Address			
E-mail	vangijse@agroparistech.fr		
Phone			

Partner 15			
Organisation	James Hutton Institue		
Name of Contact (incl. Title)	Prof Ian Toth	**Gender**:	Male
Job Title	Theme Leader for Weeds, Pests and Diseases		
Postal Address	Errol Road, Invergowrie, Dundee, DD2 5DA, UK		
E-mail	Ian.toth@hutton.ac.uk		
Phone	+44 1382 568829		

2. Executive Summary

Project aims

Pectinolytic bacteria, namely the genera *Pectobacterium* and *Dickeya,* are economically important pathogens causing potato blackleg and potato soft rot in different European countries. Main aim of this EUPHRESCO-II project was to monitor the emergence and incidence of *Dickeya* and *Pectobacterium* in the potato production system (plants and tubers) as well as in ornamentals in different countries. More specifically, the presence of different (new variants) of *Dickeya* and *Pectobacterium* species was evaluated, in particular *Dickeya solani, Pectobacterium wasabiae* and *Pectobacterium carotovorum* subsp. *brasiliense*, as well as their dissemination pathways to clean potato lots. We need to better understand the spread of different (new variants) of *Dickeya* and *Pectobacterium* species in the environment, across stocks and through generations. In order to achieve this, we need to better understand the pathogens involved and, therefore, improvements to taxonomy and diagnostics have been very important as well as measuring strain type and movement in a field situation. This EUPHRESCO-II project also includes the comparison of the virulence of *Dickeya* species, among them *D. solani* and *D. dianthicola* in the potato environment. Additionally, the characterisation of *Pectobacterium atrosepticum* isolates based on a MLSA approach on 8 housekeeping genes has been studied in Scotland.

Annual disease surveys were performed and the frequency of potato blackleg pathogens in multiple countries was assessed, including the contamination of river and water courses in the area, and related to weather data analysis and correlations between weather and species prevalence in a given season. Correlation between lab testing of seed lots and the blackleg and wilt disease incidences in the field were demonstrated in trials in Israel and in Switzerland. Special attention in Northern Europe was given to *Pectobacterium carotovorum* subsp. *brasiliense* that represents an emerging blackleg causing agent (Nunes Leite *et al*., 2014). On the one hand, the virulence of *Pectobacterium carotovorum* subsp. *brasiliense* on potato was compared with that of other *Pectobacterium* and *Dickeya* species under climatic conditions prevailing in the Netherlands. On the other hand, the survival of *Pectobacterium carotovorum* subsp. *brasiliense* (Pcb) in soil at different temperatures and moisture regimes has been assessed in trials in South Africa.

Regarding the survival of *D. solani* several experiments have been performed in Germany, as the evaluation of the survival ability of *D. solani* is an important factor for its epidemiology. For this reason, experiments have been performed for the survival of *D. solani* on different materials (wood, cotton, rubber, corroded resp. coated iron) contaminated with a defined pathogen concentration and exposed at different temperatures resp. relative air humidity. These data are very important for the risk assessment of contaminated machinery, equipment, crates, containers etc. as a pathway for the pathogen. Scientific data on this theme are not available.

Last but definitely not least, complete genome sequences of a number of *D. solani* strains, among them strain 3337 in France and a Finnish isolate, were generated and analyzed based on comparative genomics approaches.

3. Description of the main activities

Surveys on diseased plants, seed potatoes, bulbs and water samples

Natural Resources Institute Finland: As many as one hundred potato plants, with typical blackleg symptoms, were randomly collected twice in the growing season; at the time of first disease symptom appearance and later in the season or before harvest. Fields from which samples were taken were randomly selected or could also be those where producers reported outbreaks of blackleg. The prevalence of the different *Dickeya* and *Pectobacterium* species and the predominant species in the growing season were determined based on the frequency of occurrence in the samples. In addition, water samples were collected from rivers and water courses from the HG area and the locality of Himanka where large farms of ware potato are produced.
FN3PT-RD3PT: Annual surveys conducted by the FN3PT in France aimed at sampling blackleg expressing plants, collecting and characterising *Pectobacterium* spp. and *Dickeya* sp. strains.
SASA: Random samples of plants expressing blackleg or wilting symptoms were taken at growing crop inspection (GCI). In addition, a collection of 200 blackleg causing isolates (mostly *Pectobacterium atrosepticum*) was collated from stems exhibiting blackleg symptoms collected during the 2007-15 growing seasons alongside historical and reference strains of world-wide origin were characterised by multi-locus sequence analysis (MLSA) based on 8 housekeeping genes; *dnaN*, *gapA*, *gyrB*, *purA*, *recN*, *recA*, *icdA* and *dnaJ*.
HAFL/Agroscope: Analysis of latent bacterial-infected potato seed lots was performed and their correlation with blackleg disease incidence in the fields analysed. Analysis of over 100 imported and national seed lots in 2013 and 2014 was performed to get an estimation of their latent infection for the following bacteria: *Dickeya* species, *Pectobacterium atrosepticum*, *P. wasabiae* and *P. carotovorum* subsp. *brasiliense*. The analysed seed lots were multiplied on about 150 potato fields. The disease development was observed by official field inspectors during certification. Diseased potato plants showing black leg symptoms in the fields were collected and the responsible bacterial strain determined in the laboratory.
University of Helsinki: surveys of diseased potato plants and river water especially in potato growing areas was performed to study the occurrence of *D. solani* in Finland. Also the voluntary tubers were analysed to study if they contain soft rot bacteria, and could thus transfer the infection from one lot to the next, if crop rotation is not used (monoculture is fairly common in ware potato production). Also the spreading of soft rot bacteria with free-living, non-pathogenic bacteria and insects living in potato storages was analysed to get understanding of the various ways the bacteria can spread to clean potato lots. Additionally, the ability of *D. solani* to compete with the other soft rot bacteria was studied. During the project the seed potato companies and farmers wondered about red rotten potatoes, which were analysed to identify the pathogen causing this symptom.
NVWA: in 2014, a survey has been performed among bulb producers of *Hyacynthus*, *Zantedeschia* and *Tulipa* in the Netherlands, based on visual inspections and testing. In total 75 symptomatic bulb samples were included for testing.

Assessment of Virulence

WUR: In field experiments in the Netherlands, the virulence of *Pectobacterium carotovorum* subsp. *brasiliense* was compared with strains of other *Dickeya* sp. and *Pectobacterium* spp. In 2013 and 2014, seed potato tubers were vacuum-infiltrated with high densities of bacteria (10^6 cfu ml^{-1}) and planted in clay soil.
FN3PT-RD3PT: *Dickeya* virulence analyses were performed on an extensive *Dickeya* collection. Comparison tests conducted in tuber maceration bioassays involved 109 *Dickeya* strains and representative strains from all known *Dickeya*.
ILVO: in a field trial in Flanders the virulence of different variants of *Dickeya* spp. was assessed, among them *Dickeya dianthicola* GBBC 2039 and *Dickeya solani* GBBC 2040.

Assessment of D. solani survival

JKI: For the assessment of risk of spread of *D. solani* via contaminated machinery and equipment it is important to know how long the bacteria stay viable and thus can be responsible for new infections. Best approach was obtained when potato macerate was used originating from tubers that were previously

inoculated with the pathogens, as in this way the bacteria survived significantly better because of their natural environment and reflect much better the actual conditions in the practice. For this reason, the material slices were contaminated with potato macerate that had previously been gained in tubers with defined bacteria and under defined conditions. Prior to the assessment on the survival ability of *D. solani* on different materials, the aggressiveness of the isolates to be used was evaluated. The isolates DO40 (extremely aggressive), DO36 (highly aggressive) and GBBC2007 (medium aggressive) were identified for the main trials. Also the conditions for the gaining of the potato macerate were identified.

Assessment of *Pectobacterium carotovorum* subsp. *brasiliense* (Pcb) survival in soil

University of Pretoria: The soil (sand, topsoil and compost) was laid out in trays at 27˚C for two days to dry. Three different types of soil containing different organic matter contents viz, sand, loam and compost were used for the experiments. The soil was not autoclaved or pasteurised in order to maintain the integrity of the soil and so as not to affect any physical or chemical properties as well as maintaining the biological activity of the soil. Two different water regimes were used, wet and dry for each soil type in each temperature trial, this was done by maintaining half of the soil replicates at field capacity, while the other half were air dried before use and left dry. Three different temperatures were implemented for the treatments; the soil samples were incubated at 5˚C, 10°C and 25 ± 2°C in available compartments and spaces at the University of Pretoria. The treatments were subdivided into those pots that were inoculated with *Pectobacterium carotovorum* subsp. *brasiliense* (Pcb) in Ringer's solution and those treated with only Ringer's solution (control).
Placements of pots:
• 5˚C was run in the cold room in the basement of the Natural and Agricultural Sciences building.
• 10˚C was run in the conviron in the greenhouse.
• 25 ± 2°C was run in a compartment in the greenhouse.
The combination of both the temperatures and the moisture treatments gives a good representation of overwintering and oversummering conditions that can occur in different cropping regions of South Africa. The pots were arranged in a completely randomised block design with each treatment being randomly assigned a place within the trial area.
The soil will be tested for the presence of the Pcb at 1 day, 1 week, 3 weeks, 6 weeks, 9 weeks 12 weeks after the inoculation of the soil, giving enough time to simulate land lying fallow in a traditional farming system.

Assessment of epidemiological elements

HAFL/Agroscope: Field experiments were set up to explore the relevance of tuber inoculation through the lenticels, as an inoculation method, in order to assess the aggressiveness of *Pectobacterium* and *Dickeya* strains and the susceptibility of the cultivars to blackleg. Therefore, 4 to 19 cultivars were inoculated with one *D. dianthicola* strain (8823) and cv. Agria was inoculated with 5 to 6 *Dickeya* strains. Four hundred seed tubers were inoculated by soaking, then planted in the field (year 1), after which, blackleg symptoms were assessed, 12 to 16 weeks after planting. After harvest, 100 tubers from each plot were stored at 4°C and planted the following year and blackleg symptomatic plants were counted (year 2). This two year trial was repeated twice with respectively 5 and 6 *Dickeya* strains and thrice with respectively 5, 7 and 19 potato cultivars. For each trial, we tested the correlation between the expression of blackleg symptoms in year 1 and year 2.
The question was whether the vascular colonization of the potato plant by *Dickeya* differed according to the nature of the inoculum (soil- or tuber-borne) and whether this has an impact on the expression of symptoms. To better characterize the consequences of colonization of the xylem vessels by *Dickeya*, greenhouse and field trials were carried out with plants infected via mother tuber inoculation or root inoculation. The development of symptoms was tracked, as were changes in plants' transpiration rate, and plant transpiration was used as an index of the early stages of symptom expression.
FN3PT-RD3PT: Epidemiological studies conducted in a joint French-Swiss project included pluri-annual and multisite field trials (8 tuber lots over three years and 5 sites).
JHI: Field experiments have also taken place to determine the spread of *Pectobacterium* in a field situation in order to identify where and when contamination of plant s and tubers ensues. Modelling the distribution and spread of *Pectobacterium* species and the effects of climate change have also allowed national scale assessments of disease to be undertaken

Assessment of control strategies

HAFL/Agroscope: Two disinfection products, 9 bacterial strains, 4 elicitors, 1 essential oil and 15 plant extracts were tested *in vitro* for inhibitory activity against *D. dianthicola* (using liquid culture and petri dish assays). The best candidates of each category were selected for subsequent greenhouse and field trials. For these trials, (artificially) infected tubers were dipped for 15 min. in the candidate product solutions before planting. For the bacteria, pots in the greenhouse and plots in the field were in some cases additionally watered with bacterial suspensions (furrow application). For the elicitors, plants were sprayed weekly during the 2014 field trial. The bacterium Proradix was applied at two different concentrations and combined with Bion as a tuber treatment during the 2015 trial.

Correlation between testing of seed lots and the blackleg and wilt diseases in the field

Gilat Research Center: The main activities were monitoring seed lots infection with the different pectinolytic bacteria and follow the incidence of wilt, blackleg, plants desiccation and progeny tuber infection. In addition, a field trial (randomized blocks with four reps) was set up to follow the kinetics of disease symptoms.

Comparative and functional genomics of emerging *Dickeya* and *Pectobacterium* strains

INRA-UPMC Université Paris: Complete sequencing of the 3337 *D. solani* strain isolated from a diseased potato tuber in France and genomic comparison with the deeply-annotated model strain *D. dadantii* 3937 was performed inside the framework of this project. Next to this work, the draft sequences of 20 *D. solani* isolates from different countries and years of isolation were generated and compared.
University of Helsinki: The genome of a Finnish isolate of *D. solani* was generated.
JHI: Using genome sequencing of multiple *Pectobacterium* and *Dickeya* strains ANI methods have been used to improve on current taxonomic methods and to use this new information to develop specific diagnostic primers.

4. Main results

Surveys on diseased plants and water samples

Natural Resources Institute Finland: The causal agent of blackleg and soft rot in Finland has shifted from a single species to multiple species during the last three decades. At present *Dickeya solani*, *Pectobacterium atrosepticum, Pectobacterium carotovorum*, *Pectobacterium brasiliense* and *Pectobacterium wasabiae* are detected from potato in Finland. The loss from the disease has become worse because of the introduction and establishment of the aggressive species, *D. solani.* The diversity of causal agents implies that blackleg and soft rot are disease complexes (disease syndrome) and results in diagnostic complexity. Because the different species are adapted to different temperature range, blackleg and soft rot have become likely phenomena irrespective of the summer temperatures. *D. solani* dominates and over takes the other species especially in warm summers. Weather, especially temperature, determines which species predominates in a given season. Rivers in the HG area are not contaminated by either *Dickeya* or *Pectobacterium* species. So far *Dickeya* species is detected from one river from a locality 150 km away from the HG area. Only ware potato is produced in the locality. The bacteria have short survival ability in soil. The bacteria do not survive the Finnish winter in overwintering tubers. So far no proof of alternative host (survival in another host) is found for *Dickeya* and *Pectobacterium* in Finland.

FN3PT-RD3PT: Annual surveys evidenced that in France *Pectobacterium* prevailed over *Dickeya* throughout the 2003-2014 period. Strain typing evidenced the presence of *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum* (*Pcc*), *P. wasabiae (Pw), P. carotovorum* subsp. *brasiliense (Pcbr),* and the two *Dickeya* species *D. solani (D. sol)* and *D. dianthicola (D. diant)*. The incidence of the different species and subspecies varied year to year.

SASA: All Scottish seed crops were targeted and inspected twice as part of GCI during the growing seasons of 2013-2015. In 2013, 534 crops were sampled, with 509 and 548 studied in 2014 and 2015 respectively. The incidence of blackleg was found to fluctuate from year to year, with 2014 being the worst with incidence recorded at 42.3%. It is clear from analysis of blackleg causing organisms that the percentage of crops in Scotland infected by *Pectobacterium atrosepticum* remains relatively constant throughout, with approximately 95% of blackleg caused by this bacterium. The remaining 5% of disease symptoms were caused by *P. wasabiae* and *P. carotovorum subsp. carotovorum*. To date there have been only limited findings of *P. carotovorum* subsp. *brasiliense* in Scottish seed crops. Post-harvest tuber testing was also conducted on a sample of pre-basic seed crops. The number of stocks tested was 75 in 2013, 86 in 2014 and 90 in 2015. In each year a sizeable minority of PB1 stocks were found to be infected with *P. atrosepticum* and this percentage increased with each subsequent generation. No other pathogens were found on any of the samples.

HAFL: *Dickeya* spp. was the main pathogen responsible for blackleg incidence in Switzerland since the 90's up to 2012. Then a shift of bacterial population has been observed. In 2014, 80% of the analysed plant samples with blackleg symptoms in Switzerland were attributed to *P. carotovorum* subsp *brasiliense.* The monitoring of the seed lots in 2013 and 2014 showed that the reliability of the test is good for Dickeya spp. with about 5% of false negative. For *P. carotovorum* subsp. *brasiliense* a higher proportion of false positive were observed. Even a high latent infection often did not lead to a disease development in the field. This stays in contrast to *Dickeya* spp. were blackleg was always observed when seed lots with high latent infection were planted. The difference in aggressiveness of the two bacteria has to be tested in field trials. Based on the promising results of the seed testing, Swisssem decided to implement the diagnostic tool for the selection of imported and national seed lots in addition to the visual control during the growing season. The HAFL will continue with the testing service for Swisssem until 2016, then the service will be transferred to Agroscope which is responsible for the seed certification Switzerland. Agroscope will perform the analyses in cooperation with the company BIOREBA AG.

University of Helsinki: The results showed that *D. solani* was fairly common in potatoes grown in Finland in 2011, mainly in imported lots. Especially lots showing dark rot in the upper parts of the stem, as well as light rot in the whole stem, were found to be positive in *Dickeya* analysis. The river waters were collected from Southern Finland and in Southern parts of Ostrobothnia in 2012. The water samples were found to contain lot of soft rot bacteria, which were mainly identified as *Pectobacterium*. Only one river, Seinäjoki, was found positive for *D. solani*. The contamination in Seinäjoki river was present throughout the whole autumn, whereas the next summer (2013) no *Dickeya* was identified in that river anymore. A river running through eastern Helsinki was found to contain *D. zeae* in 2012. No bacteria

could be isolated from voluntary tubers although they were found in several occasions positive in PCR test, so the role of voluntary tubers in spreading bacteria has not been scientifically verified as the positive PCR result can be caused also by dead bacteria. Our results suggested that both the free-living nematodes and insects living in potato storages could spread bacteria.
NVWA: In total 75 symptomatic bulb samples were included for testing including *Hyacynthus*, *Zantedeschia* and *Tulipa.* In 43 *Zantedeschia* samples *Pectobacterium carotovorum* subsp. *carotovorum* has been isolated and identified (Table 1), whereas in 8 *Hyancinthus* samples *Dickeya solani* was found.

Table 1: Results of the survey on different bulbs performed in 2014 in the Netherlands

Host plant	*Dickeya solani*	P.c. subsp. carot	negative	unidentified	Total
Hyacint	8	0	16	1	25
Tulip	0	0	1		1
Zantedeschia	0	43	5	1	49
Total	8	43	22	2	75

Assessment of Virulence

WUR: Information was collected on the relative virulence of *Pectobacterium* and *Dickeya* species. Specific TaqMan assays *P. carotovorum* subsp. *brasiliense* and *P. wasabiae* were developed and evaluated for use by inspection services, national plant protection organizations and in research on these pathogens. Inoculation with *P. carotovorum* subsp. *brasiliense* and *Pectobacterium atrosepticum* resulted in high disease incidences (75-95%), inoculation with *D. solani* and *P. wasabiae* led to incidences between 5% and 25%, but no significant disease development was observed in treatments with *P. carotovorum* subsp. *carotovorum*, *D. dianthicola* and the water control. Co-inoculations of seed potatoes with *P. carotovorum* subsp. *brasiliense* and *D. solani* gave a similar disease incidence to inoculation with only *P. carotovorum* subsp. *brasiliense*. However, co-inoculation of *P. carotovorum* subsp. *brasiliense* with *P. wasabiae* resulted in a decrease in disease incidence compared to inoculation with only *P. carotovorum* subsp. *brasiliense*. In 2015, seed potatoes were inoculated with increasing densities of *P. carotovorum* subsp. *brasiliense*, *D. solani* or *P. atrosepticum* (10^3 – 10^6 cfu ml^{-1}). After vacuum-infiltration, even a low inoculum density resulted into a high disease incidence but if immersion without vacuum was applied, blackleg was only found at high densities. The presence of the pathogens in progeny tubers of plants derived from vacuum-infiltrated seed tubers was confirmed by TaqMan assays.
FN3PT-RD3PT: significant pathogenicity differences were observed between *Dickeya* species, with *D. solani* and *D. dianthicola* not being among the most pathogenic groups on potato. Analyses also revealed strong differences between strains within each species.
University of Helsinki: *Dickeya* was identified to be superior compared to *Pectobacterium* species in double inoculations, and the involved genes possibly coding for a bacteriocin were identified with random transposon mutagenesis. *D. solani* infections in potato tuber can be pinkish sometimes, and that is why the occurrence of pink potatoes was suspected to be caused by bacteria. PCR analysis of the tubers showed that no *Dickeya* was present in the bright pink potatoes, whereas primers for pink rot pathogen *Phytophthora erythroseptica* gave positive results.
ILVO: Starting for infected seed potatoes, all *Dickeya* variants present developed blackleg symptoms in the potato plants. Differences between the *Dickeya* variants were shown for the number of stems (parameter for maceration of the seed potato) (Figure 1) and the number of stems with blackleg symptoms (parameter for proliferation after transmission) (Figure 2).

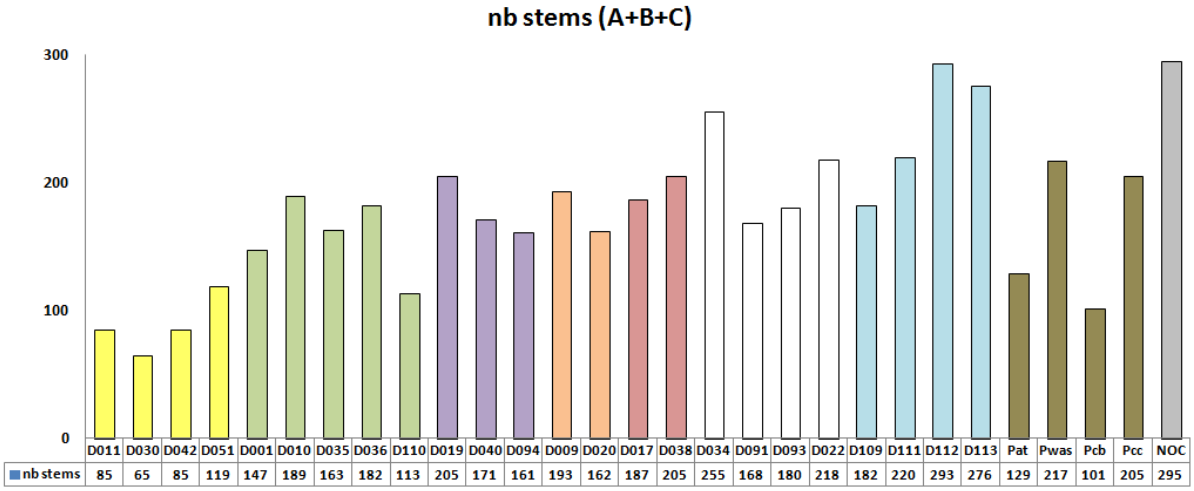

Figure 1: Differences among the Dickeya *variants in the number of stems (parameter for maceration of the seed potato)*

The results clearly demonstrated that cell density had significant influence on the disease. The highest differences among *Dickeya* variants were monitored in treatment C (Figure 3) that refers to the seed potato tubers infected with cell densities of ~10^7 cells/ liter. Infections attributed to *D. dianthicola* isolates were found in 50% of the stems whereas *D. solani* isolates resulted in 52% infected stems. Comparable infections with the ones acquired by *D. solani* were found for *D. dadantii* PD 1680 from *Oncidium* (D094), *Dickeya* sp. from river water in Schotland (D109) and *Dickeya* sp. from river water in Engeland (D111).

On the other hand, less than 10% infected stems were found in the treatment with *Dickeya* spp. GBBC 2007 from lettuce (D022), *Dickeya* spp. from river water in Finland (D112) and *Dickeya* spp. from river water in Engeland.

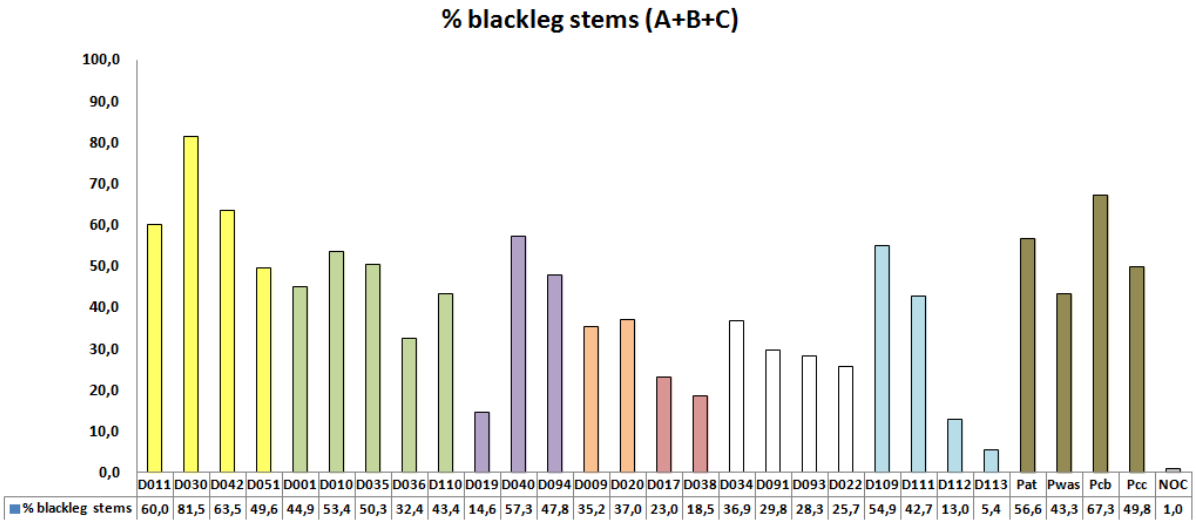

Figure 2: Differences among the Dickeya *variants in the number of stems with blackleg symptoms (parameter for proliferation after transmission)*

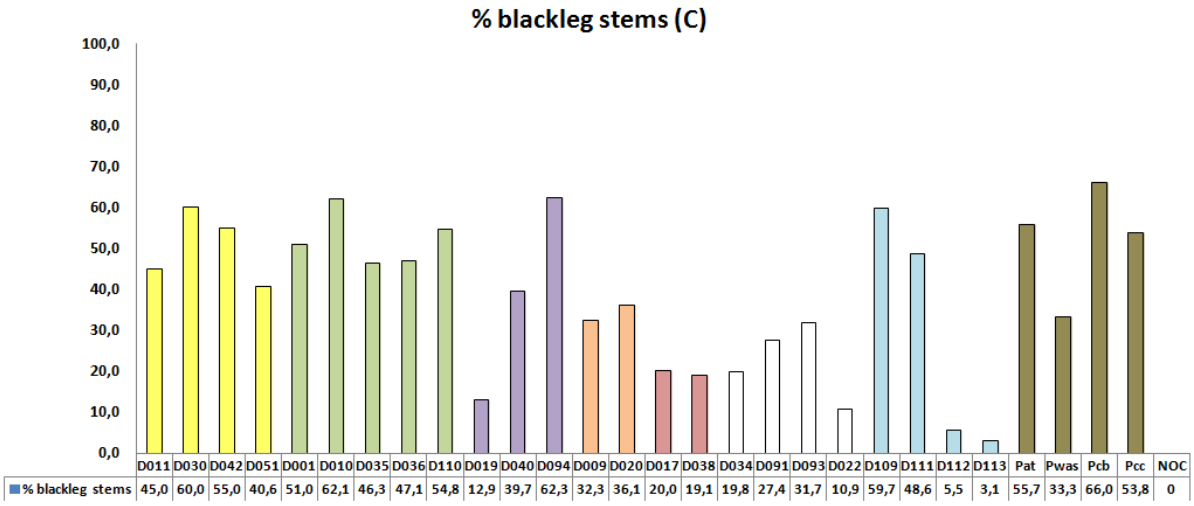

Figure 3: Differences among the Dickeya *variants in the number of stems with blackleg (parameter for proliferation after transmission) in the treatment of seed potatoes with the lowest cell densities.*

Assessment of epidemiological elements

HAFL/Agroscope: The results of trials using inoculation through the lenticels should be interpreted with caution, since they are not taking into account the infections of the progeny tubers through the vascular system of the plant. The results of trials using inoculation through the lenticels are giving a partial indication of the susceptibility of one potato cultivar to various pectinolytic bacteria strains, but can lead to wrong conclusions if the objective of the trial is to distinguish the differential susceptibilities of cultivars to those bacteria. Additionally, the trials revealed that seed- and soil- borne inocula can both induce wilting and blackleg symptoms in potato plants. It was also found that blackleg symptoms appearing during the first three weeks after plant emergence are caused mainly by a tuber-borne inoculum. Finally, it was established that the presence of a latent inoculum in the xylem vessels does not alter the plant transpiration process, as long as there are no wilting symptoms or rotting lesions.
FN3PT-RD3PT: The French-Swiss pluri-annual and multisite study evidenced strong differences in disease development between sites (and years) for the same seed lots due to pedoclimatic condition variations. Seed lots with important latent infection for *Dickeya* expressed disease symptoms in the field with a high probability at all sites. Reliability of seed tubers analyses to predict disease expression was quite consisting for *Dickeya*, but not for *P. atrosepticum.*
JHI: Field level epidemiological studies have identified the stages of crop production when Pectobacterium colonises potato plants and given clues to the source of that colonisation. Modelling has shown that blackleg disease is likely to increase in the future in Europe with climate change and also that clusters of disease on a national scale are apparent for which currently there is no explanation as to the cause.

Assessment of control strategies

HAFL/Agroscope: Disinfection products and bacterial strains showed the strongest inhibition of the growth of *D. dianthicola in vitro*. When applied as tuber treatments, NaClO and the bacterium Proradix, as well as the combination of Proradix and the elicitor Bion showed promising first results in both greenhouse and field trials, despite the observed reduced yield and emergence in the latter. Improvements in terms of treatment concentration and treatment duration should be investigated since inadequate use of the products might be the cause of reduced emergence and yield losses. Further field trials should therefore be carried out in order to validate tuber treatment with a single product as control method against the development of blackleg symptoms.

Assessment of *D. solani* survival

JKI: The initial concentrations of all three isolates (DO40, DO36 and GBBC2007) included in the potato macerate, were of the same magnitude. Isolate DO36 could be detected as the longest viable on the

materials at 5°C and a RH of ~10 %. The survival of isolate DO36 on coated iron, wood, plastic, cotton and rubber at 5°C and 10 % RH referred to for more than 15 weeks after the contamination (Table 2). Under the same trial conditions only with relative air humidity of ~90 % a completely different result could be observed. *D. solani* could be re-isolated from corroded iron after six weeks; which was the longest viability. On coated iron, wood, plastic and rubber the survival could be detected until week 3. The DO36-isolate survived on cotton for one week which is the shortest period (Table 2). It is possible that the longer re-isolation of corroded iron at high RH is related to chemical reactions on the material whereby the vitality of the extraneous plant cover also was affected. The survival ability of the DO36-isolate at 21°C and a ~10 % RH was maximal six weeks on the materials plastic, cotton and rubber. The shortest survival could be observed on wood. In this case a survival for only 24 hours could be observed. The DO36-isolate had the shortest survival rate at 21°C and ~90 % RH. The longest survival period for *D. solani* was two weeks on rubber, followed by corroded iron with one week. The shortest survival period was 24 hours on coated iron, wood, plastic and cotton (Table 2). Also at 21°C and ~90 % RH the difficulties with the extraneous plant covers on the CVP-plates were confirmed. The dense extraneous plant cover even appeared after a shorter exposition period than at a temperature of 5°C. Thus, for the further trials, it was determined to check the variants with 10 % RH first.

Table 2 Survival of the *D. solani*-isolate DO36 on different materials at 5 and 21°C under low and high RH

	Survival ability of *D. solani* in hrs or weeks				
	DO36; 5°C 10% RH; series 1 **	DO36; 5°C; 10% RH; series 2	DO36; 5°C; 90% RH	DO36; 21°C; 10% RH	Do36; 21°C; 90% RH
Corroded iron	7	< 11	6*	3	1*
Coated iron	13	> 15	3*	4	24 hrs.*
Wood	11	> 15	3	24 hrs.	24 hrs.*
Plastic	13	> 15	3*	6*	24 hrs.*
Cotton	11	> 15	1*	6*	24 hrs.*
Rubber	13	> 15	3*	6	2*

*high extraneous contamination on the plates
**the series could not be completed because of insufficient contaminated material slices

It was striking that a much shorter viability could be detected for the DO40-isolate compared with the DO36-isolate (Table 3). In the first series at 5°C and ~10 % RH already after one week a viable isolation of DO40 was impossible so that a second series was prepared. The viability of DO40 at 5°C and ~10 % RH was three weeks on rubber but only one week on corroded iron, wood and cotton. Already after one week no survival could be observed on coated iron and plastic. The longest survival period could be observed for plastic at 21°C (3 weeks). After one week no survival of DO40 was detected on corroded iron. The viability period for the DO40-isolate on all other materials was two weeks. It was shown in both series that the viability of DO40 obviously is limited soon. Possible reasons might be the following: on the one hand the initial macerate was already very decomposed by the pathogen and on the materials no sufficient food source was available for the pathogen to enable a longer survival. On the other hand it might be possible that the extraneous bacteria could develop faster in the watery initial macerate and thus food competition for DO40 could develop earlier.

Table 3 Survival of the *D. solani*-isolates DO40 and GBBC2007 on different materials at 5° C and 21 °C under low RH

	detected survival of *D. solani* until week					
	DO40; 5°C; 10% RH; series 1	DO40; 5°C; 10% RH; series 2	DO40; 21°C; 10% RH; series 1	DO40; 21°C; 10% RH; series 2	GBBC 2007; 5°C; 10% RH	GBBC 2007; 21°C; 10% RH
corroded iron	0*	1	0*	0*	> 9	0*

coated iron	0*	0	2*	0*	> 9	0*
wood	0*	1*	2*	0*	> 9	0*
plastic	0*	0*	3*	0*	> 9	0*
cotton	0*	1*	2*	0*	4	0*
rubber	0*	3*	2*	0*	4	0*

*dense extraneous contamination on the plates

After one week at 21°C and ~10 % RH GBBC2007 could not be detected as viable on none of the tested materials (Table 3). However, at 5°C GBBC2007 was detected viable on cotton and rubber up to four weeks of exposition. The trial period of nine weeks for the materials corroded iron, coated iron, wood and plastic was not yet concluded at the end of the project period. Thus it can only be detected that GBBC2007 can remain viable on these materials at 5°C and 10 % RH for more than nine weeks after the contamination.

Assessment of *Pectobacterium carotovorum* subsp. *brasiliense* (Pcb) survival in soil

University of Pretoria: The isolation results one day after inoculation showed the bacteria were present in abundance, turning the CVP media liquid after the 48-hour incubation period. Colony numbers were approximately equal for both moisture regimes and all soil types, average counts ranged between 70 and 90 colonies per plate.

The control pots containing only Ringers solution showed no Pcb present, except for one pot in the wet compost treatment and one in the wet loam treatment in the 25 ± 2°C trial. Sampling one week after inoculation had varying results, with a trend showing the bacterial survival to be greatest in the compost or loam soils and a greater survival in the wet pots in the 25 ± 2°C. The dry sand showed extremely elevated levels of bacteria while the wet sand had extremely low levels. The wet loam had the highest average colony count while the dry loam had no Pcb colonies. In the 10˚C trial the wet compost showed the greatest colony number while the wet sand showed the lowest. The number of colonies are however more similar than in the 25 ± 2°C trial, with the compost having the greatest colony count in both wet and dry soil and sand having the lowest. In the 5˚C trial the wet compost had the greatest colony count and therefore survival of the bacteria, followed by the sand and then the loam soil. Loam soil showed the lowest colony counts in this trial. The dry compost had lower levels of bacteria compared to the wet compost.

In the 5°C trial the wet compost had the greatest colony count and therefore survival of the bacteria, followed by the sand and then the loam soil. Loam soil showed the lowest colony counts in this trial. The dry compost had lower levels of bacteria compared to the wet compost. The bacteria isolated from the control pots are most likely the result of cross contamination as they were only found in two of the pots. There is however a possibility that they are naturally present in the soil and are a natural contaminant inhabiting the soil. The results show that overall the compost has the greatest average number of colonies. Survival of the bacteria in the sand and loam samples varies dramatically between temperatures and even between wet and dry treatments in the same temperature trials. The low bacterial count in the loam samples in the 0°C trial could be due to the difficulty experienced when trying to mix the soil samples, resulting in uneven distribution of the bacteria and inaccurate measurements.

Characterization of *P. atrosepticum* isolates

SASA: Analysing the concatenated sequence data from 200 strains across 8 housekeeping genes (*dnaJ*, *dnaN*, *gapA*, *gyrB*, *icdA*, *purA*, *recA* and *recN*) showed that considerable diversity existed within *P. atrosepticum*.with strains recovered in 8 major clades (A – H; Figure 4). The largest clade (A) comprised 63 strains, with strains isolated during the 1950s identical to those recovered in 2015. Most strains were isolated in Scotland but there were also isolates from Cambridge and the USA contained within this group. In all, isolates from 29 different cultivars were recovered in clade A from a wide distribution across Scotland. Similar findings were true for the other large clades (D & G). Clade D contains NCPPB 549, the type strain *for P. atrosepticum* which was isolated in 1957 from Israeli grown cv. Majestic. Isolates from South Africa and USA are also recovered in this clade with the bulk of isolates of Scottish origin. As with clade A, isolates from 30 different cultivars are contained within this group and are widely distributed across Scotland. Clade G comprises isolates from the 1950s up to the present

day recovered from Israel, England and Scotland. As with clade A & D, isolates from 23 different cultivars are contained within this group, all widely distributed across Scotland.

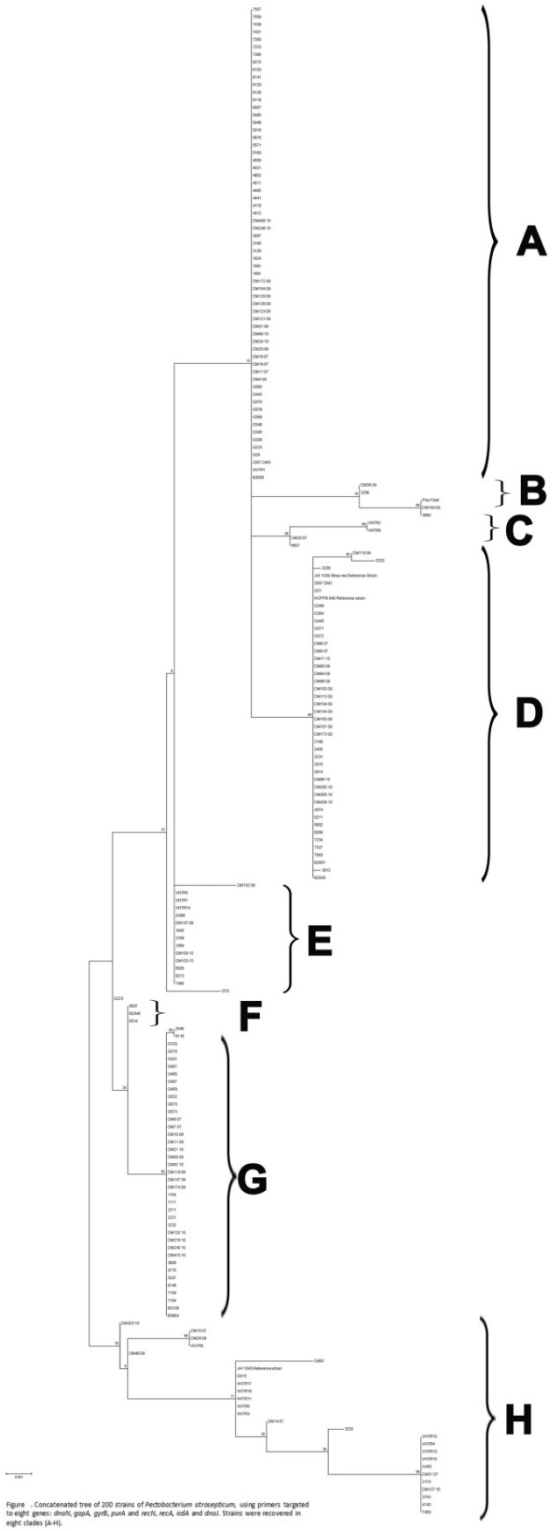

Figure 4. Concatenated tree off 200 strains of Pectobacterium atrosepticum *studied in the MLST analysis using primers targeted to 8 genes:* dnaJ, dnaN, gapA, gyrB, icdA, purA, recA *and* recN*. Strains were recovered in 8 clades (A-H).*

Correlation between testing of seed lots and the blackleg and wilt diseases in the field

Gilat Research Center: In 2014, 73 commercial imported seed lots were checked for latent *D. solani* infection. Field surveys indicated that 37% of negative seed lots had no disease symptoms in fields (-L/-F), 27.4% of positive lots had symptoms (+L/+F). With 5.5% of the seed lots false negative (-L/+F) results were obtained and with 30.1% false positive (+L/-F) (Table 4). In a field trial conducted in 2014, seed tubers from 50 lots randomly selected from the imported material (19 cultivars: 12 Sifra, 8 Mondial, 5 Allians etc.) were planted at Gilat (4 replicates X 50 tubers). Wilt and blackleg symptoms were monitored and presence of pectinolytic bacteria was checked in plants and also in progeny tubers. Plant material or DNA extracts were tested for *D. solani*, *P. atrosepticum, P. carotovorum, and P. c. bresiliense* and *P. wassabiae* by PCR/RT-PCR. *D. solani* was detected in 84% of the tested seed lots, *P. carotovorum* in 72%; *P. c. bresiliense* in 26%; *P. wassabiae* in 52% and *P. atrosepticum* in 28%. Assessment of wilt and blackleg symptoms was done on a weekly basis. Disease incidence in the various cultivars (Fig 5) ranged from 2% to 12%. Presence of bacteria were tested also in the plants derived from the imported seed lots. Plant infection with *D. solani* occurred in 92% of the lots; 12% with *P. carotovorum*, 4% *P. c. bresiliense*, 24% *P. wassabiae* and none with *P. atrosepticum*. In the progeny tubers derived from the imported seed lots, 40% were infected with *D. solani*, 100% *P. carotovorum*, 20% *P. c. bresiliense*, 72% *P. wassabiae* and 38% *P. atrosepticum* (Fig 6).

Table 4: Correlation between lab testing (RT-PCR) of commercial seed lots and the blackleg and wilt diseases in the field

Origin	**+Lab + Field**	**-Lab - Field**	**+Lab - Field**	**-Lab + Field**	**Number of seed lots**
NL	15 29.4%	20 39.2%	13 26.0%	3 5.9%	51
G	3 25.0%	0 0.0%	8 66.7%	1 8.3%	12
F	1 10.0%	7 70.0%	1 10.0%	1 10.0%	10
total	**19** **26.0%**	**27** **37.0%**	**22** **30.1%**	**5** **6.9%**	**73**

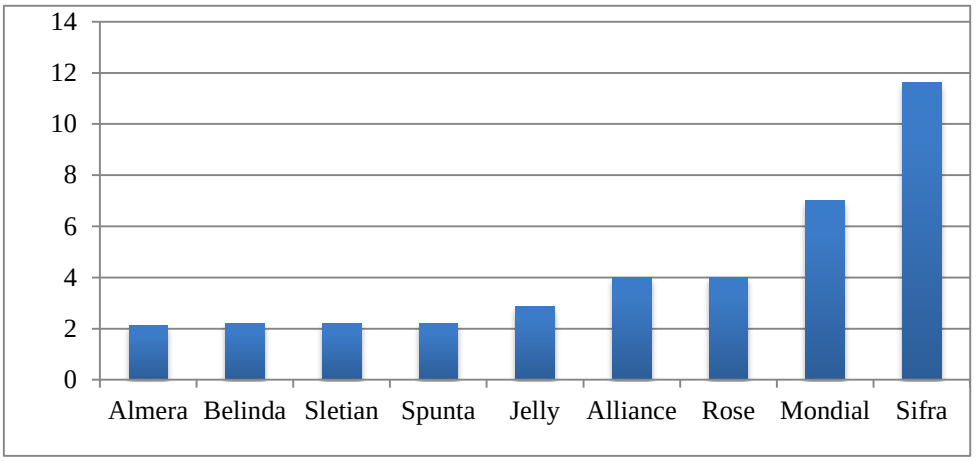

Figure 5: Disease incidence (%) in the infected cultivars

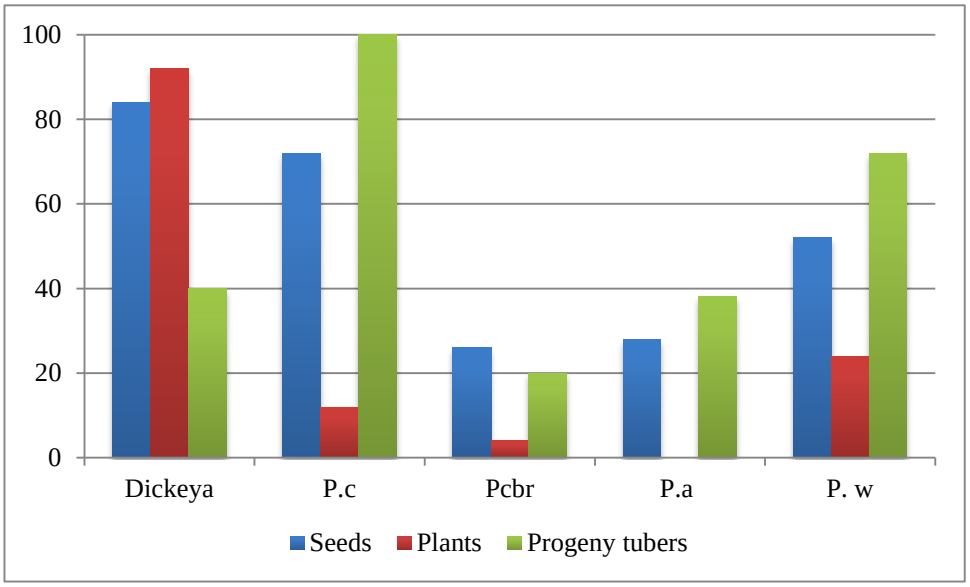

Figure 6: Percentage of infected seed lots, plants and progeny tubers (%).

Comparative and functional genomics of emerging *Dickeya* strains

INRA-UPMC Université Paris: Complete sequencing of the 3337 *D. solani* strain isolated from a diseased potato tuber in France and genomic comparison with the deeply-annotated model *D. dadantii* 3937 revealed that, while a large majority of *Dickeya* virulence genes are shared by both strains, a few hundrends genes of *D. solani* 3337, mostly regrouped in 25 genomic regions, are distinctive to *D. dadantii 3937.* These genomic regions are present in the other available draft genomes of *D. solani* strains and interestingly some of them were not found in the sequenced genomes of the other *Dickeya* species. These genomic regions regroup metabolic genes often accompanied by genes involved in transport systems. A wide metabolic analysis correlated some metabolic genes with distinctive functional traits of both *D. solani* 3337 and *D. dadantii* 3937. Three identified *D. solani* genomic regions also regroup NRPS/PKS encoding genes that might be involved in the synthesis of secondary metabolites. In addition, *D. solani* encodes a distinctive arsenal of T5SS and T6SS-related of toxin-antitoxin systems. Draft sequencing of 20 *D. solani* isolates from different countries, years of isolation or hosts reveals that most of the strains (the core-population) collected in France and Europe are very close genetically: this is a typical trait of emerging pathogens. A few of strains exhibited however a more important number of variations due to acquisition of large regions by additive and/or replacing horizontal gene transfers and one strain was clearly more divergent, constituting a distinct clade in the otherwise clonal *D. solani* species.

University of Helsinki: The genome of a Finnish isolate of *D. solani* was generated and found to contain many gene islands possibly involved in production of toxic metabolites.
JHI: ANI and comparative genomics methods have successfully led to re-categorisation of *Dickeya* and *Pectobacterium* strains and potentially to the identification of new species and subspecies. This information has allowed accurate diagnostic primers to be developed for both species.

5. Conclusions and recommendations to policy makers

Findings based on surveys

Natural Resources Institute Finland: As in many plant diseases, there is no one single solution or control method for blackleg and soft rot. Chemotherapy and thermotherapy are not practical since the disease is seed or tuber borne. It is therefore recommended to start healthy (healthy seed) and try to remain healthy through vigilant monitoring and disease surveillance. Testing the seed tuber is crucial. Most of the severe outbreaks observed in Finland are on fields where non certified seed commonly called home saved seeds are used. In Finland *Dickeya* and *Pectobacterium* are not in the list of pathogens for mandatory testing by the Food Safety Authority. Mandatory testing and practice of zero tolerance especially for *D. solani* will be useful to minimize the threat especially from this aggressive species.

FN3PT-RD3PT: Annual surveys revealed that blackleg symptoms are associated to a bacterial species complex including *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum* (*Pcc*), *P. wasabiae (Pw), P. carotovorum* subsp. *brasiliense (Pcbr),* and the two *Dickeya* species *D. solani (D. sol)* and *D. dianthicola (D. diant)*.

SASA: Over the 3 years 2013-15 approx. 30-40% of seed crops were found to have some level of blackleg present by GCI. It is clear that in the vast majority of cases (approximately 95%) blackleg is caused by *P. atrosepticum*. Although other pathogens were found (other *Pectobacterium* spp.), they were only found in small numbers and at no time were any *Dickeya* spp. detected in any plants exhibiting blackleg as identified at GCI. This is in stark contrast to other European countries where a greater mix of blackleg –causing organisms can be found and the situation appears to be more dynamic with marked shifts in the relative proportion of major pathogens found in any given year.

A limited survey of pre-basic seed crops, based on post-harvest tuber testing showed that a sizeable minority of PB1 stocks were found to be infected with *P. atrosepticum* and the percentage increased with each subsequent generation (PB1 at 2-20%; PB2 33-50%; PB3 44-65%; PB4 100%). In this part of the study *P. atrosepticum* was the only blackleg-causing pathogen detected. It is clear that *P. atrosepticum* is endemic in Scotland and that most seed crops become infected by pathogen in the 3rd or 4th field generation. It is also clear that existing measures enshrined in the Scottish seed classification scheme (but also likely to be true for classification schemes elsewhere in Europe) are limited in their ability to limit disease spread. As a consequence, a coordinated approach to tightening up disease seed classification schemes and moving towards a more rigid approach to hygiene is likely to generate real benefits across the supply chain.

University of Helsinki: The potato inspectors should be educated to suspect that rotting present in the upper parts of the stems should be suspected as blackleg caused by *D. solani*, and not necessarily as a result of aerial contamination. Cutting the stems in half reveals if the bacteria has spread from seed tuber in the central parts of the stem without visible symptoms on the outer layers of the stem. Control of insects in potato storages might reduce spreading of contamination to higher seed classes in case soft rot of tubers is evident in the storage room.

Assessment of Virulence

WUR: A rapid change in population structure of soft rot Enterobactericeae (SRE) occurred during the last years. It indicates that frequent surveys of blackleg and soft rot causing variants of the pathogens is required. Reliable diagnostic tools were developed for the different variants of SRE currently occurring in Europe.

University of Helsinki: Since *D. solani* appeared to be highly virulent, efficient in spreading in various ways and able to outcompete Pectobacteria, it should be taken seriously. If possible, all seed potato lots with high seed grades should be tested for the presence of *D. solani*.

Assessment of epidemiological elements

JHI: Field experiments have provided clues to the source of contamination of potato plants and tubers and provided information to help with field inspections and tuber quality. Modelling has highlighted the need to better understand on a national scale the localised disease outbreaks with a view to reducing disease impact on the future. New accurate classification is now available for this group of pathogens that can be used by policy makers to identify accurately the pathogens responsible for disease. Diagnostics are now available to help detect and identify these pathogens with much more accuracy than was previously available.

FN3PT-RD3PT: Extrapolation of seed tubers analyses to predict disease expression to commercial seed lots requires further work.

Assessment of D. solani survival

JKI: The trials on the survival of *D. solani* have shown that the survival period highly depends on the isolate and the environmental conditions. Despite of the use of the CVP-medium the examinations at ~90 % RH were affected by extraneous vegetation and the recovery rate for viable *D. solani* bacteria might be too low as growth on the CVP-plates may be suppressed by the extraneous bacteria. So even for the aggressive isolate DO36 the maximum survival period varied from a few hours to a maximum of 2 weeks at 21°C and from one to a maximum of six weeks at 5°C. It could also be concluded that the survival period depends on the isolates and their biological characteristics in respect to the aggressiveness. The isolate DO40 showed a very short survival period under all tested conditions. In contrast, the isolates DO36 and GBBC2007 survived at 5°C and ~10 % RH for several months. Also the comparison of the materials which were contaminated with *D. solani* showed different viability periods of the isolates. It is not possible to make a clear statement for the material with the longest survival period of *D. solani* which in turn, depends on the tested isolate and the prevailing conditions. Nevertheless, over the test series there is a tendency that *D. solani* has the worst surviving chances on corroded iron and is able to survive relatively well on rubber and plastic.

Characterization of P. atrosepticum isolates

SASA: Characterisation studies of *P. atrosepticum* showed that a degree of diversity exists between strains. However, there is little evidence that new centres of diversity are responsible for the increase in blackleg in recent years as isolates recovered from diseased plants in 2015 were found to be identical to those isolated 50 years previously.

Correlation between testing of seed lots and the blackleg and wilt diseases in the field

Gilat Research Center: A direct correlation between seed infection and disease expression in the field was observed. It is recommended to check the imported seed material prior to planting, thus providing the grower an important tool which assist in making decisions on optimal use of the material in Israel. Cultivars differ in their sensitivity to *D. solani*; e.g. Sifra and Mondial are susceptible ones.

6. Benefits from trans-national cooperation

The EUPHRESCO II *Dickeya* and *Pectobacterium* initiative has opened a new chapter bringing together over 30 experts from 17 European countries. The partnership has effectively helped for exchange of expertise and research material among member countries and institutions and has made credible contribution and benefited from cooperation. In the face of shrinking government budget and investment in agriculture and diminishing human resource capacity building, networking maximizes impact. The research network is a good example of joint effort to tackle the problem of blackleg in Europe through cooperation and coordination between diagnostic laboratories. Additionally, this transnational cooperation provides the foundation for a long-lasting cooperation which might lead to cooperation beyond the actual project. In total, benefits are enormous as sharing information on taxonomy and diagnostics will ensure a European-wide optimised and standardised system of testing.

Based on this trans-national cooperation, it has clearly demonstrated that the blackleg situation is very different among the different countries. At present, representatives of *Dickeya solani*, *Pectobacterium atrosepticum, Pectobacterium carotovorum*, *Pectobacterium brasiliense* and *Pectobacterium wasabiae* are all detected in the potato system through Europe, and beyond in some occasions. It is only by the free-exchange of information between partners that we are able to compare and contrast. The differences between countries also highlight the possible role that climate may have by selecting the pathogens which are problematic in any given region or country. Especially, exchange of knowledge on the virulence of the different variants and exchange of information on diagnostic tools is helpful to manage diseases caused by SRE. Field work has been carried out to complement rather than duplicate work carried elsewhere in Europe so that the results on a European scale can be maximised and shared. Information on modelling in one country can be extrapolated to other countries and even the whole of Europe with a view to sharing ideas and expertise about the causes of disease clustering, along with the possible causes and solutions.

7. Publications

- Ansermet Marion, Santiago Schaerer, Isabelle Kellenberger, Maud Tallant, Brice Dupuis (2016) Influence of seed- borne and soil- carried inocula of *Dickeya* spp. on potato plant transpiration and symptom expression. *European Journal of Plant Pathology,* 145(2). DOI:10.1007/s10658-016-0859-0.
- Czajkowski Robert, Perombelon M. C. M., Jafra Sylwia, Łojkowska Ewa, Potrykus Marta, van der Wolf Jan M., Śledź Wojciech. Detection, identification and differentiation of Pectobacterium and Dickeya species causing potato blackleg and tuber soft rot: a review. Annals of Applied Biology 2015, 166(1): 474-487 (doi: 10.1111/aab.12166).
- Czajkowski, R., Perombelon, M.C.M., Jafra, S., Lojkowska, E., Potrykus, M., van der Wolf, J.M. & Sledz, W. 2014. Detection, identification and differentiation of *Pectobacterium* and *Dickeya* species causing potato blackleg: a review. Annals of Applied Biology. DOI 10.1111/aab.12166.
- Degefu Y., P. Somervuo, M. Aittamaa, E. Virtanen and J. P. T. Valkonen. (2016). Evaluation of microarray in the detection of major bacterial pathogens of potato from tubers. EPPO Bulletin 46:103-111.
- Degefu Yeshitila , Marta Potrykus, Malgorzata Golanowska, Elina Virtanen1& Ewa Lojkowska (2013). A new clade of *Dickeya* spp. plays a major role in potato blackleg outbreaks in North Finland. Annals of Applied Biology 162, 231–241.
- Degefu Yeshitila (2016) Monitoring *Dickeya* and *Pectobacterium* species in the High Grade seed potato production area of Finland. Project Report to the Ministry of Agriculture and Forestry (MMM) of Finland. 12P.
- Degefu Yeshitila and Elina Virtanen (2015) a decade of monitoring, detection and characterization of *Dickeya* and Pectobacterium species in the High Grade seed potato growing area of Finland: an overview. Tuottava Peruna 2:22-24.
- Degefu Yeshitila (2015) *Dickeya* and *Pectobacterium* species: consistent threats to potato production in Europe 13p. https://portal.mtt.fi/portal/page/portal/kasper/pelto/peruna/Potatonow/tutkimus
- de Werra Patrice, Floriane Bussereau, Dominik Ziegler, Andreas Keiser (2015) First report of potato blackleg caused by *Pectobacterium carotovorum* subsp. *brasiliense* in Switzerland. *Plant Disease*, 99 (4), 551.
- de Werra Patrice, Floriane Bussereau, Isabelle Kellenberger, Brice Dupuis, Santiago Schaerer, Andreas Keiser (2015) Potato: the *Pectobacterium* Empire strikes back. *Agrarforschung Schweiz,* 6(6).
- Dubois G E, Hélias V, Schaerer S, Dupuis B. 2014. Efficient inoculation method of potato tubers with *Dickeya* spp. 19th Triennial Conference of the EAPR, July 6-11, Brussels: 237
- Garlant L, Koskinen P, Rouhiainen L, Laine P, Paulin L, Auvinen P, Holm L, Pirhonen M (2013). Genome sequence of "*Dickeya solani*", a new soft rot pathogen of potato, suggests its emergence may be related to novel combination of non-ribosomal peptide/polyketide synthetase clusters. Diversity 5(4):824-842.
- Golanowska Małgorzata, Galardini M., Bazzicalupo M., Hugouvieux-Cotte-Pattat Nicole, Mengoni Alessio, Potrykus Marta, Sławiak Monika, Łojkowska Ewa. Draft genome sequence of highly virulent strain of the plant pathogen Dickeya solani, IFB0099. Genome Announcements 2015, art. no e00109-15 (1-2) (doi: 10.1128/genomeA.00109-15).
- Hannukkala A (2011). Examples of alien pathogens in Finnish potato production - their introduction, establishment and consequences. Agricultural and Food Science 20 1: 42-61.
- Hannukkala A 2011. Recent changes in Nordic potato diseases and pathogens. In: Abstracts of the European Association for Potato Research Pathology Section Meeting 2010 on: Potato Pests and Diseases: Old Enemies, New Threats held at Carlow, Ireland, 13th-16th September 2010 / Edited by Louise Cooke. Potato Research 54: 83-84.
- Hélias V, Laurent A, Gauthier JP, Le Hingrat Y and Andrivon D. 2014. Screening *Dickeya* pathogenicity to potato shows inter- and intraspecific diversity. Potato Research 57:175.
- Hélias V, Quêtu-Laurent A, Le Hingrat Y, Andrivon D.2014 potato blackleg survey in France : overview of the associated bacterial species complex. Euphresco II meeting, November 24-25, Bern, Switzerland.
- Hélias V, Laurent A, Morel B, Le Hingrat Y., Andrivon D. 2013. Characterization of the blackleg bacterial species complex present in 2013 on potato in France. Euphresco II meeting, 22-23 November, Jerusalem, Israël.

- Hélias V, Laurent A, Morel B, Gauthier J P, Andrivon D, Le Hingrat Y. 2014. Characterization of the potato blackleg bacterial species complex in France. 19th Triennial Conference of the EAPR, July 6-11, Brussels: 110.
- Keiser A., de Werra P., Hélias V. 2013. French-Swiss concerted project 2010 -2012 assessment: Influence of seed contamination and site parameters on the disease development of *Dickeya* and *Pectobacterium* in the field.. Euphresco II meeting, 22-23 November, Jerusalem, Israël.
- Khayi S, Raoul des Essarts Y, Quêtu-Laurent A, Moumni M, Hélias V, Faure D. 2014. Genomic overview of the phytopathogen *Pectobacterium wasabiae* strain RNS 08.42.1A suggests horizontal acquisition of quorum-sensing genes. Genetica. 143: 241-252. doi:10.1007/s10709-014-9793-2.
- Khayi S, Mondy S, Beury-Cirou A, Moumni M, Hélias V, Faure D. 2014. Genome sequence of the emerging plant pathogen Dickeya solani strain RNS 08.23.3.1A. Genome Announc. 2(1): e01270-13. doi:10.1128/genomeA.01270-13.
- Koskinen JP, Laine P, Niemi O, Nykyri N, Harjunpää H, Auvinen P, Paulin L, Pirhonen M, Palva ET, Holm L (2012). Genome Sequence of *Pectobacterium* sp. SCC3193. Journal of Bacteriology 194(2):7020.
- Laurent A., Morel B., Le Hingrat Y., Andrivon D, Hélias V. 2014. Developing a 'friendly' diagnostic method to identify *Dickeya* species. 19th Triennial Conference of the EAPR, July 6-11, Brussels: 233.
- Lebiush S, Manulis-Sasson S, Erlich O, Chalupowicz L, Hazanovsky M and Tsror L. 2014. Virulence of *Dickeya solani* strains from Israel compared with strains from Europe in different potato cultivars. Potato Research 57:174.
- Motyka Agata, Żołędowska Sabina, Śledź Wojciech, Potrykus Marta, Golanowska Małgorzata, Butrymowicz J., Kołodyńska A., Łojkowska Ewa.Monitoring of seed potato plantations and surface waters for plant pathogenic bacteria from the genera Dickeya and Pectobacterium. New Biotechnology 2016, 33: S45 (doi: 10.1016/j.nbt.2016.06.881).
- Niemi O, Nykyri J, Koskinen P, Nokso-Koivisto J, Pasanen M, Broberg M, Plyusnin I, Törönen P, Holm L, Pirhonen M, Palva ET (2012). Revised phylogeny and novel horizontally acquired virulence determinants of the model soft rot phytopathogen *Pectobacterium wasabiae* SCC3193. PLOS Pathogens 8(11): e1003013.
- Nunes-Leite, L., De Haan, E.G., Krijger, M., Kastelein, P., Van der Zouwen, P.S., van den Bovenkamp, G.W., Tebaldi, N.D. & van der Wolf, J.M. 2014. First report of potato blackleg caused by *Pectobacterium carotovorum* subsp. *brasiliensis* in the Netherlands. New Disease Reports. doi.org/10.5197/j.2044-0588.2014.029.024.
- Nykyri J, Fang X, Dorati F, Bakr R, Pasanen M, Niemi O, Palva ET, Jackson R, Pirhonen M (2013). Evidence that nematodes may vector the soft rot-causing enterobacterial phytopathogens. Plant Pathology 63(4):747-757.
- Parkinson N, Pirhonen M, Elphinstone J (2014*). Dickeya aquatica* sp nov., isolated from waterways. *Int J Syst Evol Microbiol 64(7):* 2264-2266.
- Parkinson, N., Pritchard, L., Bryant R., Toth I.K. & Elphinstone, J. 2014. Epidemiology of *Dickeya dianthicola* and *Dickeya solani* in ornamental hosts and potato studied using variable number tandem repeat analysis. *European Journal of Plant Pathology* 141, 63-70.
- Pasanen M, Laurila J, Brader G, Palva ET, Ahola V, van der Wolf J, Hannukkala A, Pirhonen M (2013). Isolation of *Pectobacterium wasabiae* and atypical *P. carotovorum* subsp. *carotovorum* strains from diseased potato plants in Finland. Annals of Applied Biology 163(3):403-419.
- Pédron J, Mondy S, Raoul des Essarts Y, Van Gijsegem F, Faure D. 2014. Genomic and metabolic comparison with Dickeya dadantii 3937 reveals the emerging Dickeya solani potato pathogen to display distinctive metabolic activities and T5SS/T6SS-related toxin repertoire. *BMC Genomics* 15:283.
- Potrykus Marta, Golanowska Małgorzata, Śledź Wojciech, Żołędowska Sabina, Motyka Agata, Kołodziejska Anna, Butrymowicz Janina, Łojkowska Ewa.Biodiversity of Dickeya spp. isolated from potato plants and water sources in temperate climate. Plant Disease 2016, 100(2): 408-417 (doi:10.1094/PDIS-04-15-0439-RE).
- Potrykus Marta, Golanowska Małgorzata, Hugouvieux-Cotte-Pattat Nicole, Łojkowska Ewa. Regulators involved in Dickeya solani virulence, genetic conservation, and functional variability. Molecular Plant-Microbe Interactions 2014, 27(7): 700-711 (doi: 10.1094/MPMI-09-13-0270-R).

- Potrykus Marta, Śledź Wojciech, Golanowska Małgorzata, Sławiak Monika, Binek Aleksandra, Motyka Agata, Żołędowska Sabina, Czajkowski Robert, Łojkowska Ewa. Simultanoeous detection of major blackleg and soft rot bacterial pathogens in potato by multiplex polymerase chain reaction.
- Annals of Applied Biology 2014, 165(3): 474-487 (doi: 10.1111/aab.12156).
- Pritchard L, Humphris S, Baeyen S, Maes M, Van Vaerenbergh J, Elphinstone J, Saddler G, Toth I. Draft Genome Sequences of Four Dickeya dianthicola and Four Dickeya solani Strains.Genome Announc. 2013 1(4). pii: e00087-12.
- Pritchard L., Humphris, S., Saddler, G. S., Parkinson, N. M., Bertrand, V., Elphinstone, J. G. & Toth I. K. 2013. Detection of phytopathogens of the genus *Dickeya* using a PCR primer prediction pipeline for draft bacterial genome sequences. *Plant Pathology* 62, 587-596.
- Pritchard L, Glover R.H., Humphris S., Elphinstone J.G and Toth I.K. 2016. Genomics and taxonomy in diagnostics for food security: soft rotting enterobacterial plant pathogens. Analytical Methods 8**,** 12-24.
- Pritchard L, Humphris S, Saddler GS, Elphinstone JG, Pirhonen M, Toth IK (2013). Draft Genome Sequences of 17 isolates of the plant pathogenic bacterium Dickeya. Genome Announcements 1(6): e00978-13.
- Skelsey P., Elphinstone J. G., Saddler G. S., Wale S. J., and Toth I. K. 2015. Spatial analysis of blackleg-affected seed potato crops in Scotland. Plant Pathology 65**,** 570-576.
- Toth, I.K., Humphris S., Campbell, E. & Pritchard, L. 2015. Why genomics research on *Pectobacterium* and *Dickeya* makes a difference. *American Journal of Potato Research* 92**,** 218-222.
- Van Vaerenbergh J, S. Baeyen, P. De Vos & M. Maes. 2012. Sequence diversity in the *Dickeya* fliC gene: phylogeny of the *Dickeya* genus and TaqMan® PCR for 'D. solani', new biovar 3 variant on potato in Europe. PLoS ONE, Volume 7 | Issue 5: e35738.
- Waleron Małgorzata, Waleron Krzysztof, Łojkowska Ewa. First report of Pectobacterium carotovorum subsp. brasiliense causing soft rot on potato and other vegetables in Poland. Plant Disease 2015, 99(9): 1271 (doi:10.1094/PDIS-02-15-0180-PDN).
- van der Wolf, J.M., Nijhuis, E.H., Kowalewska, M.J., Saddler, G.S., Parkinson, N., Elphinstone, J.G., Pritchard, L., Toth, I.K., Lojkowska, E., Potrykus, M., Waleron, M., de Vos, P., Cleenwerck, I., Pirhonen, M., Garlant, L., Hélias, V., Pothier, J.F., Pflüger, V., Duffy, B., Tsror, L. & Manulis, S. 2013. *Dickeya solani* sp. nov., a pectinolytic plant pathogenic bacterium isolated from potato (*Solanum tuberosum*). *International Journal of Systematic and Evolutionary Microbiol*ogy 64, 768-774.